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LITERATURE REVIEW OF MOLECULAR METHODS FOR SIMULTANEOUS DETECTION OF PATHOGENS IN WATER

Prepared in Association with

USEPA workshop on Innovative Approaches for Detecting Microorganisms in Water
June 18-20, 2007
Andrew W. Breidenbach Environmental Research Center

Cincinnati, OH

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INTRODUCTION

This literature search was prepared under contract for USEPA's Environmental Technology Council (ETC). It supports ETC's mission for identifying needed research or technologies important to addressing decisions and/or policy making issues associated with the assessment of microorganisms in water. The search supported a USEPA workshop on Innovative Approaches for Detecting Microorganisms in Water, held June 18-20, 2007 at the Andrew W. Breidenbach Environmental Research Center in Cincinnati, OH. It is a review of molecular technologies (qPCR, microarray, microfluidics and lab-on-a-chip) for simultaneous detection of multiple waterborne pathogens in order to understand the state of the technology. The search content focuses on:

- Pathogen detection without DNA or RNA amplification
- Pathogen detection with DNA or RNA amplification
- Sample concentration methodologies
- Whole methods (concentration and detection without DNA or RNA amplification)
- Whole methods (concentration and detection with DNA or RNA amplification)
- Molecular methods that differentiate viable versus non-viable microorganisms

While the primary focus is on waterborne pathogens in potable water, the literature search includes wastewater and emerging clinical methodologies as these may be

transferable to potable water applications and reports on recent technological developments covering advances made in the last ten years.

BACKGROUND

Waterborne pathogens continue to contaminate drinking water supplies and cause waterborne disease outbreaks (WBDO) despite current regulations designed to prevent and control their spread. In the United States, from 1991-2000, 173 waterborne outbreaks and 432,733 cases of illness were reported in public and individual water systems. In 1993, an estimated 400,000 people became ill in Milwaukee, Wisconsin after drinking water contaminated with *Cryptosporidium parvum*, a waterborne pathogen that contaminates drinking water supplies. Annually, the CDC estimates that pathogen infected drinking water results in about a million new cases of illness and about a thousand deaths. Most cases, however, are not reported to health care providers because of their self-limiting nature and that do not result in WBDOs.

EPA currently regulates two indicators of microbiological drinking water quality: total coliform and turbidity. A challenge for water utilities is that current indicators of water quality do not detect all types of microbial contamination since there is a great diversity of microbial pathogens. For example, some pathogens do not co-occur with indicators of fecal pollution. Another weakness of bacterial indicators for water is their greater sensitivity to disinfection, relative to that of viruses and protozoan cysts. This sensitivity results in a lack of consistent correlations between indicator absence and the absence of pathogens. Quantitative methods are needed that can detect a broad array of microorganisms, particularly those which may occur when traditional indicators are not detected. Additionally, the turn around time for current cultural and/or detection methods for waterborne microorganisms is relatively long ($\geq$ 24 hours). Rapid or near real-time analytical methods, which give results in one to eight hours and not days, are needed.

Challenges associated with detecting pathogens in potable environmental water samples are:

- **Low densities:** Many pathogens occur at very low densities in waters which contain natural substances that interfere with microbial analyses. Certain pathogens (especially viruses) that are present at these densities have been demonstrated to cause disease and are a threat to public health.
- **Method involves large volumes of water:** In order to detect these low levels of pathogens large volumes of water need to be concentrated and tested. This usually results in co-concentration of interfering substances present in the water that limits downstream applications A closely associated problem with the concentration step are the filters used to extract pathogens from large volumes of water samples. The filters have poor recovery efficiencies with a wide standard deviation. They are also expensive making routine individual waterborne pathogen monitoring impractical. Improvements in methods are needed that will allow detection of pathogens which have very low infectious doses at low densities.
- **Simultaneous detection:** Each class of pathogens (such as viruses, bacteria, protozoa) has a distinct characteristic. They differ in size, morphology, structure, pathogenesis and susceptibility to disinfection. These differences make it difficult to isolate all the pathogens simultaneously from environmental waters before detection and identification strategies can be applied.
- **Detection of live organisms:** Many molecular methods currently available do not detect the presence of live pathogens. Methodologies must have the ability to discriminate living pathogens from those that are not viable to detect genuine health risks. The methods that do detect the presence of live pathogens are either very slow to produce results or cannot be used for all strains of a particular class of pathogens.

ETC ACTION TEAM

ETC was established to enhance communication and coordination of EPA technology activities. The Council has membership from EPA technology programs, offices, and regions and meets on a regular basis to discuss technology solutions, technology needs and program synergies. The specific action team listed below was responsible for the generation of this literature search.

Action Team: Rapid Detection of Microbial Contamination of Water: Application of Molecular Technologies to Source and Potable Water Monitoring (http://www.epa.gov/etop/forum/problem/microarray_technology.html)

ETC Team Leaders:

Keya Sen - Office of Water, Technical Support Center
513-569-7026
sen.keya@epa.gov

Sam Hayes, Office of Research and Development, National Risk Management Research Laboratory
513-569-7514
hayes.sam@epa.gov

EPA Action Team Members:

James Sinclair, Office of Water, Technical Support Center
513-569-7970
sinclair.james@epa.gov

Nicole Brinkman, Office of Research and Development, National Exposure Research Laboratory
513-569-7315
brinkman.nichole@epa.gov

Sandhya Parshionikar, Office of Water, Technical Support Center
513-569-7123
parshionikar.sandhya@epa.gov

Angela Page, Office of Research and Development, National Center for Environmental Research
202-343-9826
page.angelad@epa.gov

Marilyn Marshall, University of Arizona
520-621-1469
marshalm@u.arizona.edu

Misha Hasan, American Water Works Research Foundation
MHasan@awwarf.org

Patricia Ritchey, U.S. EPA, Region 7
913-551-7409
ritchey.patricia@epa.gov

Robert Fuerst, Office of Research and Development, National Risk Management Research Laboratory
919-541-2220
fuerst.robert@epa.gov

The literature search was performed and compiled by Analytical Consulting Services, LLC. R. Paul Schaudies, Ph.D. and Doreen A. Robinson, Ph.D. were the main contributors to its creation. The Prime Contractor was SAIC and the work was performed under contract #68-C-02-067, WA #4-129.

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CONCENTRATION AND SAMPLE PREPARATION

The relatively low abundance of pathogenic organisms in water frequently requires that samples be concentrated prior to analysis. The challenge is to concentrate the targets of interest while separating them from the other material of equivalent size and/or densities. The simplest concentration method is filtration. Filtration mechanisms range from standard flat membranes with a specific pore size to hollow fiber filtration. Another method for bulk concentration of water samples is centrifugation. In-line centrifugation is an effective means to concentrate large volumes, but it does no real separation. Unfortunately, both of these general methods concentrate particulate material and have variable collection efficiencies.

Specific concentration is achieved using specific binding elements. One approach is to use compounds such as siderophores, or other such molecules that bind a wide variety of bacterial species. Antibodies directetd at conserved extracellular molecules also provide a means of specifically removing the material of interest.

While these methods more effectively concentrate the target material, they take longer to process large volumes of water than general methods. A variety of extraction kits are commercially available for extracting nucleic acids from samples, but most are limited in terms of the volumes they can handle. Comparisons of the various kits are the topic of several of the articles below.

General Methods

Rutjes SA, Italiaander R, van den Berg HH, Lodder WJ, de Roda Husman AM. (2005) Isolation and detection of enterovirus RNA from large-volume water samples by using the NucliSens miniMAG system and real-time nucleic acid sequence-based amplification. Appl Environ Microbiol. 71(7):3734-40.

Concentration of water samples is a prerequisite for the detection of the low virus levels that are present in water and may present a public health hazard. The aim of this study was to develop a rapid, standardized molecular method for the detection of enteroviruses in large-volume surface water samples, using a concentration method suitable for the detection of infectious viruses as well as virus RNA. Concentration of water was achieved by a conventional filter adsorption-elution method and ultrafiltration, resulting in a 10,000-fold concentration of the sample. Isolation of virus RNA by a silica-based RNA extraction method was compared with the nonmagnetic and magnetic NucliSens RNA isolation methods. By using the silica-based RNA extraction method in two out of five samples, enterovirus RNA was detected, whereas four out of five samples were positive following RNA isolation with magnetic silica beads. Moreover, estimated RNA levels increased at least 100 to 500 times. Furthermore, we compared enterovirus detection by an in-house reverse transcription (RT)-PCR with a novel commercially available real-time nucleic acid sequence-based amplification (NASBA) assay. We found

that the rapid real-time NASBA assay was slightly less sensitive than our in-house RT-PCR. The advantages, however, of a commercial real-time NASBA assay, like the presence of an internal control RNA, standardization, and enormous decrease in turnaround time, makes it an attractive alternative to RT-PCR.

PMID: 16000783 [PubMed - indexed for MEDLINE]

Mumy KL, Findlay RH. (2004) Convenient determination of DNA extraction efficiency using an external DNA recovery standard and quantitative-competitive PCR. J Microbiol Methods. 57(2):259-68.

Molecular biology techniques have advanced the field of microbial ecology through the analysis of nucleic acids. Most techniques that use DNA or RNA require their extraction from environmental matrices, which can be tedious and inefficient. While a number of extraction methods, both laboratory-based and commercially available, have been developed, none of these include a convenient method to determine extraction efficiency. We have developed an external DNA recovery standard, Lambda DNA (target DNA) contained within pBR322, allowing routine determinations of DNA recovery efficiency. Target DNA was added to sediments as whole cells, total DNA extracted using commercial DNA extraction/purification kits and the amount of target DNA recovered quantified by quantitative-competitive PCR (QC-PCR). Three commercially available kits (UltraClean Soil DNA, FastDNA SPIN and Soil Master DNA Extraction) were evaluated for recovery efficiency. Recoveries for the three kits ranged from undetectable to 43.3% with average recoveries of 14.9+/-16.0%, 28.3+/-10.5% and 2.4+/-0.1% (UltraClean, FastDNA and Soil Master, respectively). Quantification of target DNA proved robust in sediments heavily polluted with polycyclic aromatic hydrocarbons and the external recovery standard could be detected following extraction and amplification from as few as 1 x 10(3) cells added to 0.5 g sediment (wet weight). The external DNA recovery standard was also added directly to the sediment as purified plasmid DNA prior to extraction. It was recovered with similar efficiency as when added as whole cells, suggesting its usefulness in estimating DNA recovery in ribosomal DNA studies. These results show that, while the commercial kits offer expedited sample processing, the extraction efficiencies vary on a sample-by-sample basis and were <100%. Therefore, quantitative DNA studies require an estimation of DNA recovery.

PMID: 15063066 [PubMed - indexed for MEDLINE]

Lemarchand K, Berthiaume F, Maynard C, Harel J, Payment P, Bayardelle P, Masson L, Brousseau R. (2005) Optimization of microbial DNA extraction and purification from raw wastewater samples for downstream pathogen detection by microarrays. J Microbiol Methods. 63(2):115-26.

Numerous waterborne pathogens are difficult to detect and enumerate with accuracy due to methodological limitations and high costs of direct culturing. The purity of DNA extracted from wastewater samples is an important issue in the sensitivity and the usefulness of molecular methods such as polymerase chain reaction (PCR) and

hybridizations on DNA microarrays. Ten different DNA extraction procedures, including physical and chemical extraction and purification steps, were examined to ascertain their relative effectiveness for extracting bacterial DNA from wastewater samples. The quality of the differentially extracted DNAs was subsequently assessed by PCR amplification and microarray hybridization. Our results showed that great differences existed among the ten procedures and only a few of the methods gave satisfactory results when applied to bacterial pathogens. This observation suggested that the extraction method needed to be carefully selected to produce significant and confident results in the detection of pathogens from environmental samples.

PMID: 15936096 [PubMed - indexed for MEDLINE]

Benoit PW, Donahue DW. (2003) Methods for rapid separation and concentration of bacteria in food that bypass time-consuming cultural enrichment. J Food Prot. 66(10):1935-48.

The rapid detection of pathogenic organisms that cause foodborne illnesses is needed to insure food safety. Conventional methods for the detection of pathogens in foods are time-consuming and labor-intensive. New advanced rapid methods (i.e., polymerase chain reaction, DNA probes) are more sensitive and selective than conventional techniques, but many of these tests are inhibited by food components, rendering them dependent on slow cultural enrichment. The need for alternative methods that will rapidly separate and concentrate bacteria directly from food samples, thereby reducing the time required for these new rapid detection techniques, is evident. Separation and concentration methods extract target bacteria from interfering food components and/or concentrate bacteria to detectable levels. This review describes several methods used to separate and/or concentrate bacteria in food samples. Several methods discussed here, including centrifugation and immunomagnetic separation, have been successfully used, individually and in combination, to rapidly separate and/or concentrate bacteria from food samples in less time than is required for cultural enrichment.

PMID: 14572237 [PubMed - indexed for MEDLINE]

McFeters GA, Pyle BH, Lisle JT, Broadaway SC. (1999) Rapid direct methods for enumeration of specific, active bacteria in water and biofilms. Symp Ser Soc Appl Microbiol. 85(28):193S-200S.

Conventional methods for detecting indicator and pathogenic bacteria in water may underestimate the actual population due to sublethal environmental injury, inability of the target bacteria to take up nutrients and other physiological factors which reduce bacterial culturability. Rapid and direct methods are needed to more accurately detect and enumerate active bacteria. Such a methodological advance would provide greater sensitivity in assessing the microbiological safety of water and food. The principle goal of this presentation is to describe novel approaches we have formulated for the rapid and simultaneous detection of bacteria plus the determination of their physiological activity in water and other environmental samples. The present version of our method involves the

concentration of organisms by membrane filtration or immunomagnetic separation and combines an intracellular fluorochrome (CTC) for assessment of respiratory activity plus fluorescent-labelled antibody detection of specific bacteria. This approach has also been successfully used to demonstrate spatial and temporal heterogeneities of physiological activities in biofilms when coupled with cryosectioning. Candidate physiological stains include those capable of determining respiratory activity, membrane potential, membrane integrity, growth rate and cellular enzymatic activities. Results obtained thus far indicate that immunomagnetic separation can provide a high degree of sensitivity in the recovery of seeded target bacteria (*Escherichia coli* O157:H7) in water and hamburger. The captured and stained target bacteria are then enumerated by either conventional fluorescence microscopy or ChemScan(R), a new instrument that is very sensitive and rapid. The ChemScan(R) laser scanning instrument (Chemunex, Paris, France) provides the detection of individual fluorescently labelled bacterial cells using three emission channels in less than 5 min. A high degree of correlation has been demonstrated between results obtained with the ChemScan and traditional plate counts of mixed natural bacterial populations in water. The continuing evolution of these methods will be valuable in the rapid and accurate analysis of environmental samples.

PMID: 11543584 [PubMed - indexed for MEDLINE]

Stevens KA, Jaykus LA. (2004) Bacterial separation and concentration from complex sample matrices: a review. Crit Rev Microbiol. 30(1):7-24.

The use of many rapid detection technologies could be expanded if the bacteria were separated, concentrated, and purified from the sample matrix before detection. Specific advantages of bacterial concentration might include facilitating the detection of multiple bacterial strains; removal of matrix-associated assay inhibitors; and provision of adequate sample size reduction to allow for the use of representative food sample sizes and/or small media volumes. Furthermore, bacterial concentration could aid in improving sampling techniques needed to detect low levels of pathogens or sporadic contamination, which may perhaps reduce or even eliminate the need for cultural enrichment prior to detection. Although bacterial concentration methods such as centrifugation, filtration, and immunomagnetic separation have been reported for food systems, none of these is ideal and in many cases a technique optimized for one food system or microorganism is not readily adaptable to others. Indeed, the separation and subsequent concentration of bacterial cells from a food sample during sample preparation continues to be a stumbling block in the advancement of molecular methods for the detection of foodborne pathogens. The purpose of this review is to provide a detailed understanding of the science, possibilities, and limitations of separating and concentrating bacterial cells from the food matrix in an effort to further improve our ability to harness molecular methods for the rapid detection of foodborne pathogens.

PMID: 15116760 [PubMed - indexed for MEDLINE]

Långmark J, Ashbolt NJ, Szewzyk U, Stenström TA. (2001) Adequacy of *in situ* glass slides and direct sand extractions to assess the microbiota within sand columns used for drinking water treatment. Can J Microbiol. 47(7):601-7.

Abstract: Historically, Cholodny-Rossi buried glass slide techniques have been used to study the microbiota of subsurface environments, yet the bias of such a technique has not been compared against direct sand extraction using modern in situ probing. Over a period of 34 wk, four separate 4-m-deep sand columns receiving raw lake water were examined to compare direct extraction of sand filter biofilm material against in situ glass slide biofilms. Significantly different DAPI direct counts and fluorescent in situ hybridization signals for major phylogenetic groups were observed. Not only were lower proportions (P < 0.001) of EUB338-probed DAPI cells observed on in situ glass slides, but also fewer gamma-Proteobacteria (12%-21%) and more alpha-Proteobacteria (16%-33%) when compared to direct sand extracts. Hence, investigators of the microbial ecology of even simple sand biofilms must consider the inherent biases from "accepted" methods and seek further independent methods to identify those which may be most accurate.

PubMed: 11547879 [PubMed - indexed for MEDLINE]

Bacteria

Karpiscak MM, Sanchez LR, Freitas RJ, Gerba CP. (2001) Removal of bacterial indicators and pathogens from dairy wastewater by a multi-component treatment system. Water Sci Technol. 44(11-12):183-90.

Microbial removal by a multi-component treatment system for dairy and municipal wastewater is being studied in Arizona, USA. The system consists of paired solids separators, anaerobic lagoons, aerobic ponds and constructed wetlands cells. The organisms under study include: total coliform, fecal coliform, enterovirus, *Listeria monocytogenes*, *Clostridium perfringens*, coliphage, *Giardia lamblia* and *Cryptosporidium parvum*. Organism removal rates from dairy wastewater varied from 13.2 per cent for fecal coliform to 94.9 per cent for coliphage. It appears that the much higher turbidity of the dairy wastewater, nearly 1,300 NTU, decreased the treatment systems' ability to remove some microbial indicators and pathogens. Information from this study can be used to determine the adequacy of multi-component treatment systems for the control of wastewater-borne pathogens, both in municipal treatment systems as well as in confined animal feeding operations (CAFO). This information also can assist municipalities and the CAFO industry in the implementation of rational and efficient treatment strategies for appropriate reuse of wastewaters.

PMID: 11804092 [PubMed - indexed for MEDLINE]

Truesdail SE, Lukasik J, Farrah SR, Shah DO, Dickinson RB. (1998) Analysis of bacterial deposition on metal (hydr)oxide-coated sand filter media. J Colloid Interface Sci. 203(2):369-78.

The aim of this study was to investigate the importance of surface potential in microbial deposition onto modified granular surfaces. Recent experimental and theoretical work has indicated that surfaces coated with metal oxides and hydroxide rich oxide/hydroxide mixtures ((hydr)oxides) have the potential to increase the capture efficiencies of commercial filtration systems. This study quantitatively compared different metal (hydr)oxide coatings in their abilities to enhance bacterial deposition. Specifically, the deposition rates of bacterial strains *Streptococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli* were compared for Ottawa sand and surface coatings consisting of aluminum (hydr)oxide, iron (hydr)oxide, and mixed iron and aluminum (hydr)oxide. The metal-(hydr)oxide-modified granular media enhanced. bacterial deposition relative to the noncoated Ottawa sand. The electropositive surfaces, the aluminum and the mixed (hydr)oxides, had similar average kinetic rate constants, five times larger than the rate constants observed for the untreated Ottawa sand. The measured kinetic rate constants for the positively charged systems of aluminum (hydr)oxide and mixed (hydr)oxide collectors suggested that the overall rate of deposition was limited by the transport of bacteria to the granular surface rather than the rate of attachment. For systems where the collector surfaces were negatively charged, as in the cases of Ottawa sand and the iron (hydr)oxide coating, large energy barriers to attachment were predicted from DLVO theory but these barriers did not totally inhibit bacterial deposition. The deposition results could not be fully explained by DLVO theory and suggested the importance of other factors such as collector charge heterogeneity, motility, and bacterial surface appendages in enhanced deposition.

PMID: 9705775 [PubMed - as supplied by publisher]

Viruses

Schwab KJ, De Leon R, Sobsey MD. (1996) Immunoaffinity concentration and purification of waterborne enteric viruses for detection by reverse transcriptase PCR. Appl Environ Microbiol. 62(6):2086-94

To assess the risks from viral contamination of drinking-water supplies, there is a clear need for methods to directly detect viral pathogens. In this study, we developed a broad-spectrum immunocapture method for concentration and purification of enteric viruses. The method involved indirect antibody capture (AbCap) of intact viruses followed by release of virion genomic RNA and reverse transcriptase PCR for amplification and oligoprobe hybridization for detection. The procedure involved concentrating enteric viruses from large volumes of water by standard filtration-elution techniques with IMDS filters and 1 liter of 1% beef extract-0.05 M glycine (BE/G) as an eluate. The BE/G eluate was concentrated and purified by polyethylene glycol (PEG) precipitation, Pro-Cipitate (a commercially available protein precipitating reagent) precipitation, and a second PEG precipitation to a volume of approximately 500 mu l. Aliquots of the second PEG precipitate were further processed by RNA extraction, AbCap, or cell culture analysis for infectious viruses. The AbCap method was applied to 11 field samples of fecally contaminated surface water. Of the 11 samples, 9 were positive for enteric viruses by AbCap method 4 of 11 samples were positive for enteric viruses by direct RNA extraction of a small aliquot of the second PEG concentrate; and 4

of 11 samples were positive for enteric viruses by measurement of cell culture infectivity. The results of enteric viruses were compared with those for standard bacterial and coliphage indicators of fecal contamination.

PMID: 8787407 [PubMed - indexed for MEDLINE]

Leisinger M, Metzler A. (2000) Use of silica as a carrier to recover and prepare waterborne enteric viruses for detection by RT-PCR. Zentralbl Hyg Umweltmed. (4):283-96.

A rapid, efficient and inexpensive method was developed to concentrate poliovirus type 1 (PV1), rotavirus (RV) and hepatitis A virus (HAV) from artificially spiked samples of tap and surface water. The method consists of adsorbing the viruses to silicon dioxide (SiO2) in the presence of 0.5 mM AlCl3 and adjustment of the pH to 3.5. The silica-adsorbed virus was collected by low speed centrifugation. Viral RNA was then extracted with guanidium thiocyanate (GT), and environmental nucleases and inhibitors of reverse transcriptase and Taq polymerase were further eliminated from concentrates by sequential treatment with GT, ethanol and acetone. Subsequent RT-PCR allowed the detection of as few as 1 to 10 TCID50 of PV1, RV, and HAV in seeded 1 liter samples of tap water. The same protocol was then used with effluents from two local sewage treatment plants. These samples, found to be free of HAV, were most commonly contaminated with enteroviruses and rotaviruses. Addition of 1000 TCID50 of HAV, PV1 or RV to a second 1 liter sample, taken at the same time from the corresponding surface waters allowed detection of the input virus without discernible inhibition by amplification inhibitors. The newly established method seems amenable to scaling up and promising for virus monitoring in different water types. The method is rapid and results can be obtained within 24 to 36 hours.

PMID: 9638882 [PubMed - indexed for MEDLINE]

Jothikumar N, Khanna P, Paulmurugan R, Kamatchiammal S, Padmanabhan P. (1995) A simple device for the concentration and detection of enterovirus, hepatitis E virus and rotavirus from water samples by reverse transcription-polymerase chain reaction. J Virol Methods. 55(3):401-15.

A simultaneous concentration of enteroviruses, hepatitis E virus, and rotavirus from drinking water samples through a filtration column filled with granular activated carbon (GAC) was achieved. Urea-arginine phosphate buffer (UAPB) as an eluent at pH 9.0 was used for effective desorption and elution of viruses from GAC. Further concentration of viruses with magnesium chloride enabled nucleic acid extraction, cDNA synthesis, amplification with a specific set of primers for enterovirus, hepatitis E virus and rotavirus. Polymerase chain reaction (PCR) products were then confirmed by Southern transfer and hybridization with the relevant probes. The efficacy of the protocol was established with 100 1 of water samples seeded with poliovirus-1, providing 74% recovery in granular activated carbon based UAPB-RT-PCR. The GAC-based method for

concentration of viruses from water samples was preferred, despite its somewhat lower efficacy compared to 80% in membrane filter based UAPB-RT-PCR protocol, due to the specific requirements of short-time and savings in cost of analyses. The protocol was used for the detection of waterborne viruses from 24 drinking water sources in urban areas of New Delhi. Direct isolation of viruses from water samples revealed that the 4 samples were positive for enteroviruses, two for hepatitis E virus, and 10 samples for rotavirus. One sample was positive for both hepatitis E virus and rotavirus, and another for all the 3 types of viruses.

PMID: 8609205 [PubMed - indexed for MEDLINE]

Tansuphasiri U, Vathanophas K, Pariyanonda A, Kittigul L, Utrarachkij F, Diraphat P, Siripanichgon K, Punchitton S, Chitpirom K, Cheaochantanakij N. (2000) Rapid detection of polioviruses in environmental water samples by one-step duplex RT-PCR. Southeast Asian J Trop Med Public Health. 31(1):47-56.

This study describes the rapid detection of polioviruses in environmental waters by a simple reverse transcriptase-polymerase chain reaction (RT-PCR) using two primer pairs for differentiation of poliovirus from non-polio enteroviruses in a single reaction by a one-step method, combining RT and PCR in a single tube. The detection by agarose gel electrophoresis yielded 2 bands of 153-bp and 293-bp for poliovirus tested without the need for further hybridization. The detection sensitivity of this one-step duplex RT-PCR, as measured with RNA extracted by heat treatment from supernatant of infected cell extracts, was 10(-1) 50% tissue culture effective doses (TCID50). This assay was used to evaluate the ability of sample concentration by membrane filter-based adsorption and elution, and purification by a simple RNA isolation based on guanidine isothiocyanate-phenol-chloroform extraction; the system yielded a detection limit of 5 x 10(-1) TCID50 seeded in 5 liters of tap water. This protocol was applied to the poliovirus detection in environmental water collected from 2 communities in Bangkok, Thailand during February and May 1998. Of 100 samples tested, 2 water samples collected from the same open sewage pipeline at one location were positive for polioviruses and one sample collected from another sewage pipeline was positive for non-polio enterovirus while a further 97 water samples were negative for both polioviruses and non-polio enteroviruses. With poliovirus detection by cell culture technique, none of the 100 samples tested was positive for poliovirus type 1, 2 or 3. RT-PCR was more sensitive, rapid, simple and cost-effective than the cell culture technique since the two water samples which were positive for polioviruses by RT-PCR failed to be detected by cell culture. Sequence data of 293-bp amplicons from positive samples were compared with those of reference poliovirus strains in the Genbank and the EMBL databases and identity to the sequence of type 1 strain Sabin was found to be 99%.

PMID: 11023064 [PubMed - indexed for MEDLINE]

Schwab KJ, De Leon R, Sobsey MD. (1995) Concentration and purification of beef extract mock eluates from water samples for the detection of enteroviruses,

hepatitis A virus, and Norwalk virus by reverse transcription-PCR. Appl Environ Microbiol. 61(2):531-7.

In this study we developed a concentration and purification procedure to facilitate reverse transcription (RT)-PCR detection of enteric viruses in water sample concentrates obtained by conventional filter adsorption-elution methods. One liter of beef extract-glycine eluate with or without humic acid and seeded with poliovirus type 1, hepatitis A virus, and Norwalk virus was used as a model system, and the eluent was further processed for RT-PCR compatibility. The sample concentration and purification procedures which we used included polyethylene glycol precipitation, Pro-Cipitate precipitation, a second polyethylene glycol precipitation, spin column chromatography, and ultrafiltration. The sample volumes were reduced from 1 liter to 20 to 50 microliters, and the samples were purified enough so that viruses could be detected by the RT-PCR. The ability to detect low levels of enteric viruses by molecular techniques was compared directly with the ability to detect enteric viruses by cell culture infectivity procedures. As little as 3 PFU of poliovirus type 1 in an initial 1 liter of mock eluate was detected by the RT-PCR.

PMID: 7574592 [PubMed - indexed for MEDLINE]

Ma JF, Naranjo J, Gerba CP. (1994) Evaluation of MK filters for recovery of enteroviruses from tap water. Appl Environ Microbiol. 60(6):1974-7.

The MK filter is an electropositively charged filter that can be used to concentrate enteroviruses from large volumes (400 to 1,000 liters) of water. This filter is less expensive than the commonly used 1MDS electropositive filter. In this study, we compared the recovery of poliovirus 1 (PV1) and that of coxsackievirus B3 (CB3) from 378 liters of tap water, using both the MK and the 1MDS filters. Viruses were eluted from the filters with 3% beef extract buffered with 0.05 M glycine (pH 9.5) and reconcentrated via organic flocculation. At high virus inputs (approximately 10(6) PFU), the overall recovery (after elution and reconcentration) of PV1 and CB3 from tap water with the MK filter was less than that achieved with the 1MDS filter ($P < 0.05$). The recoveries of PV1 from tap water with the MK and 1MDS filters were 73.2% +/- 26% ($n = 5$ trials) and 90.2% +/- 5.9% ($n = 5$ trials), respectively. The recoveries of CB3 from tap water with the MK and 1MDS filters were 32.8% +/- 34.5% ($n = 4$ trials) and 95.8% +/- 12.0% ($n = 4$ trials), respectively. This study indicated that the MK filter consistently provided lower recovery, with wider variability, of PV1 and CB3 from tap water than the 1MDS filter.

PMID: 8031090 [PubMed - indexed for MEDLINE]

Roepke DC, Halvorson DA, Goyal SM, Kelleher CJ. (1989) An adsorption-elution technique for the recovery of influenza virus from water. Avian Dis. 33(4):649-53.

A virus adsorption-elution (viradel) procedure was modified and evaluated for the concentration of influenza virus from water. Influent pH, flow rate, eluent pH and composition, and a second-step concentration method were evaluated. The viradel procedure in combination with a chicken erythrocyte adsorption technique resulted in up to 3200-fold concentration of influenza virus from 100 liters of tap water.

PMID: 2619660 [PubMed - indexed for MEDLINE]

Shieh YS, Baric RS, Sobsey MD. (1997) Detection of low levels of enteric viruses in metropolitan and airplane sewage. Appl Environ Microbiol. 63(11):4401-7.

To detect less prevalent viruses, such as wild-type polioviruses in sewage from a highly immunized community, a method was developed to efficiently recover viruses and remove PCR inhibitors. The method consisted of initial separation of solids from liquid, followed by solvent extractions, polyethylene glycol precipitations, Sephadex G-200 chromatography, and guanidinium isothiocyanate (GIT) extraction. To elute viruses from the separated solids, 0.5 M threonine (pH 7.5) was as efficient as 3% beef extract but conferred no PCR inhibition. In samples that were concentrated approximately 1,000-fold, 21% of the initially seeded viruses were recovered. When poliovirus type 3 (PV3) Sabin strain at low levels and PV1 LSc strain at high levels were seeded in raw sewage, PV3 was specifically detected in the final sample concentrates at sensitivities of 14 PFU by direct PCR and 0.7 PFU by GIT extraction-PCR. While applying the method to international airplane sewage, which contains high levels of solids as well as commercial sanitizers, 44% (7 of 16) of the samples were found to harbor enteroviruses by both cell culture infectivity and pan-enterovirus PCR analyses. Nucleotide sequencing of the PCR products revealed that multiple enterovirus genotypes were amplified from each final sewage concentrate, whereas the fewer virus genotypes detected by cell culture infectivity were probably the better growing strains. By this method, we demonstrated that air travel may contribute to the intercontinental dissemination of enteric pathogens.

PMID: 9361427 [PubMed - indexed for MEDLINE]

Tsai YL, Sobsey MD, Sangermano LR, Palmer CJ. (1993) Simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-polymerase chain reaction. Appl Environ Microbiol. 59(10):3488-91.

A rapid and simple method was developed to detect enteroviruses and hepatitis A virus (HAV) in sewage and ocean water. Sewage samples were concentrated by Centriprep-100 and Centricon-100 at 1,000 x g. Samples collected from estuary and near-shore surf zone ocean water in Southern California were concentrated by vortex flow filtration and microconcentration. Reverse transcriptase-polymerase chain reaction (RT-PCR), with enterovirus primers or HAV capsid-specific primers, was used to detect enteroviruses or HAV in all concentrated samples. A nonradioactive internal probe was used to confirm the amplified products. Results of seeding experiments indicated that at 4 degrees C, HAV was more persistent than poliovirus in seawater and both HAV and

poliovirus persisted longer at 4 degrees C than at 25 degrees C. RT-PCR was at least 500-fold more sensitive than cell culture. Results were obtained within 5 h by RT-PCR, in contrast with the 5 days to 3 weeks required for cell culture.

PMID: 7504433 [PubMed - indexed for MEDLINE]

Scott TM, Lukasik J, Farrah SR. (2002) Improved method for recovery of bacteriophage from large volumes of water using negatively charged microporous filters. Can J Microbiol. 48(4):305-10.

Current virus-recovery procedures using negatively charged microporous filters provide an inexpensive, reliable method for the recovery and detection of enteroviruses from water and wastewater; however, adjustment of the test samples to pH 3.5 to promote enterovirus adsorption results in significant inactivation of bacteriophage and an inability to simultaneously recover them from large volumes of water using this procedure. Procedures specifically designed for the detection of bacteriophage are currently in use but generally are only effective for small volumes of water. Positively charged filters can be used to recover both enteroviruses and bacteriophage from large volumes of water at neutral pH; however, the filters are expensive. The addition of manganese chloride to test solutions at pH 3.5 prior to filtration through negatively charged Filterite filters allowed for sampling of larger volumes of water by reducing the inactivation of bacteriophage and increasing the recovery of PRD1, MS2, and naturally isolated bacteriophage by a factor of four or five when compared with recoveries from solutions without MnCl2. This method provides an inexpensive, reliable alternative to large-volume bacteriophage recovery procedures that use positively charged filters at neutral pH.

PMID: 12030702 [PubMed - indexed for MEDLINE]

Lukasik J, Scott TM, Andryshak D, Farrah SR. (2000) Influence of salts on virus adsorption to microporous filters. Appl Environ Microbiol. 66(7):2914-20.

We investigated the direct and indirect effects of mono-, di-, and trivalent salts (NaCl, MgCl(2), and AlCl(3)) on the adsorption of several viruses (MS2, PRD-1, phiX174, and poliovirus 1) to microporous filters at different pH values. The filters studied included Millipore HA (nitrocellulose), Filterite (fiberglass), Whatman (cellulose), and 1MDS (charged-modified fiber) filters. Each of these filters except the Whatman cellulose filters has been used in virus removal and recovery procedures. The direct effects of added salts were considered to be the effects associated with the presence of the soluble salts. The indirect effects of the added salts were considered to be (i) changes in the pH values of solutions and (ii) the formation of insoluble precipitates that could adsorb viruses and be removed by filtration. When direct effects alone were considered, the salts used in this study promoted virus adsorption, interfered with virus adsorption, or had little or no effect on virus adsorption, depending on the filter, the virus, and the salt. Although we were able to confirm previous reports that the addition of aluminum chloride to water enhances virus adsorption to microporous filters, we found that the enhanced adsorption was associated with indirect effects rather than direct effects. The increase in viral

adsorption observed when aluminum chloride was added to water was related to the decrease in the pH of the water. Similar results could be obtained by adding HCl. The increased adsorption of viruses in water at pH 7 following addition of aluminum chloride was probably due to flocculation of aluminum, since removal of flocs by filtration greatly reduced the enhancement observed. The only direct effect of aluminum chloride on virus adsorption that we observed was interference with adsorption to microporous filters. Under conditions under which hydrophobic interactions were minimal, aluminum chloride interfered with virus adsorption to Millipore, Filterite, and 1MDS filters. In most cases, less than 10% of the viruses adsorbed to filters in the presence of a multivalent salt and a compound that interfered with hydrophobic interactions (0.1% Tween 80 or 4 M urea).

PMID: 10877786 [PubMed - indexed for MEDLINE]

Farrah SR, Preston DR, Toranzos GA, Girard M, Erdos GA, Vasuhdivan V. (1991) Use of modified diatomaceous earth for removal and recovery of viruses in water. Appl Environ Microbiol. 57(9):2502-6.

Diatomaceous earth was modified by in situ precipitation of metallic hydroxides. Modification decreased the negative charge on the diatomaceous earth and increased its ability to adsorb viruses in water. Electrostatic interactions were more important than hydrophobic interactions in virus adsorption to modified diatomaceous earth. Filters containing diatomaceous earth modified by in situ precipitation of a combination of ferric chloride and aluminum chloride adsorbed greater than 80% of enteroviruses (poliovirus 1, echovirus 5, and coxsackievirus B5) and coliphage MS2 present in tap water at ambient pH (7.8 to 8.3), even after filtration of 100 liters of tap water. Viruses adsorbed to the filters could be recovered by mixing the modified diatomaceous earth with 3% beef extract plus 1 M NaCl (pH 9).

PMID: 1768124 [PubMed - indexed for MEDLINE]

Borrego JJ, Cornax R, Preston DR, Farrah SR, McElhaney B, Bitton G. (1991) Development and application of new positively charged filters for recovery of bacteriophages from water. Appl Environ Microbiol. 57(4):1218-22.

Electronegative and electropositive filters were compared for the recovery of indigenous bacteriophages from water samples, using the VIRADEL technique. Fiber glass and diatomaceous earth filters displayed low adsorption and recovery, but an important increase of the adsorption percentage was observed when the filters were treated with cationic polymers (about 99% adsorption). A new methodology of virus elution was developed in this study, consisting of the slow passage of the eluent through the filter, thus increasing the contact time between eluent and virus adsorbed on the filters. The use of this technique allows a maximum recovery of 71.2% compared with 46.7% phage recovery obtained by the standard elution procedure. High percentages (over 83%) of phage adsorption were obtained with different filters from 1-liter aliquots of the samples, except for Virosorb 1-MDS filters (between 1.6 and 32% phage

adsorption). Phage recovery by using the slow passing of the eluent depended on the filter type, with recovery ranging between 1.6% for Virosorb 1-MDS filters treated with polyethyleneimine and 103.2% for diatomaceous earth filters treated with 0.1% Nalco.
PMID: 2059044 [PubMed - indexed for MEDLINE]

Preston DR, Vasudevan TV, Bitton G, Farrah SR, Morel JL. (1998) Novel approach for modifying microporous filters for virus concentration from water. Appl Environ Microbiol. 54(6):1325-9.

Electronegative microporous filters composed of epoxyfiberglass (Filterite) were treated with cationic polymers to enhance their virus-adsorbing properties. This novel and inexpensive approach to microporous filter modification entails soaking filters in an aqueous solution of a cationic polymer such as polyethyleneimine (PEI) for 2 h at room temperature and then allowing the filters to air dry overnight on absorbent paper towels. PEI-treated filters were evaluated for coliphage (MS2, T2, and phi X174) and enterovirus (poliovirus type 1 and coxsackievirus type B5) adsorption from buffer at pH 3.5 to 9.0 and for indigenous coliphages from unchlorinated secondary effluent at ambient pH. Adsorbed viruses were recovered with 3% beef extract (pH 9). Several other cationic polymers were used to modify epoxyfiberglass filters and were evaluated for their ability to concentrate viruses from water. Zeta potentials of disrupted filter material indicated that electronegative epoxyfiberglass filters were made more electropositive when treated with cationic polymers. In general, epoxyfiberglass filters treated with cationic polymers were found to adsorb a greater percentage of coliphages and enteroviruses than were untreated filters.
PMID: 2843091 [PubMed - indexed for MEDLINE]

Cryptosporidium and Other Protozoans

Carey CM, Lee H, Trevors JT. (2004) Biology, persistence and detection of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocyst. Water Res. 38(4):818-62.

Cryptosporidium parvum and *Cryptosporidium hominis* are obligate enteric protozoan parasites which infect the gastrointestinal tract of animals and humans. The mechanism(s) by which these parasites cause gastrointestinal distress in their hosts is not well understood. The risk of waterborne transmission of *Cryptosporidium* is a serious global issue in drinking water safety. Oocysts from these organisms are extremely robust, prevalent in source water supplies and capable of surviving in the environment for extended periods of time. Resistance to conventional water treatment by chlorination, lack of correlation with biological indicator microorganisms and the absence of adequate methods to detect the presence of infectious oocysts necessitates the development of consistent and effective means of parasite removal from the water supply. Additional research into improving water treatment and sewage treatment practices is needed, particularly in testing the efficiency of ozone in oocyst inactivation. Timely and efficient detection of infectious *C. parvum* and *C. hominis* oocysts in environmental samples

requires the development of rapid and sensitive techniques for the concentration, purification and detection of these parasites. A major factor confounding proper detection remains the inability to adequately and efficiently concentrate oocysts from environmental samples, while limiting the presence of extraneous materials. Molecular-based techniques are the most promising methods for the sensitive and accurate detection of *C. parvum* and *C. hominis*. With the availability of numerous target sequences, RT-PCR will likely emerge as an important method to assess oocyst viability. In addition, a multiplex PCR for the simultaneous detection of *C. parvum*, *C. hominis* and other waterborne pathogens such as *Giardia lamblia* would greatly benefit the water industry and protect human health.

PMID: 14769405 [PubMed - indexed for MEDLINE]

Hallier-Soulier S, Guillot E. (2003) An immunomagnetic separation-reverse transcription polymerase chain reaction (IMS-RT-PCR) test for sensitive and rapid detection of viable waterborne *Cryptosporidium parvum*. Environ Microbiol. 5(7):592-8.

The public health problem posed by the waterborne parasite *Cryptosporidium parvum* incited the water supply industry to develop very accurate analytical tools able to assess the presence of viable oocysts in drinking water. In this study, we report the development of a viability assay for *C. parvum* oocysts based on immunomagnetic separation and reverse transcription polymerase chain reaction (IMS-RT-PCR). The detection limit of the IMS-RT-PCR assay, which targets the hsp70 heat shock-induced mRNA, was in the range of ten viable oocysts per 100-l tap water samples. Purified *Cryptosporidium parvum* oocysts were exposed to heating, freezing and three chemical disinfection treatments namely, chlorination, chlorine dioxide treatment and ozonation under conventional doses used in water treatment plants, then detected by IMS-PCR and IMS-RT-PCR. The results obtained by IMS-PCR showed that none of the treatments had an effect on oocyst detection. The inactivation of oocysts by boiling resulted in no RT-PCR signal. Chlorine as well as chlorine dioxide did not influence oocyst viability as determined by IMS-RT-PCR. Ozone more effectively inactivated oocysts. The IMS-RT-PCR assay in conjunction with IMS-PCR marks the development of a combined detection and viability test which can be used for drinking water quality control as well as for reliable evaluation of treatment efficiency.

PMID: 12823191 [PubMed - indexed for MEDLINE]

Quintero-Betancourt W, Peele ER, Rose JB. (2002) *Cryptosporidium parvum* and *Cyclospora cayetanensis*: a review of laboratory methods for detection of these waterborne parasites. J Microbiol Methods. 49(3):209-24.

Cryptosporidium and *Cyclospora* are obligate, intracellular, coccidian protozoan parasites that infest the gastrointestinal tract of humans and animals causing severe diarrhea illness. In this paper, we present an overview of the conventional and more novel techniques that are currently available to detect *Cryptosporidium* and *Cyclospora* in

water. Conventional techniques and new immunological and genetic/molecular methods make it possible to assess the occurrence, prevalence, virulence (to a lesser extent), viability, levels, and sources of waterborne protozoa. Concentration, purification, and detection are the three key steps in all methods that have been approved for routine monitoring of waterborne oocysts. These steps have been optimized to such an extent that low levels of naturally occurring *Cryptosporidium* oocysts can be efficiently recovered from water. The filtration systems developed in the US and Europe trap oocysts more effectively and are part of the standard methodologies for environmental monitoring of *Cryptosporidium* oocysts in source and treated water. Purification techniques such as immunomagnetic separation and flow cytometry with fluorescent activated cell sorting impart high capture efficiency and selective separation of oocysts from sample debris. Monoclonal antibodies with higher avidity and specificity to oocysts in water concentrates have significantly improved the detection and enumeration steps. To date, PCR-based detection methods allow us to differentiate the human pathogenic *Cryptosporidium* parasites from those that do not infect humans, and to track the source of oocyst contamination in the environment. Cell culture techniques are now used to examine oocyst viability. While fewer studies have focused on *Cyclospora cayetanensis*, the parasite has been successfully detected in drinking water and wastewater using current methods to recover *Cryptosporidium* oocysts. More research is needed for monitoring of *Cyclospora* in the environment. Meanwhile, molecular methods (e.g. molecular markers such as intervening transcribed spacer regions), which can identify different genotypes of *C. cayetanensis*, show good promise for detection of this emerging coccidian parasite in water.

PMID: 11869786 [PubMed - indexed for MEDLINE]

Fontaine M, Guillot E. (2003) An immunomagnetic separation-real-time PCR method for quantification of *Cryptosporidium parvum* in water samples. J Microbiol Methods. 54(1):29-36.

The protozoan parasite *Cryptosporidium parvum* is known to occur widely in both raw and drinking water and is the cause of waterborne outbreaks of gastroenteritis throughout the world. The routinely used method for the detection of *Cryptosporidium* oocysts in water is based on an immunofluorescence assay (IFA). It is both time-consuming and nonspecific for the human pathogenic species *C. parvum*. We have developed a TaqMan polymerase chain reaction (PCR) test that accurately quantifies *C. parvum* oocysts in treated and untreated water samples. The protocol consisted of the following successive steps: Envirochek capsule filtration, immunomagnetic separation (IMS), thermal lysis followed by DNA purification using Nanosep centrifugal devices and, finally, real-time PCR using fluorescent TaqMan technology. Quantification was accomplished by comparing the fluorescence signals obtained from test samples with those from standard dilutions of *C. parvum* oocysts. This IMS-real-time PCR assay permits rapid and reliable quantification over six orders of magnitude, with a detection limit of five oocysts for purified oocyst solutions and eight oocysts for spiked water samples. Replicate samples of spiked tap water and Seine River water samples (with approximately 78 and 775 oocysts) were tested. *C. parvum* oocyst recoveries, which

ranged from 47.4% to 99% and from 39.1% to 68.3%, respectively, were significantly higher and less variable than those reported using the traditional US Environmental Protection Agency (USEPA) method 1622. This new molecular method offers a rapid, sensitive and specific alternative for *C. parvum* oocyst quantification in water.

PMID: 12732419 [PubMed - indexed for MEDLINE]

Stine SW, Vladich FD, Pepper IL, Gerba CP. (2005) Development of a method for the concentration and recovery of microsporidia from tap water. J Environ Sci Health A Tox Hazard Subst Environ Eng. 40(5):913-25.

Microsporidia are obligate intracellular parasites. Microsporidian spores infect a wide variety of hosts, including humans. The spores may be found in infected hosts' urine and feces, thus waterborne transmission is possible. This study details method development for the detection of microsporidia in tap water. In this study, filtration, centrifugation, purification, and detection parameters were optimized for the detection of microsporidia. The Pall-Gelman Envirocheck sampling capsule (Pall Gelman, Ann Arbor, MI) was chosen as the filter element. Optimal centrifugal force for spore recovery was 1500 x g. Additionally, it was determined that eluting microsporidia spores in a detergent elution buffer solution had a detrimental effect on spore recovery. A direct examination of the concentrate resulted in a greater recovery with less variability than subjecting the sample concentrate to a Percoll-sucrose density gradient purification step. The staining method employed for the detection spores was Calcofluor white (Sigma, St. Louis, MO). Percent recoveries for 10 L tap water samples (n = 5) using the Envirocheck sampling capsule without a density gradient purification step were 26.1+/-13.4 compared to 25+/-13.8 for samples subjected to a density gradient purification step.

PMID: 15887563 [PubMed - indexed for MEDLINE]

Ochiai Y, Takada C, Hosaka M. (2005) Detection and discrimination of *Cryptosporidium parvum* and *C. hominis* in water samples by immunomagnetic separation-PCR. Appl Environ Microbiol. 71(2):898-903.

Cryptosporidium parvum and *C. hominis* have been the cause of large and serious outbreaks of waterborne cryptosporidiosis. A specific and sensitive recovery-detection method is required for control of this pathogen in drinking water. In the present study, nested PCR-restriction fragment length polymorphism (RFLP), which targets the divergent Cpgp40/15 gene, was developed. This nested PCR detected only the gene derived from *C. parvum* and *C. hominis* strains, and RFLP was able to discriminate between the PCR products from *C. parvum* and *C. hominis*. To evaluate the sensitivity of nested PCR, *C. parvum* oocysts inoculated in water samples of two different turbidities were recovered by immunomagnetic separation (IMS) and detected by nested PCR and fluorescent antibody assay (FA). Genetic detection by nested PCR and oocyst number confirmed by FA were compared, and the results suggested that detection by nested PCR depends on the confirmed oocyst number and that nested PCR in combination with IMS has the ability to detect a single oocyst in a water sample. We applied an agitation

procedure with river water solids to which oocysts were added to evaluate the recovery and detection by the procedure in environmental samples and found some decrease in the rate of detection by IMS.

PMID: 15691946 [PubMed - indexed for MEDLINE]

Simmons OD 3rd, Sobsey MD, Heaney CD, Schaefer FW 3rd, Francy DS. (2001) Concentration and detection of *Cryptosporidium* oocysts in surface water samples by Method 1622 using ultrafiltration and capsule filtration. Appl Environ Microbiol. 67(3):1123-7.

The protozoan parasite *Cryptosporidium parvum* is known to occur widely in both source and drinking water and has caused waterborne outbreaks of gastroenteritis. To improve monitoring, the U.S. Environmental Protection Agency developed method 1622 for isolation and detection of *Cryptosporidium* oocysts in water. Method 1622 is performance based and involves filtration, concentration, immunomagnetic separation, fluorescent-antibody staining and 4',6-diamidino-2-phenylindole (DAPI) counterstaining, and microscopic evaluation. The capsule filter system currently recommended for method 1622 was compared to a hollow-fiber ultrafilter system for primary concentration of *C. parvum* oocysts in seeded reagent water and untreated surface waters. Samples were otherwise processed according to method 1622. Rates of *C. parvum* oocyst recovery from seeded 10-liter volumes of reagent water in precision and recovery experiments with filter pairs were 42% (standard deviation [SD], 24%) and 46% (SD, 18%) for hollow-fiber ultrafilters and capsule filters, respectively. Mean oocyst recovery rates in experiments testing both filters on seeded surface water samples were 42% (SD, 27%) and 15% (SD, 12%) for hollow-fiber ultrafilters and capsule filters, respectively. Although *C. parvum* oocysts were recovered from surface waters by using the approved filter of method 1622, the recovery rates were significantly lower and more variable than those from reagent grade water. In contrast, the disposable hollow-fiber ultrafilter system was compatible with subsequent method 1622 processing steps, and it recovered *C. parvum* oocysts from seeded surface waters with significantly greater efficiency and reliability than the filter suggested for use in the version of method 1622 tested.

PMID: 11229901 [PubMed - indexed for MEDLINE]

Combination Methods

Watt PM, Johnson DC, Gerba CP. (2002) Improved method for concentration of *Giardia, Cryptosporidium,* and poliovirus from water. J Environ Sci Health A Tox Hazard Subst Environ Eng. 37(3):321-30.

Methods for the concentration of enteric viruses and the protozoan parasites, *Giardia* and *Cryptosporidium*, from drinking water currently require the use of two different types of filters. Electropositive or electronegative microporous filters (0.2-0.45 microm nominal porosity) are used for the collection of enteroviruses, while polypropylene spun-fiber filters (1 microm porosity) and small pleated cartridge filters are used for the collection of protozoan parasites from water. Since the filter mechanically traps the protozoa by size exclusion, a microporous filter with an appropriately small nominal porosity could possibly be used for co-collection of both protozoa and enteroviruses. This study compared the concentration efficiencies of a polypropylene fiber cartridge (DPPPY) filter and two different microporous filters (Filterite and IMDS) with poliovirus (type 1), with respect to their ability to concentrate *Giardia* and *Cryptosporidium* from water. *Giardia* cysts and *Cryptosporidium* oocysts were added to 4001 of either tap water or tertiary treated wastewater and passed through the test filter. The protozoa were eluted from the polypropylene filter by hand-washing in a detergent solution. Viruses and protozoa were eluted from the microporous filter by two consecutive back-washes with a 1.5% beef extract, 0.1% Tween 80 solution. The eluent was then centrifuged to remove the parasites and the supernatant assayed for viruses. The overall efficiency was greater for the Filterite filter (40.4% for Giardia; 36.6% for *Cryptosporidium*) when compared to the spun fiber filter (10.1% for Giardia; 16.0% for *Cryptosporidium*). The Filterite filters were easier and faster to process than the polypropylene spun fiber filters. There was no significant difference in the recovery of protozoa from 1MDS and DPPPY filters. Co-collection of viruses and protozoan parasites from water onto the same filter is possible and can reduce the time and cost of routine water monitoring.

PMID: 11929071 [PubMed - indexed for MEDLINE]

VIABILITY DETERMINATION

Viability is an important aspect of waterborne pathogen detection because dead pathogens do not present a health threat. Methods are needed that can identify single viable cells within hours. The classic measurement of viability is to culture an organism. Culture remains the most sensitive method, however, culture methods are slow and not all target organisms can be cultured. Measurement of certain chemicals, such as ATP and NADH+ indicate that organisms are viable. ATP is generally measured by the use of a photon generating enzyme, luciferease, isolated from the fire fly. Luciferase based measurements generally require in excess of 100,000 viable bacteria to get a useful result. Spectrophotometric measurements of absorption can distinguish NAD from NADH. In some cases bacteria are stimulated to synthesize a heat shock protein followed by the isolation and amplification of the specific messenger RNA for this response. This method is reported to have a sensitivity of 100 cfu/ml. A significant remaining challenge is both to identify the organism as well as the viability state. A promising approach involves an amplification of strain specific messenger RNA.

Johnson-White B, Lin B, Ligler FS. (2007) Combination of immunosensor detection with viability testing and confirmation using the polymerase chain reaction and culture. Anal Chem. 79(1):140-6.

Rapid and accurate differential determination of viable versus nonviable microbes is critical for formulation of an appropriate response after pathogen detection. Sensors for rapid bacterial identification can be used for applications ranging from environmental monitoring and homeland defense to food process monitoring, but few provide viability information. This study combines the rapid screening capability of the array biosensor using an immunoassay format with methods for determination of viability. Additionally, cells captured by the immobilized antibodies can be cultured following fluorescence imaging to further confirm viability and for cell population expansion for further characterization, e.g., strain identification or antibiotic susceptibility testing. Finally, we demonstrate analysis of captured bacteria using the polymerase chain reaction (PCR). PCR results for waveguide-captured cells were 3 orders of magnitude more sensitive than the fluorescence immunoassay and can also provide additional genetic information on the captured microbes. These approaches can be used to rapidly detect and distinguish viable versus nonviable and pathogenic versus nonpathogenic captured organisms, provide culture materials for further analysis on a shorter time scale, and assess the efficacy of decontamination or sterilization procedures.

PMID: 17194131 [PubMed - indexed for MEDLINE]

Zhao W, Yao S, Hsing IM. (2006) A microsystem compatible strategy for viable Escherichia coli detection. Biosens Bioelectron. 2006 Jan 15;21(7):1163-70.

This study delineates a microsystem compatible strategy that enables the rapid determination of *Escherichia coli* viability for the application in food and

water monitoring. This approach differentiates the living cells from the dead ones by detecting the presence of a "viability indicator", i.e. mRNAs of a common *E. coli* GroEL heat shock protein (hsp). Our method starts with a stimulated and controlled transcription of hsp mRNA under an elevated temperature (47 degrees C) for 20min. Following that, the short-life mRNA is rapidly extracted using streptavidin-modified magnetic particles containing biotin-labeled DNA probes complementary to a specific region of the mRNA. The quantification of mRNA by gel electrophoresis and Ag/Au-based electrochemical detection is done after the amplification of mRNAs by reverse transcription-polymerase chain reaction (RT-PCR). Heat shock temperatures and durations that have profound effect to the mRNA transcription were studied and it was found that the mRNA undergoes a rapid minute-by-minute self-degradation after the environment resumes room temperature. Issues such as the DNA contamination that interfere the magnetic particle-based mRNA extraction technique were tackled. A sensitive Ag/Au-based electrochemical analysis method was used to detect the RT-PCR products and a cell concentration as low as 10(2)cfu/ml can be achieved by the electrochemical method, but not by the conventional gel electrophoresis. The strategy demonstrated in this study can be readily implemented in a microsystem and is a step forward for the realization of an integrated bioanalytical microsystem (lab on a chip) for the viable cell detection.

PMID: 15927460 [PubMed - indexed for MEDLINE]

Bernard L, Courties C, Duperray C, Schafer H, Muyzer G, Lebaron P. (2001) A new approach to determine the genetic diversity of viable and active bacteria in aquatic ecosystems. Cytometry. 43(4):314-21.

BACKGROUND: Discrimination among viable, active, and inactive cells in aquatic ecosystems is of great importance to understand which species participate in microbial processes. In this study, a new approach combining flow cytometry (FCM), cell sorting, and molecular analyses was developed to compare the diversity of viable cells determined by different methods with the diversity of total cells and active cells. METHODS: Total bacteria were determined by SYBR-II staining. Viable bacteria were determined in water samples from different sites by plate count techniques and by the direct viable count (DVC) method. Substrate-responsive cells (i.e., DVC(+) cells) were distinguished from nonresponsive cells (i.e., DVC(-) cells) by FCM and sorted. The genetic diversity of the sorted cell fraction was compared with the diversity of the total microbial community and with that of the culturable cell fraction by denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified 16S rDNA fragments. The same approach was applied to a seawater sample enriched with nutrients. In this case, actively respiring cells (CTC+) were also enumerated by FCM, sorted, and analyzed by DGGE. RESULTS: The diversity of viable cells varied depending on the methods (traditional culture or DVC) used for viability assessment. Some phylotypes detected in the fraction of viable cells were not detectable at the community level (from total DNA). Similar results were found for actively respiring cells. Inversely, some phylotypes found at the community level were not found in viable and active cell-sorted fractions. It suggests that diversity determined at the community

level includes nonactive and nonviable cells. CONCLUSION: This new approach allows investigation of the genetic diversity of viable and active cells in aquatic ecosystems. The diversity determined from sorted cells provides relevant ecological information and uncultured organisms can also be detected. New investigations in the field of microbial ecology such as the identification of species able to maintain cellular activity under environmental changes or in the presence of toxic compounds are now possible.

PMID: 11260599 [PubMed - indexed for MEDLINE]

Guy RA, Kapoor A, Holicka J, Shepherd D, Horgen PA. (2006) A rapid molecular-based assay for direct quantification of viable bacteria in slaughterhouses. J Food Prot. 69(6):1265-72.

A rapid test for microbial quantification in carcass and environmental swabs that does not require enrichment and provides results in less than 4 h is described here. Steps in the assay include the rapid concentration of bacteria on sponge swabs by vacuum filtration followed by real-time PCR detection. The assay has been applied for the detection of coliforms, *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* on carcass swabs and environmental samples in a slaughterhouse-processing line. Comparison of this rapid method with standard culture techniques for coliform counts on beef and pork carcass swabs revealed higher numbers of bacteria (2- to 50-fold) by the rapid test compared with the plate counts. This was due to the detection of all bacteria (live, dead, and non-culturable forms) in the rapid assay. To allow detection of only viable bacteria, concentrated samples were treated with ethidium monoazide (EMA) prior to DNA extraction and real-time PCR detection, thereby preventing the amplification of DNA from bacteria with damaged cell walls and allowing only the DNA from bacteria with intact membranes to be detected. EMA treatment resulted in a significant reduction ($P < 0.001$) in the number of coliforms detected compared to real-time PCR without EMA treatment. In beef swabs, the counts obtained in EMA real-time PCR were not significantly different ($P < 0.08$) from the culture counts and the correlation coefficient between the two assays was 0.7385. A lower correlation coefficient (0.402) was obtained with pork swabs. The assay described herein has the potential to be applied on a routine basis to slaughterhouse lines for the detection of indicator organisms or specific pathogens.

PMID: 16786844 [PubMed - indexed for MEDLINE]

Baeumner AJ, Leonard B, McElwee J, Montagna RA. (2004) A rapid biosensor for viable *B. anthracis* spores. Anal Bioanal Chem. 380(1):15-23.

A simple membrane-strip-based biosensor assay has been combined with a nucleic acid sequence-based amplification (NASBA) reaction for rapid (4 h) detection of a small number (ten) of viable *B. anthracis* spores. The biosensor is based on identification of a unique mRNA sequence from one of the anthrax toxin genes, the protective antigen (pag), encoded on the toxin plasmid, pXO1, and thus provides high specificity toward *B. anthracis*. Previously, the anthrax toxins activator (atxA) mRNA had been used in our

laboratory for the development of a biosensor for the detection of a single *B. anthracis* spore within 12 h. Changing the target sequence to the pag mRNA provided the ability to shorten the overall assay time significantly. The vaccine strain of *B. anthracis* (Sterne strain) was used in all experiments. A 500-microL sample containing as few as ten spores was mixed with 500 microL growth medium and incubated for 30 min for spore germination and mRNA production. Thus, only spores that are viable were detected. Subsequently, RNA was extracted from lysed cells, selectively amplified using NASBA, and rapidly identified by the biosensor. While the biosensor assay requires only 15 min assay time, the overall process takes 4 h for detection of ten viable *B. anthracis* spores, and is shortened significantly if more spores are present. The biosensor is based on an oligonucleotide sandwich-hybridization assay format. It uses a membrane flow-through system with an immobilized DNA probe that hybridizes with the target sequence. Signal amplification is provided when the target sequence hybridizes to a second DNA probe that has been coupled to liposomes encapsulating the dye sulforhodamine B. The amount of liposomes captured in the detection zone can be read visually or quantified with a hand-held reflectometer. The biosensor can detect as little as 1 fmol target mRNA (1 nmol L(-1)). Specificity analysis revealed no cross-reactivity with 11 organisms tested, among them closely related species such as *B. cereus, B. megaterium, B. subtilis, B. thuringiensis, Lactococcus lactis, Lactobacillus plantarum,* and *Chlostridium butyricum*. Also, no false positive signals were obtained from nonviable spores. We suggest that this inexpensive biosensor is a viable option for rapid, on-site analysis providing highly specific data on the presence of viable *B. anthracis* spores.

PMID: 15309363 [PubMed - indexed for MEDLINE]

Lee JL, Levin RE. (2006) Use of ethidium bromide monoazide for quantification of viable and dead mixed bacterial flora from fish fillets by polymerase chain reaction. J Microbiol Methods. 67(3):456-62.

Ethidium bromide monoazide (EMA) was utilized to selectively allow conventional PCR amplification of target DNA from viable but not dead cells from a broth culture of bacterial mixed flora derived from cod fillets. The universal primers designated DG74 and RW01 that amplify a 370-bp sequence of a highly conserved region of all eubacterial 16S rDNA were used for the PCR. The use of 10 microg/ml or less of EMA did not inhibit the PCR amplification of DNA derived from viable bacteria. The minimum amount of EMA to completely inhibit the PCR amplification of DNA derived from dead bacterial cells was 0.8 microg/ml. Amplification of target DNA from only viable cells in a suspension with dead cells was selectively accomplished by first treating the cells with 1 microg/ml of EMA. A standard curve was generated relating the intensity of fluorescence of DNA bands to the log of CFU of mixed bacterial cultures for rapidly assessing the number of genomic targets per PCR derived from the number of CFU. A linear range of DNA amplification was exhibited from 1 x 10(2) to 1 x 10(5) genomic targets per PCR. The viable/dead cell discrimination with the EMA-PCR method was evaluated by comparison with plate counts following freezing and thawing. Thawing frozen cell suspensions initially containing 1 x 10(5) CFU/ml at 4, 20, and 37 degrees C yielded a 0.8 log reduction in the number of viable cells determined by both plate counts

and EMA-PCR. In contrast, thawing for 5 min at 70 degrees C resulted in a 5 log reduction in CFU derived from plate counts (no CFU detected) whereas the EMA-PCR procedure resulted in only a 2.8 log reduction in genomic targets, possibly reflecting greater damage to enzymes or ribosomes at 70 degrees C to a minority of the mixed population compared to membrane damage.

PMID: 17183624 [PubMed - indexed for MEDLINE]

Birch L, Dawson CE, Cornett JH, Keer JT. (2001) A comparison of nucleic acid amplification techniques for the assessment of bacterial viability. Lett Appl Microbiol. 33(4):296-301.

AIMS: The ability to determine the presence and viability status of bacteria by molecular methods could offer significant advantages to the food, environmental and health sectors, in terms of improved speed and sensitivity of detection. METHODS AND RESULTS: In this study, we have assessed three amplification techniques, PCR, RT-PCR and NASBA, for their ability to detect nucleic acid persistence in an *E. coli* strain following heat-killing. NASBA offered the greatest sensitivity of the three methods tested. The presence of residual DNA and mRNA could be detected by PCR and NASBA, respectively, for up to 30 h postdeath, by which time cell death had been confirmed by culture methods. Thus a single quantitative measurement based on nucleic acid amplification did not permit unequivocal determination of cell viability. CONCLUSIONS, SIGNIFICANCE AND IMPACT OF THE STUDY: The correlation between cell viability and persistence of nucleic acids must be well characterized for a particular analytical situation before molecular techniques can be substituted for traditional culture methods.

PMID: 11559404 [PubMed - indexed for MEDLINE]

Baeumner AJ, Cohen RN, Miksic V, Min J. (2003) RNA biosensor for the rapid detection of viable *Escherichia coli* in drinking water. Biosens Bioelectron. 18(4):405-13.

A highly sensitive and specific RNA biosensor was developed for the rapid detection of viable *Escherichia coli* as an indicator organism in water. The biosensor is coupled with protocols developed earlier for the extraction and amplification of mRNA molecules from *E. coli* [Anal. Biochem. 303 (2002) 186]. However, in contrast to earlier detection methods, the biosensor allows the rapid detection and quantification of *E. coli* mRNA in only 15-20 min. In addition, the biosensor is portable, inexpensive and very easy to use, which makes it an ideal detection system for field applications. Viable *E. coli* are identified and quantified via a 200 nt-long target sequence from mRNA (clpB) coding for a heat shock protein. For sample preparation, a heat shock is applied to the cells prior to disruption. Then, mRNA is extracted, purified and finally amplified using the isothermal amplification technique nucleic acid sequence-based amplification (NASBA). The amplified RNA is then quantified with the biosensor. The biosensor is a membrane-based DNA/RNA hybridization system using liposome amplification. The various biosensor

components such as DNA probe sequences and concentration, buffers, incubation times have been optimized, and using a synthetic target sequence, a detection limit of 5 fmol per sample was determined. An excellent correlation to a much more elaborate and expensive laboratory based detection system was demonstrated, which can detect as few as 40 *E. coli* cfu/ml. Finally, the assay was tested regarding its specificity; no false positive signals were obtained from other microorganisms or from nonviable *E. coli* cells.
PMID: 12604258 [PubMed - indexed for MEDLINE]

Keer JT, Birch L. (2003) Molecular methods for the assessment of bacterial viability. J Microbiol Methods. 53(2):175-83.

A significant number of pathogenic microorganisms can be found in environmental reservoirs (air, water, soil). It is important to assess the viability status of these organisms to determine whether they pose a threat to public health. Classical methods for determining viability are time consuming. Hence, molecular methods have been developed to address this problem. Molecular methods offer speed, sensitivity and specificity. Both DNA and RNA have been analysed using molecular amplification methods such as polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR) and nucleic acid sequence-based amplification (NASBA). However, due to the variable persistence of nucleic acids in cells post-death, the correlation between presence of DNA and RNA and viability is not clear-cut. Similarly, the choice of target and sensitivity of the method can significantly affect the validity of the viability assay. This review assesses the molecular methods currently available and evaluates their ability to assess cell viability with emphasis on environmental pathogens.
PMID: 12654489 [PubMed - indexed for MEDLINE]

van der Vliet GM, Schepers P, Schukkink RA, van Gemen B, Klatser PR. (1994) Assessment of mycobacterial viability by RNA amplification. Antimicrob Agents Chemother. 38(9):1959-65.

We investigated whether the presence of intact RNA is a valuable indicator of viability of mycobacteria with *Mycobacterium smegmatis*. *M. smegmatis* was exposed to various concentrations of rifampin and ofloxacin suspended in broth for different periods of time. The NASBA nucleic acid amplification system was used because of its rapid, sensitive, and specific detection of 16S rRNA. During drug exposure, the viability of the mycobacteria, expressed by the number of CFU, was compared with the presence of 16S rRNA as determined by NASBA and with the presence of DNA coding for 16S rRNA as determined by PCR. Both NASBA and PCR were shown to have a detection limit of approximately 5 x 10(2) CFU/ml. The intensity of the NASBA signal corresponded well with the number of CFU, and the lack of NASBA signal coincided with a loss of viability, which was reached after 3 days of exposure to bactericidal concentrations of both drugs. The presence of mycobacterial DNA, as determined by the intensity of the PCR signal, and the viability of *M. smegmatis* were not related, but an increase in the number of cells and intensity of PCR signal correlated well. Bacterial viability may thus

be assessed by a rapid, sensitive, and specific, and semiquantitative technique by using NASBA. This system of viability testing provides the potential for rapid evaluation of drug susceptibility testing.

PMID: 7529012 [PubMed - indexed for MEDLINE]

Lebuhn M, Effenberger M, Garces G, Gronauer A, Wilderer PA. (2004) Evaluating real-time PCR for the quantification of distinct pathogens and indicator organisms in environmental samples. Water Sci Technol. 50(1):263-70.

We evaluated quantitative real-time PCR (qPCR) and RTqPCR (for RNA species) for their ability to quantify microorganisms and viruses in problematic environmental samples such as cattle manure, digester material, wastewater and soil. Important developments included a standard spiking approach which compensated for methodological bias and allowed sample-to-sample comparison and reliable quantification. Programme CeTe was developed to calculate endogenous concentrations of target organisms (nucleic acid copies) for each sample separately from the generated standard curves. The approach also permitted assessment of the detection limit of the complete method, including extraction. It varied from sample to sample, due to different extraction efficiencies and variable co-extraction of PCR inhibitors. False negative results were thereby avoided. By using this approach we were able to optimise a DNA extraction protocol from the different tested sample types. Protocols for the extraction of RNA species from environmental samples were also optimised. DNA was (almost) not degraded after lethal shock (autoclaving) in the sterile environment. In contrast, the parallel selective cultivation and qPCR results for various microbial parameters from an anaerobic digester chain suggested that DNA from decaying organisms was readily recycled in metabolically active environments. It may, therefore, be used to determine viable organisms in samples exhibiting substantial metabolic turnover. It is proposed that our standard spiking approach, including data evaluation by the program CeTe, should be considered in future standardisation and norms for the quantification of nucleic acid containing organisms in environmental and product samples.

PMID: 15318520 [PubMed - indexed for MEDLINE]

Cools I, Uyttendaele M, D'Haese E, Nelis HJ, Debevere J. (2006) Development of a real-time NASBA assay for the detection of *Campylobacter jejuni* cells. J Microbiol Methods. 66(2):313-20.

The objectives of this study were the development of a real-time NASBA assay for the detection of *Campylobacter jejuni* mRNA and the evaluation of its potential to determine the viability of the detected *C. jejuni* cells. A set of specific primers and probes was chosen to amplify the mRNA of the tuf-gene and the GTPase-gene. Only the tuf-assay was able to detect as low as 10(2) cells per NASBA reaction and was specific for *Campylobacter*. However, as the assay was able to detect dead cells, it cannot be used to demonstrate the viability of *C. jejuni* cells. The tuf-gene mRNA is not a good viability indicator due to its stability.

PMID: 16443295 [PubMed - indexed for MEDLINE]

Lleo MM, Pierobon S, Tafi MC, Signoretto C, Canepari P. (2000) mRNA detection by reverse transcription-PCR for monitoring viability over time in an *Enterococcus faecalis* viable but nonculturable population maintained in a laboratory microcosm. Appl Environ Microbiol. 66(10):4564-7.

The viable but nonculturable (VBNC) state is a survival strategy adopted by bacteria when they are exposed to hostile environmental conditions. It has been shown that VBNC forms of bacteria are no longer capable of growing on conventional bacteriological media but conserve pathogenic factors and/or genes. It is thus necessary to develop methods capable of detecting nonculturable bacteria and of establishing their viability when the microbiological quality of environments is monitored. In this study we demonstrated that a gene was expressed during the VBNC state in a low-nutrient-concentration microcosm through detection of *Enterococcus faecalis* pbp5 mRNA by reverse transcription-PCR over a 3-month period. The presence of mRNA correlated with metabolic activity and resuscitation capability, indicating the viability of the VBNC cells.

PMID: 11010918 [PubMed - indexed for MEDLINE]

Min J, Baeumner AJ. (2002) Highly sensitive and specific detection of viable *Escherichia coli* in drinking water. Anal Biochem. 303(2):186-93.

A highly sensitive and specific assay method was developed for the detection of viable *Escherichia coli* as an indicator organism in water, using nucleic acid sequence-based amplification (NASBA) and electrochemiluminescence (ECL) analysis. Viable *E. coli* were identified via a 200-nt-long target sequence from mRNA (clpB) coding for a heat shock protein. In the detection assay, a heat shock was applied to the cells prior to disruption to induce the synthesis of clpB mRNA and the mRNA was extracted, purified, and finally amplified using NASBA. The amplified mRNA was quantified with an ECL detection system after hybridization with specific DNA probes. Several disruption methods were investigated to maximize total RNA extracted from viable cells. Optimization was also carried out regarding the design of NASBA primer pairs and detection probes, as well as reaction and detection conditions. Finally, the assay was tested regarding sensitivity and specificity. Analysis of samples revealed that as few as 40 *E. coli* cells/mL can be detected, with no false positive signals resulting from other microorganisms or nonviable *E. coli* cells. Also, it was shown that a quantification of *E. coli* cells was possible with our assay method.

PMID: 11950218 [PubMed - indexed for MEDLINE]

Coombes BK, Mahony JB. (2000) Nucleic acid sequence based amplification (NASBA) of *Chlamydia pneumoniae* major outer membrane protein (ompA) mRNA with bioluminescent detection. Comb Chem High Throughput Screen. 3(4):315-27.

Chlamydia pneumoniae has been associated with chronic conditions such as atherosclerosis and coronary heart disease but the precise role of this intracellular bacteria in the pathogenesis of these diseases is not well defined. Several techniques have been developed for detection of *C. pneumoniae* in atheromatous lesions, however it remains unclear whether persistent forms of the organism and/or actively replicating bacteria contribute to associated pathology. The aim of this study was to utilize nucleic acid sequence based amplification (NASBA) technology together with a highly sensitive aequorin bioluminescent hybridization assay for the detection of *C. pneumoniae* ompA mRNA transcripts. A NASBA targeting the ompA gene of *C. pneumoniae* was developed, and the sensitivity was evaluated using both *C. pneumoniae* ompA RNA generated in vitro, and purified *C. pneumoniae* inclusion forming units (IFU). C. pneumoniae NASBA was capable of detecting between 100 and 1000 ompA RNA molecules and could detect 0.2 IFU of *C. pneumoniae* using the aequorin bioluminescent assay. The sensitivity of the bioluminescent assay was at least 10-fold higher than Northern blot detection. The linearity of NASBA amplification was assessed in time-course amplification experiments with different input numbers of RNA molecules. When NASBA products were analyzed during the linear phase of amplification, the dynamic range of bioluminescent detection extended over 8-log units of input RNA copy number. NASBA amplification coupled with bioluminescent detection may prove to be a useful molecular tool for the detection, quantitation and analysis of differentially expressed chlamydial genes during various stages of infection and disease pathology or for other mRNAs of interest in different disease processes.

PMID: 10974144 [PubMed - indexed for MEDLINE]

Shelton DR, Higgins JA, Van Kessel JA, Pachepsky YA, Belt K, Karns JS. (2004) Estimation of viable *Escherichia coli* O157 in surface waters using enrichment in conjunction with immunological detection. J Microbiol Methods. 58(2):223-31.

The use of a minimal lactose enrichment broth (MLB) in conjunction with immunomagnetic electrochemiluminescence detection (IM-ECL) was evaluated for the estimation of viable *Escherichia coli* O157 populations in surface water samples. In principle, *E. coli* O157 populations (C(initial *E. coli* O157)) can be derived from enrichment data according to the equation: C(initial *E. coli* O157) = C(initial coliforms) x C(final *E. coli* O157)/C(final coliforms)), assuming that the growth rates and lag times of water-borne *E. coli* O157 and collective coliforms are sufficiently comparable, or at least consistent. We have previously described a protocol for determining C(final *E. coli* O157) in MLB-enriched water samples. In the present study, 80% of coliforms (red/pink colonies on MacConkey Agar) grew in MLB, indicating that this provides reasonably accurate estimates of C(initial coliforms). Estimates of C(final coliforms) were determined from turbidity data. Initial E. coli O157 populations (C(initial *E. coli* O157)) were calculated for 33 Baltimore watershed samples giving a positive IM-ECL response. The majority of samples contained *E. coli* O157 concentrations of < 1 cell per 100 ml. These data indicate that *E. coli* O157 are present in surface water samples but at very low levels. Growth rates for MLB-enriched coliforms were highly variable (k= 0.47 +/- 0.13

h(-1), n= 72). There was no correlation between growth rates and any measured water parameter, suggesting that coliform populations in water samples are spatially and temporally unique. Although variability in growth rates was expected to yield some low values, the fact that most *E. coli* O157 concentrations were < 1 suggests that other factor(s) were also responsible. Studies with *E. coli* O157:H7 and wild-type *E. coli* suggest that increased lag times due to starvation were at least partially responsible for the observed data. Based on estimates of C(initial coliforms) and k(coliforms), MLB was evaluated for sensitivity and quantitativeness. Simulated populations of *E. coli* O157:H7 at stationary phase varied from ca. 10(3) to 10(8) cells ml(-1) enrichment culture. Although not suitable for quantitation, MLB enrichment in conjunction with IM-ECL can detect as few as one viable water-borne *E. coli* O157 cell per 100 ml surface water. Experiments are in progress to evaluate alternative media for sensitivity and quantitative detection of enterohemorrhagic *E. coli*.

PMID: 15234520 [PubMed - indexed for MEDLINE]

Baudart J, Coallier J, Laurent P, Prevost M. (2002) Rapid and sensitive enumeration of viable diluted cells of members of the family *Enterobacteriaceae* in freshwater and drinking water. Appl Environ Microbiol. 68(10):5057-63.

Water quality assessment involves the specific, sensitive, and rapid detection of bacterial indicators and pathogens in water samples, including viable but nonculturable (VBNC) cells. This work evaluates the specificity and sensitivity of a new method which combines a fluorescent in situ hybridization (FISH) approach with a physiological assay (direct viable count [DVC]) for the direct enumeration, at the single-cell level, of highly diluted viable cells of members of the family *Enterobacteriaceae* in freshwater and drinking water after membrane filtration. The approach (DVC-FISH) uses a new direct detection device, the laser scanning cytometer (Scan RDI). Combining the DVC-FISH method on a membrane with Scan RDI detection makes it possible to detect as few as one targeted cell in approximately 10(8) nontargeted cells spread over the membrane. The ability of this new approach to detect and enumerate VBNC enterobacterial cells in freshwater and drinking water distribution systems was investigated and is discussed.

PMID: 12324357 [PubMed - indexed for MEDLINE]

PATHOGEN DETECTION WITHOUT AMPLIFICATION

The majority of detection approaches that do not require amplification are optical methods, including surface plasmon resonance, ellipsometry, and surface enhanced Raman scattering. Alternative approaches involve electrical detection of a binding event.

Structural recognition provides the predominant means of organism isolation, fixation, or identification. Structural recognition can be through the use of antibodies, DNA/RNA/or PNA aptamers, phage display etc. The detection of this event can be sensed by a variety of techniques that sense a measurable difference following binding. This can be either direct, or through the interaction of a secondary binding element that contains the reporter element.

Binding elements provide the specificity of the assay. The tightness of fit, characterized by the avidity and affinity, in combination with the reporting element, determine sensitivity. The binding elements can be in solution (flow cytometry) or they can be immobilized on a surface. Multiplexing can be accomplished either in a array format, or with multiple groups of beads, each with different binding elements. The use of nanotechnology has improved the surface properties of some materials, and nanofabrication techniques have provided enhanced structures for the binding elements.

Sippy N, Luxton R, Lewis RJ, Cowell DC. (2003) Rapid electrochemical detection and identification of catalase positive micro-organisms. Biosens Bioelectron. 18(5-6):741-9.

The rapid detection and identification of bacteria has application in a number of fields, e.g. the food industry, environmental monitoring and biomedicine. While in biomedicine the number of organisms present during infection is multiples of millions in the other fields it is the detection of low numbers of organisms that is important, e.g. an infective dose of *Escherichia coli* O157:H7 from contaminated food is less than 100 organisms. A rapid and sensitive technique has been developed to detect low numbers of the model organism *E. coli* O55, combining Lateral Flow Immunoassay (LFI) for capture and amperometry for sensitive detection. Nitrocellulose membranes were used as the solid phase for selective capture of the bacteria using antibodies to *E. coli* O55. Different concentrations of *E. coli* O55 in Ringers solution were applied to LFI strips and allowed to flow through the membrane to an absorbent pad. The capture region of the LFI strip was placed in close contact with the electrodes of a Clarke cell poised at +0.7 V for the detection of hydrogen peroxide. Earlier research identified that the consumption of hydrogen peroxide by bacterial catalase provided a sensitive indicator of aerobic and facultative anaerobic microorganisms numbers. Modification and application of this technique to the LFI strips demonstrated that the consumption of 8 mM hydrogen peroxide was correlated with the number of microorganisms presented to the LFI strips in the range of 2 x 10(1)-2 x 10(7) colony forming units (cfu). Capture efficiency was dependent on the number of organisms applied and varied from 71% at 2 x 10(2) cfu to 25% at 2 x 10(7) cfu. The procedure was completed in less than 10 min and could detect

less than 10 cfu captured from a 200 microl sample applied to the LFI strip. The approached adopted provides proof of principle for the basis of a new technological approach to the rapid, quantitative and sensitive detection of bacteria that express catalase activity.

PMID: 12706587 [PubMed - indexed for MEDLINE]

Chemburu S, Wilkins E, Abdel-Hamid I. (2005) Detection of pathogenic bacteria in food samples using highly-dispersed carbon particles. Biosens Bioelectron. 21(3):491-9.

There is an unmet need for detection methods that can rapidly and sensitively detect food borne pathogens. A flow through immunoassay system utilizing highly dispersed carbon particles and an amperometric technique has been developed and optimized. A sandwich immunoassay format was utilized in which pathogenic cells were captured by antibodies immobilized onto activated carbon particles, and labeled with horseradish peroxidase (HRP) conjugated antibodies. Flow of the peroxidase substrates resulted in an amperometric signal that is proportional to the number of captured cells. Factors influencing the analytical performance of the system, such as the quantity of carbon particles and concentrations of capture antibody, enzyme labeled antibody, and enzyme substrates, were investigated and optimized. Detection and quantification of *Escherichia coli*, *Listeria monocytogenes* and *Campylobacter jejuni* were demonstrated with low detection limits of 50, 10, and 50 cells/ml, respectively, and an overall assay time of 30 min. Milk and chicken extract samples were spiked with various concentrations of these pathogens and were used to challenge the system. The system design is flexible enough to allow its application to the detection of viruses and proteins.

PMID: 16076439 [PubMed - indexed for MEDLINE]

Mathew FP, Alocilja EC. (2005) Porous silicon-based biosensor for pathogen detection. Biosens Bioelectron. 20(8):1656-61.

A porous silicon-based biosensor for rapid detection of bacteria was fabricated. Silicon (0.01 ohmcm, p-type) was anodized electrochemically in an electrochemical Teflon cell containing ethanoic hydrofluoric acid solution to produce sponge-like porous layer of silicon. Anodizing conditions of 5 mA/cm2 for 85 min proved best for biosensor fabrication. A single-tube chemiluminescence-based assay, previously developed, was adapted to the biosensor for detection of *Escherichia coli*. Porous silicon chips were functionalized with a dioxetane-Polymyxin B (cell wall permeabilizer) mixture by diffusion and adsorption on to the porous surface. The reaction of beta-galactosidase enzyme from *E. coli* with the dioxetane substrate generated light at 530 nm. Light emission for the porous silicon biosensor chip with *E. coli* was significantly greater than that of the control and planar silicon chip with *E. coli* ($P<0.01$). Sensitivity of the porous silicon biosensor was determined to be 101-102 colony forming units (CFU) of *E. coli*. The porous silicon-based biosensor was fabricated and functionalized to successfully detect *E. coli* and has potential applications in food and environmental testing.

PMID: 15626624 [PubMed - indexed for MEDLINE]

Smith HV. (1998) Detection of parasites in the environment. Parasitology. 117 Suppl:S113-41.

The environmental route of transmission is important for many protozoan and helminth parasites, with water, soil and food being particularly significant. Both the potential for producing large numbers of transmissive stages and their environmental robustness (with the ability to survive in moist microclimates for prolonged periods of time) pose persistent threats to public and veterinary health. Increased demands made on natural resources increase the likelihood of encountering environments and produce contaminated with parasites. In the last 30 years, endemic and epidemic waterborne and foodborne outbreaks in developed countries have led to a reappraisal of conventional isolation and detection methods. While these methods have proved invaluable in our understanding of environmental transmission routes for helminths, they have been less effective for the parasitic protozoa. Robust, efficient detection, viability and typing methods are required to assess risk and to further epidemiological understanding. Greater awareness of parasite contamination of our environment and its impact on health has precipitated the development of better detection methods. Currently, nowhere is this more apparent than with *Cryptosporidium*, with a broad range of immunological, microscopical and molecular methods available. The upsurge in molecular techniques, particularly the polymerase chain reaction, for determining occurrence and viability have brought with them the added benefits of increased sensitivity and specificity, yet many methods still have to be shown to address these issues consistently in the field. Rapid commercialization of reagents and standardization of methods provide consistency. The advances identified in non-destructive and destructive methods for the protozoa have application for helminths and emerging pathogens and should determine the importance of the matrices involved in the environmental transmission of parasites, further safeguarding public and veterinary health.

PMID: 10660936 [PubMed - indexed for MEDLINE]

Bergwerff AA, van Knapen F. (2006) Surface plasmon resonance biosensors for detection of pathogenic microorganisms: strategies to secure food and environmental safety. J AOAC Int. 89(3):826-31.

This review describes the exploitation of exclusively optical surface plasmon resonance (SPR) biosensors for the direct and indirect detection of pathogenic microorganisms in food chains and the environment. Direct detection is, in most cases, facilitated by the use of defined monoclonal or polyclonal antibodies raised against (a part of) the target pathogenic microorganisms. The antibodies were immobilized to a solid phase of the sensor to capture the microbe from the sample. Alternatively, antibodies were used in an inhibition-like assay involving incubation with the target organism prior to analysis of nonbound antibodies. The free immunoglobins were screened on a sensor surface coated with either purified antigens or with Fc or Fab

binding antibodies. Discussed examples of these approaches are the determination of *Escherichia coli* O1 57:H7, *Salmonella* spp., and *Listeria monocytogenes*. Another direct detection strategy involved SPR analysis of polymerase chain reaction products of Shiga toxin-2 genes reporting the presence of *E. coli* O157:H7 in human stool. Metabolic products have been exploited as biomarkers for the presence of a microbial agent, such as enterotoxin B and a virulence factor for the occurrence of *Staphylococcus aureus* and *Streptococcus suis*, respectively. Indirect detection, on the other hand, is performed by analysis of a humoral immune response of the infected animal or human. By immobilization of specific antigenic structures, infections with Herpes simplex and human immunodeficiency viruses, *Salmonella* and *Treponema pallidum* bacteria, and *Schistosoma* spp. parasites were revealed using human, avian, and porcine sera and avian eggs. Bound antibodies were easily isotyped using an SPR biosensor to reveal the infection history of the individual. Discussed studies show the recent recognition of the suitability of this type of instrument for (rapid) detection of health-threatening microbes to food and environmental microbial safety.

PMID: 16792081 [PubMed - indexed for MEDLINE]

Johnson PE, Lund ML, Shorthill RW, Swanson JE, Kellogg JL. (2001) Real time biodetection of individual pathogenic microorganisms in food and water. Biomed Sci Instrum. 37:191-6.

The primary objective of this research is to examine the feasibility of using an innovative technique based on laser-induced fluorescence coupled with flow cytometry to detect pathogenic microorganisms in food or water in real time. Our initial application is the rapid detection of *E. coli* O157:H7 in ground beef. The research performed demonstrated conclusively that this approach is feasible, and that the technique has key advantages over current alternatives including: it is (1) able to totally examine a large volume of food or water in real time, (2) capable of detecting single microorganisms (alternative techniques require in excess of 10(4) microorganisms), (3) intrinsically automatic, and (4) sensitive only to the selected bacteria. We have demonstrated the feasibility of detecting individual *E. coli* bacteria with a breadboard system. The performance of this system allows for rapid detection of individual specific pathogenic microorganisms. Two of the most significant commercial applications of this technique are the detection of infectious microorganisms in contaminated food and water. Food-borne microbial pathogens account for approximately 7 million illnesses and 9,000 deaths in the U.S. annually, with an estimated economic loss of at least $6 billion [1]. In addition, this method has the potential for a broad range of other commercial applications, including the detection of small numbers of molecules, such as the ultrasensitive detection of explosives and groundwater contaminants.

PMID: 11347387 [PubMed - indexed for MEDLINE]

Murphy L. (2006) Biosensors and bioelectrochemistry. Curr Opin Chem Biol. 10(2):177-84.

This review describes recent developments in the field of biosensors and bioelectrochemistry. Nanoparticles have been used to improve sensor performance and to develop biosensors based on new detection principles. Their use has extended into all areas of biosensor and bioelectrochemistry research. Other active areas of biosensor development include DNA sensing, immunosensing, direct electron transfer between an electrode and a redox protein or enzyme, and in vivo sensors.

PMID: 16516536 [PubMed - indexed for MEDLINE]

Nistor C, Osvik A, Davidsson R, Rose A, Wollenberger U, Pfeiffer D, Emneus J, Fiksdal L. (2002) Detection of *Escherichia coli* in water by culture-based amperometric and luminometric methods. Water Sci Technol. 45(4-5):191-9.

The application of amperometric biosensor- and chemiluminiscence based methods for rapid detection of viable *E. coli* in water has been investigated. An amplification of the amperometric signal by a factor of 4 was obtained when the cellobiose dehydrogenase (CDH) biosensor was used instead of a plain graphite electrode for detection of b-galactosidase (b-GAL) activity at 22.5 degrees C. A linear correlation was demonstrated for detection time (DT) vs. initial concentrations (logarithmic units) of *E. coli* IT1 and *E. coli* in environmental samples, respectively, by use of the CDH biosensor or a chemiluminometric technique. The study has shown that an *E. coli* concentration > or = 10(4) cfu/100 mL in environmental samples was determined by the CDH biosensor within one working day. However, further reduction of the DT can be obtained, e.g. by increasing the signal amplification factor using other biosensors.

PMID: 11936634 [PubMed - indexed for MEDLINE]

Kwakye S, Goral VN, Baeumner AJ. (2006) Electrochemical microfluidic biosensor for nucleic acid detection with integrated minipotentiostat. Biosens Bioelectron. 21(12):2217-23.

An electrochemical microfluidic biosensor with an integrated minipotentiostat for the quantification of RNA was developed based on nucleic acid hybridization and liposome signal amplification. Specificity of the biosensor was ensured by short DNA probes that hybridize with the target RNA or DNA sequence. The reporter probe was coupled to liposomes entrapping the electrochemically active redox couple potassium ferri/ferrohexacyanide. The capture probes were coupled to superparamagnetic beads that were isolated on a magnet in the biosensor. Upon capture, the liposomes were lysed to release the electrochemical markers that were detected on an interdigitated ultramicroelectrode array in the biosensor just downstream of the magnet. The current was measured, stored and displayed by miniaturized instrumentation (miniEC). The accuracy of the miniEC was evaluated by comparing its performance to a standard bench-top electrochemical workstation in static and dynamic DC amperometric experiments. In both sets of experiments, the inexpensive miniEC performance was comparable in signal strength to that of the electrochemical workstation. In fact, the miniEC achieved a detection limit of 0.01 microM combined ferri/ferrohexacyanide concentration which was

10 x lower than that of the standard lab-bench system. The response time of the miniEC system was the same for low concentrations taking about 10 s to steady state. It was, however, slower at higher concentrations, taking 5 s versus only 1 s for the bench-top system. Finally, the functionality of the miniEC was successfully demonstrated with the detection of Dengue virus RNA.

PMID: 16386889 [PubMed - indexed for MEDLINE]

Pyun JC, Beutel H, Meyer JU, Ruf HH. (1998) Development of a biosensor for *E. coli* based on a flexural plate wave (FPW) transducer. Biosens Bioelectron. 13(7-8):839-45.

To fulfill the need for rapid, cost-effective and sensitive methods for the detection of bacteria in medical diagnostics, food technology, biotechnology and environmental monitoring, a development of a bacterial sensor was initiated. Our approach of a biosensor for *E. coli* is based on an acousto-gravimetric flexural plate wave (FPW) transducer (gravimetric detection limit of less than 6 ng in a 32 microns thick sensitive layer in aqueous media), and an immunoaffinity layer on the transducer membrane for the molecular recognition of the target bacteria. An intermediate layer of covalently coupled poly (acrylic acid) yielded a major reduction of the non-specific binding to the metal surface. Such a biosensor, using antibodies against *E. coli* K12 and *E. coli* 15 outer surface antigens, yielded a detection range of 3.0 x 10(5) to 6.2 x 10(7) cells/ml for samples with the corresponding bacteria. To increase the sensitivity further, an amplification method using microspheres coupled with antibodies against *E. coli* was tested as a sandwich assay, and up to now a five-fold amplification of the signal has been achieved.

PMID: 9828380 [PubMed - indexed for MEDLINE]

Abdel-Hamid I, Ivnitski D, Atanasov P, Wilkins E. (1999) Flow-through immunofiltration assay system for rapid detection of *E. coli* O157:H7. Biosens Bioelectron. 14(3):309-16.

A flow-through amperometric immunofiltration assay system based on disposable porous filter-membranes for rapid detection of *Escherichia coli* O157:H7 has been developed. The analytical system utilizes flow-through, immunofiltration and enzyme immunoassay techniques in conjunction with an amperometric sensor. The parameters affecting the immunoassay such as selection of appropriate filter membranes, membrane pore size, antibody binding capacity and the concentrations of immunoreagents were investigated and optimized. Non-specific adsorption of the enzyme conjugate was investigated and minimized. A sandwich scheme of immunoassay was employed and the immunofiltration system allows to specifically and directly detect *E. coli* cells with a lower detection limit of 100 cells/ml. The working range is from 100 to 600 cells/ml with an overall analysis time of 30 min. No pre-enrichment was needed. This immunosensor can be easily adapted for assay of other microorganisms and may be a basis for a new class of highly sensitive bioanalytical devices for rapid quantitative detection of bacteria.

PMID: 10230031 [PubMed - indexed for MEDLINE]

Van Poucke SO, Nelis HJ. (1997) Limitations of highly sensitive enzymatic presence-absence tests for detection of waterborne coliforms and *Escherichia coli*. Appl Environ Microbiol. 63(2):771-4.

This study presents evidence for the unfeasibility of enzymatic presence-absence tests to detect one total coliform or one *Escherichia coli* organism in 100 ml of drinking water within a working day. The results of field trials with prototype chemiluminometric procedures indicated that the sensitivity-boosting measures that are essential to achieve the required speed compromise the specificity of the tests.
PMID: 9023956 [PubMed - indexed for MEDLINE]

Armon R, Kott Y. (1993) A simple, rapid and sensitive presence/absence detection test for bacteriophage in drinking water. J Appl Bacteriol. 74(4):490-6.

A rapid, simple and sensitive direct bacteriophage presence detection method for 500 ml drinking water samples has been developed. The method includes a glass device consisting of a jar containing the water sample and an immersible probe filled with solidified soft agar containing bacterial host cells. Host bacteria in logarithmic phase were added to the experimental volume and the probe was submerged. The entire device was incubated in a water bath at 36 degrees C. Plaques of somatic bacteriophage infecting *Escherichia coli* strain CN13, could be detected within 3 h. Male-specific bacteriophages infecting *E. coli* F+ amp were detected within 6 h. Bacteriophage infecting the anaerobe *Bacteroides fragilis* subsp. *fragilis* HSP40 were detected after 8 h. Application of this device and the associated technique, enabled a one-step detection of 1 pfu of *E. coli* or *Bact. fragilis* specific bacteriophage in 500 ml drinking water samples.
PMID: 8486556 [PubMed - indexed for MEDLINE]

Stanek JE, Falkinham JO 3rd. (2001) Rapid coliphage detection assay. J Virol Methods. 91(1):93-8.

A rapid coliphage detection assay was developed, based on the phage-induced release of beta-galactosidase from cells of *Escherichia coli*. The assay could detect as few as five coliphage per sample without an overnight incubation period. The range of acceptable assay parameters was identified.
PMID: 11164490 [PubMed - indexed for MEDLINE]

Pal S, Alocilja EC, Downes FP. (2007) Nanowire labeled direct-charge transfer biosensor for detecting *Bacillus* species. Biosens Bioelectron. 22(9-10):2329-36.

A direct-charge transfer (DCT) biosensor was developed for the detection of the foodborne pathogen, *Bacillus cereus*. The biosensor was fabricated using antibodies as the sensing element and polyaniline nanowire as the molecular electrical transducer. The sensor design consisted of four membrane pads, namely, sample application, conjugate, capture and absorption pads. Two sets of polyclonal antibodies, secondary antibodies conjugated with polyaniline nanowires and capture antibodies were applied to the conjugate and the capture pads of the biosensor, respectively. The detection technique was based on capillary flow action which allowed the liquid sample to move from one membrane to another. The working principle involved antigen-antibody interaction and direct electron charge flow to generate a resistance signal that was being recorded. Detection from sample application to final results was completed in 6 min in a reagentless process. Experiments were conducted to find the best performance of the biosensors by varying polyaniline types and concentrations. Polyaniline protonated with hydrochloric acid, emeraldine salt and polyaniline protonated with perchloric acid were the three kinds of polyaniline used in this study. The biosensor sensitivity in pure cultures of B. cereus was found to be 10(1) to 10(2)CFU/ml. Results indicated that using emeraldine salt at a concentration of 0.25 g/ml gave the best biosensor performance in terms of sensitivity. The biosensor was also found to be specific in detecting the presence of *B. cereus* in a mixed culture of different *Bacillus* species and other foodborne pathogens. The speed, sensitivity and ease-of-use of this biosensor make it a promising device for rapid field-based diagnosis towards the protection of our food supply chain. The phenotypic and genotypic similarities between *B. cereus* and *Bacillus anthracis* will also allow this biosensor to serve as an excellent model for the detection of *B. anthracis*.

PMID: 17320373 [PubMed - in process]

Lepeuple AS, Gilouppe S, Pierlot E, De Roubin MR. (2004) Rapid and automated detection of fluorescent total bacteria in water samples. Int J Food Microbiol. 92(3):327-32.

Traditional methods for the detection and enumeration of bacteria in water samples are growth-based and require several days to obtain the result. New techniques which reduce the time of analysis have been developed. The objective of this work was to test a rapid method for the detection and enumeration of total viable bacteria using direct fluorescent labelling and detection by laser scanning. This method (referred to as TVC for Total Viable Count) was compared to the R2A culture method and the cyano-ditolyl-tetrazolium chloride (CTC) staining method for the analysis of samples before the final chlorination (after GAC filtration) and drinking water samples. For the comparison of TVC and CTC, the outcome depends on the water type: for samples after GAC filtration, TVC counts were significantly lower than CTC counts by up to 2 log10 orders of magnitude. For chlorinated water samples, TVC counts were not significantly different from CTC counts. The comparison of TVC and R2A showed that TVC counts could be lower than R2A counts or equivalent depending on the type of water. For drinking water, the TVC method proved to yield results equivalent to those of the R2A method. The TVC method requires much shorter time frame than others. It is also simple to use and allows the analysis of large volumes (100 ml) of drinking water.

PMID: 15145591 [PubMed - indexed for MEDLINE]

Johnson PE, Deromedi AJ, Lebaron P, Catala P, Cash J. (2006) Fountain Flow cytometry, a new technique for the rapid detection and enumeration of microorganisms in aqueous samples. Cytometry A. 69(12):1212-21.

BACKGROUND: Pathogenic microorganisms are known to cause widespread waterborne disease worldwide. There is an urgent need to develop a technique for the real-time detection of pathogens in environmental samples at low concentrations, <10 microorganisms/ml, in large sample volumes, > or =100 ml. METHODS: A novel method, Fountain Flow cytometry, for the rapid and sensitive detection of individual microorganisms in aqueous samples is presented. Each sample is first incubated with a fluorescent label and then passed as a stream in front of a laser, which excites the label. The fluorescence is detected with a CCD imager as the sample flows toward the imager along its optical axis. The feasibility of Fountain Flow cytometry (FFC) is demonstrated by the detection of *Escherichia coli* labeled with ChemChrome CV6 and SYBR Gold in buffer and natural river water. RESULTS: Detections of labeled *E. coli* were made in aqueous suspensions with an efficiency of 96% +/- 14% down to a concentration approximately 200 bacteria/ml. CONCLUSIONS: The feasibility of FFC is demonstrated by the detection of *E. coli* in buffer and natural river water. FFC should apply to the detection of a wide range of pathogenic microorganisms including amoebae.

PMID: 17089372 [PubMed - indexed for MEDLINE]

Ferrari BC, Stoner K, Bergquist PL. (2006) Applying fluorescence based technology to the recovery and isolation of *Cryptosporidium* and *Giardia* from industrial wastewater streams. Water Res. 40(3):541-8.

As increasing water shortages continue, water re-use is posing new challenges with treated wastewater becoming a significant source of non-potable water. Rapid detection strategies that target waterborne pathogens of concern to industry are gaining importance in the assessment of water quality. This study reports on the ability to recover spiked *Cryptosporidium* and *Giardia* from a variety of industrial wastewater streams of varied water quality. Incorporation of an internal quality control used commonly in finished water enabled quantitative assessments of pathogen loads and we describe successful analysis of pre- and part-treated wastewater samples from four industrial sites. The method used combined calcium carbonate flocculation followed by flow cytometry and epifluorescence microscopy. Our focus will now aim at characterising the ambient parasites isolated from industrial wastewater with the objective of developing a suite of highly specific platform detection technologies targeted to industrial needs.

PMID: 16426657 [PubMed - indexed for MEDLINE]

Escoriza MF, VanBriesen JM, Stewart S, Maier J, Treado PJ. (2006) Raman spectroscopy and chemical imaging for quantification of filtered waterborne bacteria. J Microbiol Methods. 66(1):63-72.

Rapid and reliable assessment of pathogenic microbial contamination in water is critically important. In the present work we evaluated the suitability of Raman Spectroscopy and Chemical Imaging as enumeration techniques for waterborne pathogens. The prominent C-H stretching band observed between 2800-3000 cm(-1) of the spectrum is used for quantification purposes. This band provides the highest intensity of the bacterial-spectrum bands facilitating the detection of low number of microorganisms. The intensity of the Raman response correlates with number of cells present in drops of sample water on aluminum-coated slides. However, concentration of pathogens in drinking and recreational water is low, requiring a concentration step, i.e., filtering. Subsequent evaluation of filtering approaches for water sampling for Raman detection showed significant background signal from alumina and silver membranes that reduces method sensitivity. Samples concentrated by filtration show good correlation between Raman spectroscopy and other quantification methods including turbidity (R(2)=0.92), plate counts (R(2)=0.87) and dry weight (R(2)=0.97). Background interferences did not allow for evaluation of this relationship at low cell concentrations.

PMID: 16325947 [PubMed - indexed for MEDLINE]

Sinclair JL (2000) Enumeration of *Cryptosporidium* spp. in water with US EPA Method 1622. J AOAC Internat. 83(5):1108-14.

Abstract: The occurrence of *Cryptosporidium parvum* or other pathogenic *Cryptosporidium* species in water must be known in order to assess risk and determine the treatment needed to reduce *Cryptosporidium* oocysts to acceptable levels in finished drinking water. Because *Cryptosporidium* oocyst occurrence may be sparse, methods must concentrate a large volume of water and correctly identify oocysts in the concentrate. The U.S. Environmental Protection Agency Information Collection Rule (ICR) protozoan method gives low and variable recoveries of *Cryptosporidium* oocysts, making risk assessment difficult. Therefore, a method giving better oocyst recovery and more consistent results was needed. Method 1622 was developed with existing materials and procedures, and improvements were made in filtration, cleanup, and detection. Absolute porosity filters were used, with cleanup by immunomagnetic separation and detection by direct fluorescent antibody stain with 4',6-diamidino-2-phenylindole (DAPI) staining for additional cell structures. Both the level and consistency of oocyst recovery were improved compared to recovery with the ICR method.

Chung J, Vesey G, Gauci M, Ashbolt NJ (2004) Fluorescence resonance energy transfer (FRET)-based specific labeling of *Cryptosporidium* oocysts for detection in environmental samples. Cytometry A. 60(1):97-106.

Abstract: BACKGROUND: Accurate detection and quantification of *Cryptosporidium* oocysts in water are a challenge to the water industry. This article demonstrates a way to fluorescently label *Cryptosporidium* oocysts, based on fluorescence resonance energy transfer (FRET). Labeled oocysts can then be applied to environmental waters and their movement followed by flow cytometric detection and enumeration of the FRET-labeled oocysts, as demonstrated here with environmental water samples. METHODS: *Cryptosporidium* oocysts were labeled with three fluorochromes, FITC, Texas red, and Cy7, that through FRET yielded a Stokes shift of approximately 272 nm with excitation from a standard argon laser emitting at 488 nm. Defined flow cytometric settings and gatings were used to select FITC/green (530-nm), Texas red/red (650-nm), and Cy7/infrared (780-nm) fluorescing particles with light scatter properties similar to oocysts. Water concentrates were seeded with 10 tri-labeled oocysts and were analyzed using flow cytometry. Unseeded water concentrates were also analyzed. RESULTS: Analysis of unseeded water concentrates detected no autofluorescent particle similar to the labeled oocysts. Labeled oocysts were detected successfully with up to 85% recovery in water concentrates spiked with 10 tri-labeled oocysts. CONCLUSIONS: Low numbers of FRET-labeled oocysts can be quantified and clearly distinguished from autofluorescing background in environmental water concentrates.

Campbell GA, Mutharasan R (2007) A method of measuring *Escherichia coli* 0157:H7 at 1 cell/mL in 1 liter sample using antibody functionalized piezoelectric-excited millimeter-sized cantilever sensor. Environ Sci Tech. 41(5):1668-74.

Abstract: Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors immobilized with antibody specific to *Escherichia coli* (EC) 0157:H7 is used to detect EC at 1 cell/mL in 1 mL and 1 L samples in a batch and flow mode, respectively. Two sensor designs were used. The first design (PEMC-a) has both the piezoelectric and non-piezoelectric layer anchored, while in the second design (PEMC-b) had only the piezoelectric layer anchored. PEMC-a, used in batch mode with 1 mL sample, showed limit of detection at 10 cells/ mL using the second bending mode at 85.5 kHz in air. PEMC-b exhibited resonant frequencies at 186.5, 883.5, and 1778.5 kHz in air and 162.5, 800.0, and 1725.5 kHz in sample flow conditions. A one-liter sample containing 1000 EC cells was introduced at 1.5, 2.5, 3, and 17 ml/min, and the change in resonant frequency was monitored. The total frequency change observed for the mode at 800 kHz and sample flow rates of 1.5, 2.5, 3, and 17 mL/min were 2230+/-11, 3069 +/-47, 4686+/-97, and 7188+/-52 Hz, respectively. Each detection experiment was confirmed by exposing the sensor to a low pH solution followed by a phosphate buffered saline (PBS) rinse, which caused the release of the attached EC. The final frequency change observed was nearly identical to the value prior to EC attachment. Kinetic analysis showed that the observed binding rate constant at 1.5, 2.5, 3 mL/min were 0.009, 0.015, and 0.021 min(-1), respectively. The significance of these results is that very low concentration of pathogens in large sample volumes can be measured in a short time period without the need for filtration or enrichment.

Maraldo D, Rijal K, Campbell G, Mutharasan R. (2007) Method for label-free detection of femtogram quantities of biologics in flowing liquid samples. Anal Chem. 79(7):2762-70.

Abstract: Rapid (approximately 10 min) measurement of very low concentration of pathogens (approximately 10 cells/mL) and protein (approximately fg/mL) has widespread use in medical diagnostics, monitoring biothreat agents, and in a broader context as a research method. For low-level pathogen, we currently use culture enrichment methods and, thus, rapid analysis is not possible. For low protein concentration, no direct method is currently available. We report here a novel macrocantilever design whose high-order resonant mode near 1 MHz exhibits mass detection sensitivity of 10 cells/mL for cells and 100 fg/mL for protein. The sensor is 1x3 mm and uses a piezoelectric layer for both actuation and sensing resonance. Sample is flowed (approximately 1 mL/min) past the antibody-immobilized sensor, and as antigen binds to the sensor, resonance frequency decreases in proportion to antigen concentration. The sensor showed selectivity to the pathogen even though copious nonpathogenic variant was simultaneously present.

PMID: 17309231 [PubMed - indexed for MEDLINE]

Campbell GA, Mutharasan R. (2007) Method of measuring *Bacillus anthracis* spores in the presence of copious amounts of *Bacillus thuringiensis* and *Bacillus cereus*. Anal Chem. 79(3):1145-52.

Abstract: A sensitive and reliable method for the detection of *Bacillus anthracis* (BA; Sterne strain 7702) spores in presence of large amounts of *Bacillus thuringiensis* (BT) and *Bacillus cereus* (BC) is presented based on a novel PZT-anchored piezoelectric excited millimeter-sized cantilever (PAPEMC) sensor with a sensing area of 1.5 mm2. Antibody (anti-BA) specific to BA spores was immobilized on the sensing area and exposed to various samples of BA, BT, and BC containing the same concentration of BA at 333 spores/mL, and the concentration of BT + BC was varied in concentration ratios of (BA:BT + BC) 0:1, 1:0, 1:1, 1:10, 1:100, and 1:1000. In each case, the sensor responded with an exponential decrease in resonant frequency and the steady-state frequency changes reached were 14 +/- 31 (n = 11), 2742 +/- 38 (n = 3), 3053 +/- 19 (n = 2), 2777 +/- 26 (n = 2), 2953 +/- 24 (n = 2), and 3105 +/- 27 (n = 2) Hz, respectively, in 0, 27, 45, 63, 154, and 219 min. The bound BA spores were released in each experiment, and the sensor response was nearly identical to the frequency change during attachment. These results suggest that the transport of BA spores to the antibody immobilized surface was hindered by the presence of other *Bacillus* species. The observed binding rate constant, based on the Langmuir kinetic model, was determined to be 0.15 min-1. A hindrance factor (alpha) is defined to describe the reduced attachment rate in the presence of BT + BC and found to increase exponentially with BT and BC concentration. The hindrance factor increased from 3.52 at 333 BT + BC spores/mL to 11.04 at 3.33 x 105 BT + BC spores/mL, suggesting that alpha is a strong function of BT and BC concentration. The

significance of these results is that anti-BA functionalized PEMC sensors are highly selective to *Bacillus anthracis* spores and the presence of other *Bacillus* species, in large amounts, does not prevent binding but impedes BA transport to the sensor.

PMID: 17263347 [PubMed - indexed for MEDLINE]

Campbell GA, Mutharasan R. (2006) Detection of *Bacillus anthracis* spores and a model protein using PEMC sensors in a flow cell at 1 mL/min. Biosens Bioelectron. 22(1):78-85.

Abstract: Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors of 4mm(2) sensing area were immobilized with antibody specific to *Bacillus anthracis* (anti-BA) spores or bovine serum albumin (anti-BSA). Detection of pathogen (*Bacillus anthracis* (BA) at 300 spores/mL) and BSA (1 mg/mL) were investigated under both stagnant and flow conditions. Two flow cell designs were evaluated by characterizing flow-induced resonant frequency shifts. One of the flow cells labeled SFC-2 (hold-up volume of 0.3 mL), showed small fluctuations (+/-20 Hz) around a common resonant frequency response of 217 Hz in the flow rate range of 1-17 mL/min. The total resonant frequency change obtained for the binding of 300 spores/mL in 1h was 90+/-5 Hz (n=2), and 162+/-10 Hz (n=2) under stagnant and flow conditions, respectively. Binding of antibodies, anti-BA and anti-BSA, were more rapid under flow than under stagnant conditions. The sensor was repeatedly exposed to BSA with an intermediate release step. The first and second responses to BSA were nearly identical. The total resonant frequency response to BSA was 388+/-10 (n=2) Hz under flow conditions. Kinetic analysis is carried out to quantify the effect of flow rate on antibody immobilization and the two types of detection experiments.

PMID: 16423521 [PubMed - indexed for MEDLINE]

Leung A, Rijal K, Shankar PM, Mutharasan R. (2006) Effects of geometry on transmission and sensing potential of tapered fiber sensors. Biosens Bioelectron. 21(12):2202-9.

Abstract: Geometry of tapered fiber sensors critically affects the response of an evanescent field sensor to cell suspensions. Single-mode fibers (nominally at 1300 nm) were tapered to symmetric or asymmetric tapers with diameters in the range of 3-20 microm, and overall lengths of 1-7 mm. Their transmission characteristics in air, water and in the presence of *Escherichia coli* (JM101 strain) at concentrations of 100, 1000, 7000 and 7 million cells/mL were measured in the 400-800 nm range and gave rich spectral data that lead to the following conclusions. (1) No change in transmission was observed due to *E. coli* with tapers that showed no relative change in transmission in water compared to air. (2) Tapers that exhibited a significant difference in transmission in water compared to air gave weak response to the presence of the *E. coli*. Of these, tapers with low waist diameters (6 microm) showed sensitivity to *E. coli* at 7000 cells/mL and higher concentration. (3) Tapers that showed modest difference in water transmission compared to air, and those that had small waist diameters gave excellent response to *E.*

coli at 100-7000 cells/mL. In addition, mathematical modeling showed that: (1) at low wavelength (470 nm) and small waist diameter (6 microm), transmission with water in the waist region is higher than in air. (2) Small changes in waist diameter (approximately 0.05 microm) can cause larger changes in transmission at 470 nm than at 550 nm at waist diameter of 6 microm. (3) For the same overall geometry, a 5.5 microm diameter taper showed larger refractive index sensitivity compared to a 6.25 microm taper at 470 nm.

PMID: 16406569 [PubMed - indexed for MEDLINE]

Rijal K, Leung A, Shankar PM, Mutharasan R. (2005) Detection of pathogen *Escherichia coli* O157:H7 AT 70 cells/mL using antibody-immobilized biconical tapered fiber sensors. Biosens Bioelectron. 21(6):871-80.

Abstract: Optical fibers (core diameter 8 microm, cladding diameter 125 microm) was tapered to a waist diameter in the range of 8-12 microm, and then a monoclonal antibody to the pathogen, *Escherichia coli* O157:H7 was covalently bonded to the surface of the tapered region. Using 470 nm light, the taper was exposed to various concentrations (7 x 10(7), 7 x 10(5), 7 x 10(3), and 70 cells/mL) of the pathogen, and the sensor showed changes in transmitted light as the antigen attached to the antibody on the taper surface. The response was equal and opposite when the pathogen was released from the surface using a low pH buffer. The magnitude of the change was inversely proportional to the concentration of the pathogen. The sensor showed good sensitivity at as low a concentration as 70 cells/mL. The antibody-immobilized taper sensor was also exposed to a mixture of the pathogen and a non-pathogenic variant (JM101) at 0%, 50% and 70% by concentration. The sensor showed good selectivity to the pathogenic antigen. A first order attachment kinetic model is proposed to quantify the rate of attachment of pathogen to the sensor surface. The kinetic rate constant (k) of *E. coli* O157:H7 to the fiber was found to vary in the range of (2.5-6.1) x 10(-9) min(-1) (cells/mL)(-1).

PMID: 16257655 [PubMed - indexed for MEDLINE]

Campbell GA, Mutharasan R. (2006) Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors detect *Bacillus anthracis* at 300 spores/mL. Biosens Bioelectron. 21(9):1684-92.

Abstract: Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors consisting of a piezoelectric and a borosilicate glass layer with a sensing area of 2.48 mm2 were fabricated. Antibody specific to *Bacillus anthracis* (BA, Sterne strain 7702) spores was immobilized on PEMC sensors, and exposed to spores (300 to 3x10(6) spores/mL). The resonant frequency decreased at a rate proportional to the spore concentration and reached a steady state frequency change of 5+/-5 Hz (n=3), 92+/-7 Hz (n=3), 500+/-10 Hz (n=3), 1030+/-10 Hz (n=2), and 2696+/-6 Hz (n=2) corresponding to 0, 3x10(2), 3x10(3), 3x10(4), and 3x10(6) spores/mL, respectively. The reduction in resonant frequency is proportional to the change in cantilever mass, and thus the observed changes are due to the attachment of spores on the sensor surface. Selectivity of the antibody-functionalized sensor was determined with samples of BA (3x10(6)/mL) mixed with

Bacillus thuringiensis (BT; 1.5x10(9)/mL) in various volume ratios that yielded BA:BT ratios of 1:0, 1:125, 1:250, 1:500 and 0:1. The corresponding resonance frequency decreases were, respectively, 2345, 1980, 1310, 704 and 10 Hz. Sample containing 100% BT spores (1.5x10(9)/mL and no BA) gave a steady state frequency decrease of 10 Hz, which is within noise level of the sensor, indicating excellent selectivity. The observed binding rate constant for the pure BA and BT-containing samples ranged from 0.105 to 0.043 min-1 in the spore concentration range 300 to 3x10(6)/mL. These results show that detection of *B. anthracis* spore at a very low concentration (300 spores/mL) and with high selectivity in presence of another *Bacillus* spore (BT) can be accomplished using piezoelectric-excited millimeter-sized cantilever sensors.

PMID: 16169715 [PubMed - indexed for MEDLINE]

Campbell GA, Mutharasan R (2005) Detection of pathogen *Escherichia coli* O157:H7 using self-excited PZT-glass microcantilevers. Biosens Bioelectron. 21(3):462-73.

Abstract: Composite self-excited PZT-glass cantilevers (5 and 3 mm in length, 1.8 and 2.0 mm wide) were fabricated and their resonance characteristics were determined in air and at 1 mm liquid immersion. In air, resonance occurred at 65.8 and 63.4 kHz for the two cantilevers used in this paper. Monoclonal antibody (MAb) specific to the pathogen *Escherichia coli* (*E. coli*) O157:H7 was immobilized at the cantilever glass tip, and then exposed to pathogen in the concentration range of 7x10(2) to 7x10(7)bacteria/mL. Resonance of the second mode decreased due to pathogen attachment in accordance with a proposed kinetic model. The specific attachment rate constant was found to be 3x10(-9) to 5x10(-9) min-1 (cell/mL)-1. Exposure to a mixed population containing both a pathogenic and non-pathogenic strain showed that the antibody-immobilized cantilever is highly selective, thus demonstrating its usefulness for detecting water-borne pathogens.

PMID: 16076436 [PubMed - indexed for MEDLINE]

Zuckerman U, Tzipori S. (2006) Portable continuous flow centrifugation and method 1623 for monitoring of waterborne protozoa from large volumes of various water matrices. J Appl Microbiol. 100(6):1220-7.

Abstract: AIMS: The aims of this study were to validate a portable continuous flow centrifuge (PCFC) as an alternative concentration step of US-EPA Method 1623 and to demonstrate it's efficacy for recovery of low numbers of protozoa from large volumes of various water matrices. METHODS AND RESULTS: Recoveries of *Cryptosporidium parvum* oocysts, *Giardia intestinalis* cysts and *Encephalitozoon intestinalis* spores spiked into 10-1000 l volumes of various water matrices were evaluated during in-house and collaborative trials. Spiked protozoa were either approved standards or diluted stock samples enumerated according to USEPA Method 1623. *Cryptosporidium* recoveries exceeded method 1623 criteria and substantially high recoveries were observed for *Giardia* and *E. intestinalis*. CONCLUSIONS: Portable continuous flow centrifuge methodology exceeded method 1623 acceptance criteria for *Cryptosporidium* and could

be easily adopted for other protozoa. SIGNIFICANCE AND IMPACT OF THE STUDY: The PCFC could be adopted as an alternative user-friendly concentration method for *Cryptosporidium* and for monitoring of large volumes of source and tap water for accidental or deliberate contamination with protozoa and potentially with other enteric pathogens. It is anticipated that PCFC would also be equal or superior to filtration for protozoa monitoring in wastewater and effluents.

PMID: 16696669 [PubMed - indexed for MEDLINE]

Gehring AG, Albin DM, Bhunia AK, Reed SA, Tu SI, Uknalis J. (2006) Antibody microarray detection of *Escherichia coli* O157:H7: Quantification, assay limitations, and capture efficiency. Anal Chem. 78(18):6601-7.

A sandwich fluorescent immunoassay in a microarray format was used to capture and detect *E. coli* O157:H7. Here, we explored quantitative aspects, limitations, and capture efficiency of the assay. When biotinylated capture antibodies were used, the signal generated was higher (over 5-fold higher with some cell concentrations) compared to biotinylated protein G-bound capture antibodies. By adjusting the concentration of reporter antibody, a linear fluorescent response was observed from approximately 3.0 x 10(6) to approximately 9.0 x 10(7) cells/mL, and this was in agreement with the number of captured bacteria as determined by fluorescence microscopy. Capture efficiency calculations revealed that, as the number of bacteria presented for capture decreased, capture efficiency increased to near 35%. Optimization experiments, with several combinations of capture and reporter antibodies, demonstrated that the amount of bacteria available for capture (10(6) versus 10(8) cells/mL) affected the optimal combination. The findings presented here indicate that antibody microarrays, when used in sandwich assay format, may be effectively used to capture and detect *E. coli* O157:H7.

PMID: 16970339 [PubMed - indexed for MEDLINE]

Thirumalapura NR, Ramachandran A, Morton RJ, Malayer JR. (2006) Bacterial cell microarrays for the detection and characterization of antibodies against surface antigens. J Immunol Methods. 309(1-2):48-54.

Bacterial cell surface antigens interact with the host immune system resulting in the production of antibodies. Detection of antibodies against surface antigens has applications in diagnosis of many bacterial infections, assessment of immune status and epidemiological studies. We developed a microarray platform, for antibody detection, by printing Gram-negative and Gram-positive whole bacterial cells on nitrocellulose coated glass substrates. Antibody binding was detected using fluorophore labeled secondary antibodies. The sensitivity of antibody detection was found to be 0.1 microg/ml. Using bacterial cell microarrays it was also possible to successfully detect antibodies against Francisella tularensis in canine serum samples declared positive for tularemia based on microagglutination antibody titer. Use of bacterial cells as the antigen source in immunoassays has the advantages of simulating in vivo presentation of surface antigens and also eliminating the need for antigen purification. The microarray format gives the

added advantage of simultaneous detection of antibodies against multiple bacteria employing only small amounts of samples and reagents.
PMID: 16423364 [PubMed - indexed for MEDLINE]

Dufva M, Christensen CB. (2005) Diagnostic and analytical applications of protein microarrays. Expert Rev Proteomics. 2(1):41-8.

DNA microarrays have changed the field of biomedical sciences over the past 10 years. For several reasons, antibody and other protein microarrays have not developed at the same rate. However, protein and antibody arrays have emerged as a powerful tool to complement DNA microarrays during the past 5 years. A genome-scale protein microarray has been demonstrated for identifying protein-protein interactions as well as for rapid identification of protein binding to a particular drug. Furthermore, protein microarrays have been shown as an efficient tool in cancer profiling, detection of bacteria and toxins, identification of allergen reactivity and autoantibodies. They have also demonstrated the ability to measure the absolute concentration of small molecules. Besides their capacity for parallel diagnostics, microarrays can be more sensitive than traditional methods such as enzyme-linked immunosorbent assay, mass spectrometry or high-performance liquid chromatography-based assays. However, for protein and antibody arrays to be successfully introduced into diagnostics, the biochemistry of immunomicroarrays must be better characterized and simplified, they must be validated in a clinical setting and be amenable to automation or integrated into easy-to-use systems, such as micrototal analysis systems or point-of-care devices.
PMID: 15966851 [PubMed - indexed for MEDLINE]

Li B, Jiang L, Song Q, Yang J, Chen Z, Guo Z, Zhou D, Du Z, Song Y, Wang J, Wang H, Yu S, Wang J, Yang R. (2005) Protein microarray for profiling antibody responses to *Yersinia pestis* live vaccine. Infect Immun. 73(6):3734-9.

A protein microarray representing 149 *Yersinia pestis* proteins was developed to profile antibody responses in EV76-immunized rabbits. Antibodies to 50 proteins were detected. There are 11 proteins besides F1 and V antigens to which the predominant antibody response occurred, and these proteins show promise for further evaluation as candidates for subunit vaccines and/or diagnostic antigens.
PMID: 15908403 [PubMed - indexed for MEDLINE]

Pavlickova P, Knappik A, Kambhampati D, Ortigao F, Hug H. (2003) Microarray of recombinant antibodies using a streptavidin sensor surface self-assembled onto a gold layer. Biotechniques. 34(1):124-30.

We have developed a sensitive method for the detection of recombinant antibody-antigen interactions in a microarray format. The biochip sensor platform used in this study is based on an oriented streptavidin monolayer that provides a biological interface

with well-defined surface architecture that dramatically reduces nonspecific binding interactions. All the antibody or antigen probes were biotinylated and coupled onto streptavidin-coated biochip surfaces (1 microL total volume). The detection limits for the immobilized probes on the microarray surface were 0.5 microgram/mL (200 fmol/spot) for the peptide antigen and 0.1 microgram/mL (3 fmol/spot) for the recombinant antibodies. Optimal concentrations for the detection of the Cy5-labeled protein target were in the range of 20 micrograms/mL. Protein microchips were used to measure antibody-antigen kinetics, to find optimal temperature conditions, and to establish the shelf life of recombinant antibodies immobilized on the streptavidin surface. For recombinant antibody fragments with a kDa of 10-100 nM, we have established an easy and direct immunoassay. In addition, we developed an indirect method for antibody detection with no need for expensive and time-consuming antibody purifications and modifications. Such a method was shown to be useful for large-scale screening of recombinant antibody fragments directly after their functional expression in bacteria. Our data demonstrate that recombinant antibody fragments are suitable components in the construction of antibody chips.

PMID: 12545549 [PubMed - indexed for MEDLINE]

Delehanty JB, Ligler FS. (2002) A microarray immunoassay for simultaneous detection of proteins and bacteria. Anal Chem. 74(21):5681-7.

We report the development and characterization of an antibody microarray biosensor for the rapid detection of both protein and bacterial analytes under flow conditions. Using a noncontact microarray printer, biotinylated capture antibodies were immobilized at discrete locations on the surface of an avidin-coated glass microscope slide. Preservation of capture antibody function during the deposition process was accomplished with the use of a low-salt buffer containing sucrose and bovine serum albumin. The slide was fitted with a six-channel flow module that conducted analyte-containing solutions over the array of capture antibody microspots. Detection of bound analyte was subsequently achieved using fluorescent tracer antibodies. The pattern of fluorescent complexes was interrogated using a scanning confocal microscope equipped with a 635-nm laser. This microarray system was employed to detect protein and bacterial analytes both individually and in samples containing mixtures of analytes. Assays were completed in 15 min, and detection of cholera toxin, staphylococcal enterotoxin B, ricin, and *Bacillus globigii* was demonstrated at levels as low as 8 ng/mL, 4 ng/mL, 10 ng/mL, and 6.2 x 10(4) cfu/mL, respectively. The assays presented here are very fast, as compared to previously published methods for measuring antibody-antigen interactions using microarrays (minutes versus hours).

PMID: 12433105 [PubMed - indexed for MEDLINE]

PATHOGEN DETECTION WITHOUT AMPLIFICATION USING MICROARRAYS

Microarrays provide a platform to immobilize up to millions of specific binding elements in a discrete area defined by specific X, Y coordinates. The multiplicity of sequences that can be displayed using an array format allows for the identification of multiple organisms, as well as the ability to look for multiple signatures from each organism. The ability to look for multiple signatures in a highly parallel fashion changes the paradigm of what questions are asked. Specific strain level sequences can provide high level resolution of what particular organism(s) are present, while other locations on the array can be dedicated to characterization questions such as, what virulence factors or antibiotic resistance genes are present. The array can also be used to identify the presence of messenger RNA indicating viability.

Many methods have been used to immobilize binding elements on a surface. They include masking, etching, etc. High density microarrays frequently have design limitations and can cost hundreds of dollars each. Lower density arrays such as membrane-based formats are a lower cost option. Numerous platforms exist for low, medium or high density arrays.

Bijlsma JJ, Burghout P, Kloosterman TG, Bootsma HJ, de Jong A, Hermans PW, Kuipers OP. (2007) Development of genomic array footprinting for identification of conditionally essential genes in *Streptococcus pneumoniae*. Appl Environ Microbiol. 73(5):1514-24.

Streptococcus pneumoniae is a major cause of serious infections such as pneumonia and meningitis in both children and adults worldwide. Here, we describe the development of a high-throughput, genome-wide technique, genomic array footprinting (GAF), for the identification of genes essential for this bacterium at various stages during infection. GAF enables negative screens by means of a combination of transposon mutagenesis and microarray technology for the detection of transposon insertion sites. We tested several methods for the identification of transposon insertion sites and found that amplification of DNA adjacent to the insertion site by PCR resulted in nonreproducible results, even when combined with an adapter. However, restriction of genomic DNA followed directly by in vitro transcription circumvented these problems. Analysis of parallel reactions generated with this method on a large mariner transposon library showed that it was highly reproducible and correctly identified essential genes. Comparison of a mariner library to one generated with the in vivo transposition plasmid pGh:ISS1 showed that both have an equal degree of saturation but that 9% of the genome is preferentially mutated by either one. The usefulness of GAF was demonstrated in a screen for genes essential for surviving zinc stress. This identified a gene encoding a putative cation efflux transporter, and its deletion resulted in an inability to grow under high-zinc conditions. In conclusion, we developed a fast, versatile, specific, and high-throughput method for the identification of conditionally essential genes in *S. pneumoniae*.

PMID: 17261526 [PubMed - indexed for MEDLINE]

Ahn S, Kulis DM, Erdner DL, Anderson DM, Walt DR. (2006) Fiber-optic microarray for simultaneous detection of multiple harmful algal bloom species. Appl Environ Microbiol. 72(9):5742-9.

Harmful algal blooms (HABs) are a serious threat to coastal resources, causing a variety of impacts on public health, regional economies, and ecosystems. Plankton analysis is a valuable component of many HAB monitoring and research programs, but the diversity of plankton poses a problem in discriminating toxic from nontoxic species using conventional detection methods. Here we describe a sensitive and specific sandwich hybridization assay that combines fiber-optic microarrays with oligonucleotide probes to detect and enumerate the HAB species *Alexandrium fundyense*, *Alexandrium ostenfeldii*, and *Pseudo-nitzschia australis*. Microarrays were prepared by loading oligonucleotide probe-coupled microspheres (diameter, 3 mum) onto the distal ends of chemically etched imaging fiber bundles. Hybridization of target rRNA from HAB cells to immobilized probes on the microspheres was visualized using Cy3-labeled secondary probes in a sandwich-type assay format. We applied these microarrays to the detection and enumeration of HAB cells in both cultured and field samples. Our study demonstrated a detection limit of approximately 5 cells for all three target organisms within 45 min, without a separate amplification step, in both sample types. We also developed a multiplexed microarray to detect the three HAB species simultaneously, which successfully detected the target organisms, alone and in combination, without cross-reactivity. Our study suggests that fiber-optic microarrays can be used for rapid and sensitive detection and potential enumeration of HAB species in the environment.

PMID: 16957189 [PubMed - indexed for MEDLINE]

Hu Z, Zhang A, Storz G, Gottesman S, Leppla SH. (2006) An antibody-based microarray assay for small RNA detection. Nucleic Acids Res. 34(7):e52.

Detection of RNAs on microarrays is rapidly becoming a standard approach for molecular biologists. However, current methods frequently discriminate against structured and/or small RNA species. Here we present an approach that bypasses these problems. Unmodified RNA is hybridized directly to DNA microarrays and detected with the high-affinity, nucleotide sequence-independent, DNA/RNA hybrid-specific mouse monoclonal antibody S9.6. Subsequent reactions with a fluorescently-labeled anti-mouse IgG antibody or biotin-labeled anti-mouse IgG together with fluorescently labeled streptavidin produces a signal that can be measured in a standard microarray scanner. The antibody-based method was able to detect low abundance small RNAs of *Escherichia coli* much more efficiently than the commonly-used cDNA-based method. A specific small RNA was detected in amounts of 0.25 fmol (i.e. concentration of 10 pM in a 25 microl reaction). The method is an efficient, robust and inexpensive technique that allows quantitative analysis of gene expression and does not discriminate against short or structured RNAs.

PMID: 16614443 [PubMed - indexed for MEDLINE]

Kostic T, Weilharter A, Sessitsch A, Bodrossy L. (2005) High-sensitivity, polymerase chain reaction-free detection of microorganisms and their functional genes using 70-mer oligonucleotide diagnostic microarray. Anal Biochem. 346(2):333-5.

Traditional application of the DNA microarrays to study gene expression is being expanded to accommodate the rising need for high throughput, parallel microbial diagnostics. Accessing the microbial diversity in environmental and clinical samples presents a major challenge in terms of both specificity and sensitivity. Specificity requirements for microbial diagnostic microarrays (MDMs) applied in environmental microbiology are the parallel and reliable detection of many microorganisms at the species, genus, or even higher taxonomic levels, whereas for the clinical MDMs species, subspecies and strain levels are to be targeted. The relevant measure for sensitivity in this case is defined as the lowest relative abundance of the target group detectable within the analyzed community. The current detection limit of MDMs lies in the range of 1–5%. The option for the parallel detection of multiple marker genes (e.g., genes responsible for microbial pathogenesis, antibiotic resistance, and functional genes involved in certain pathways) is an additional bonus for MDMs, broadening their application potential.

PMID: 16169510 [PubMed - indexed for MEDLINE]

Ahn S, Walt DR. (2005) Detection of *Salmonella* spp. using microsphere-based, fiber-optic DNA microarrays. Anal Chem. 77(15):5041-7.

Salmonella spp. are one of the most problematic food pathogens in public health, as they are responsible for food poisoning associated with contamination of meat, poultry, and eggs. Thus, rapid and sensitive detection of *Salmonella* spp. is required to ensure food safety. In this study, a fiber-optic DNA microarray using microsphere-immobilized oligonucleotide probes specific for the *Salmonella* invA and spvB genes was developed for detection of *Salmonella* spp. Microarrays were prepared by randomly distributing DNA probe-functionalized microspheres (3.1-microm diameter) into microwells created by etching optical fiber bundles. Hybridization of the probe-functionalized microspheres to target DNA from *Salmonella* was performed and visualized using Cy3-labeled secondary probes in a sandwich-type assay format. In this study, 10(3)-10(4) cfu/mL of the target organism could be detected after 1-h hybridization without any additional amplification. The DNA microarray showed no cross-reactivity with other common food pathogens, including *E. coli* and *Y. enterocolitica*, and could even detect *Salmonella* spp. from cocktails of bacterial strains with only moderate loss of sensitivity due to nonspecific binding. This work suggests that fiber-optic DNA microarrays can be used for rapid and sensitive detection of *Salmonella* spp. Since fiber-optic microarrays can be prepared with different probes, this approach could also enable the simultaneous detection of multiple food pathogens.

PMID: 16053320 [PubMed - indexed for MEDLINE]

Kelly JJ, Siripong S, McCormack J, Janus LR, Urakawa H, El Fantroussi S, Noble PA, Sappelsa L, Rittmann BE, Stahl DA. (2005) DNA microarray detection of nitrifying bacterial 16S rRNA in wastewater treatment plant samples. Water Res. 39(14):3229-38.

A small scale DNA microarray containing a set of oligonucleotide probes targeting the 16S rRNAs of several groups of nitrifying bacteria was developed for the monitoring of wastewater treatment plant samples. The microarray was tested using reference rRNAs from pure cultures of nitrifying bacteria. Characterization of samples collected from an industrial wastewater treatment facility demonstrated that nitrifying bacteria could be detected directly by microarray hybridization without the need for PCR amplification. Specifically, the microarray detected *Nitrosomonas* spp. but did not detect Nitrobacter. The specificity and sensitivity of direct detection was evaluated using on-chip disseciation analysis, and by two independent analyses-an established membrane hybridization format and terminal restriction fragment length polymorphism fingerprinting (T-RFLP). The latter two analyses also revealed *Nitrospira* and *Nitrobacter* to be contributing populations in the treatment plant samples. The application of DNA microarrays to wastewater treatment systems, which has been demonstrated in the current work, should offer improved monitoring capabilities and process control for treatment systems, which are susceptible to periodic failures.

PMID: 16009395 [PubMed - indexed for MEDLINE]

Anthony RM, Schuitema AR, Oskam L, Klatser PR. (2005) Direct detection of *Staphylococcus aureus* mRNA using a flow through microarray. J Microbiol Methods. 60(1):47-54.

The direct detection of mRNAs from bacterial cultures on a DNA array without amplification and labelling would greatly extend the range of applications suitable for microarray analysis. Here we describe the direct detection of 23S rRNA and seven mRNA species from total *Staphylococcus aureus* RNA prepared using commercially available RNA purification columns followed by fluorescent detection on a flow through microarray. RNA hybridisation was detected using paired secondary labelled probes directly 5' and 3' to immobilised 60 mers. In this way, we were able to detect the effect of 30-min exposure to antimicrobials on mRNA levels within 3 h after column purification of total RNA without the need for enzymatic manipulation. Specifically the expression of mecA was confirmed in a highly resistant strain and induction of katA and ile-tRNA synthetase genes after exposure to mupirocin could be detected.

PMID: 15567224 [PubMed - indexed for MEDLINE]

Radke SM, Alocilja EC. (2005) A high density microelectrode array biosensor for detection of *E. coli* O157:H7. Biosens Bioelectron. 20(8):1662-7.

A high density microelectrode array biosensor was developed for the detection of *Escherichia coli* O157:H7. The biosensor was fabricated from (100) silicon with a 2

microm layer of thermal oxide as an insulating layer, an active area of 9.6 mm2 and consists of an interdigitated gold electrode array. The sensor surface was functionalised for bacterial detection using heterobifunctional crosslinkers and immobilised polyclonal antibodies to create a biological sensing surface. Bacteria suspended in solution became attached to the immobilised antibodies when the biosensor was tested in liquid samples. The change in impedance caused by the bacteria was measured over a frequency range of 100 Hz-10 M Hz. The biosensor was evaluated for *E. coli* O157:H7 detection in pure culture and inoculated food samples. The biosensor was able to discriminate between cellular concentrations of 10(4)-10(7)CFU/mL and has applications in detecting pathogens in food samples.

PMID: 15626625 [PubMed - indexed for MEDLINE]

PATHOGEN DETECTION WITH AMPLIFICATION

There are basically three types of amplification for the detection of pathogenic organisms in water. The first is replication of the target organisms using classical microbiological culture methods. The second involves various enzymatic cascade systems. The third is amplification of RNA or DNA. Confounding issues of culture-based amplification are that many target organisms are considered "unculturable" under standard conditions. Even organisms that grow well in such conditions almost never grow at identical rates, thus, the end product has very little resemblance to the ratios of organisms in the original sample. .

Methods that do not rely on nucleic acid based amplification generally require some type of cascade mechanism, either using an enzyme inherent in the organism, or a sandwich type structural recognition assay. Sensitivity and selectivity are the main concerns in non-nucleic acid amplification methods. Unlike PCR that requires a significant structural recognition event for every round of amplification, cascade events generally increase unchecked and therefore are more susceptible to false positive events.

Classical PCR provides a high level of selectivity and sensitivity for detection of microorganisms in water. Real-time PCR has an advantage over classical PCR it that it provides a level of quantization of the number of copies of starting material. Genetic elements used to identify a particular organism can either be a unique sequence not known to exist elsewhere, or at least not within the sample type under evaluation. Ribosomal genes are frequently selected because they occur numerous times in the genome thereby providing an increase in sensitivity. A popular method used to increase selectivity of PCR reactions is to use "nested" primers. This is when a set of two or more primers are spaced in between another set of primers. The outside set of primers increases the available template for the internal set of primers.

An interesting isothermal approach is loop-mediated isothermal amplification (LAMP). This approach provides results in less than 30 minutes and has the sensitivity of classical PCR. The test has been commercialized by Eiken Chemical company in Japan.

A key aspect of nucleic acid based detection and identification is the ability to ask very broad as well as very specific questions about a given sample. One can look for ribosomal sequences that are highly conserved to ask the question, "Are bacteria present in my sample?" At the same time, one can also look for a sequence that provides strain level characterization.

Noble RT, Weisberg SB. (2005) A review of technologies for rapid detection of bacteria in recreational waters. J Water Health. 3(4):381-92.

Monitoring of recreational beaches for fecal indicator bacteria is currently performed using culture-based technology that can require more than a day for laboratory analysis,

during which time swimmers are at risk. Here we review new methods that have the potential to reduce the measurement period to less than an hour. These methods generally involve two steps. The first is target capture, in which the microbial group of interest (or some molecular/chemical/or biochemical signature of the group) is removed, tagged or amplified to differentiate it from the remaining material in the sample. We discuss three classes of capture methods: 1) Surface and whole-cell recognition methods, including immunoassay techniques and molecule-specific probes; 2) Nucleic acid methods, including polymerase chain reaction (PCR), quantitative PCR (Q-PCR), nucleic acid sequence based amplification (NASBA) and microarrays; and 3) Enzyme/substrate methods utilizing chromogenic or fluorogenic substrates. The second step is detection, in which optical, electrochemical or piezoelectric technologies are used to quantify the captured, tagged or amplified material. The biggest technological hurdle for all of these methods is sensitivity, as EPA's recommended bathing water standard is less than one cell per ml and most detection technologies measure sample volumes less than 1 ml. This challenge is being overcome through addition of preconcentration or enrichment steps, which have the potential to boost sensitivity without the need to develop new detector technology. The second hurdle is demonstrating a relationship to health risk, since most new methods are based on measuring cell structure without assessing viability and may not relate to current water quality standards that were developed in epidemiology studies using culture-based methods. Enzyme/substrate methods may be the first rapid methods adopted because they are based on the same capture technology as currently-approved EPA methods and their relationship to health risk can be established by demonstrating equivalency to existing procedures. Demonstration of equivalency may also be possible for some surface and whole-cell recognition methods that capture bacteria in a potentially viable state. Nucleic acid technologies are the most versatile, but measure nonviable structure and will require inclusion in epidemiological studies to link their measurement with health risk.

PMID: 16459844 [PubMed - indexed for MEDLINE]

Fode-Vaughan KA, Wimpee CF, Remsen CC, Collins ML. (2001) Detection of bacteria in environmental samples by direct PCR without DNA extraction. Biotechniques. 31(3):598, 600, 602-4

Cultured cells and environmental samples were used directly in PCRs without the isolation of DNA. Serial dilution was used to eliminate the inhibitory effect of materials in natural samples. Primers specific for pmoA, which encodes a subunit of the particulate methane monooxygenase, were used to detect and quantify methanotrophic bacteria by direct most probable number PCR. Phototrophic bacteria were detected in environmental samples by direct PCR with primers specific for pufM, and members of the bacterial domain were detected with primers for 16S rDNA. Direct PCR provides a rapid, simple, and sensitive methodfor detecting and quantifying bacteria in environmental samples. Detection of methanotrophic bacteria can be applied to monitoring bioremediation.

PMID: 11570503 [PubMed - indexed for MEDLINE]

Labrenz M, Brettar I, Christen R, Flavier S, Botel J, Hofle MG. (2004) Development and application of a real-time PCR approach for quantification of uncultured bacteria in the central Baltic Sea. Appl Environ Microbiol. 70(8):4971-9.

We have developed a highly sensitive approach to assess the abundance of uncultured bacteria in water samples from the central Baltic Sea by using a noncultured member of the "Epsilonproteobacteria" related to *Thiomicrospira denitrificans* as an example. Environmental seawater samples and samples enriched for the target taxon provided a unique opportunity to test the approach over a broad range of abundances. The approach is based on a combination of taxon- and domain-specific real-time PCR measurements determining the relative *T. denitrificans*-like 16S rRNA gene and 16S rRNA abundances, as well as the determination of total cell counts and environmental RNA content. It allowed quantification of *T. denitrificans*-like 16S rRNA molecules or 16S rRNA genes as well as calculation of the number of ribosomes per *T. denitrificans*-like cell. Every real-time measurement and its specific primer system were calibrated using environmental nucleic acids obtained from the original habitat for external standardization. These standards, as well as the respective samples to be measured, were prepared from the same DNA or RNA extract. Enrichment samples could be analyzed directly, whereas environmental templates had to be preamplified with general bacterial primers before quantification. Preamplification increased the sensitivity of the assay by more than 4 orders of magnitude. Quantification of enrichments with or without a preamplification step yielded comparable results. *T. denitrificans*-like 16S rRNA molecules ranged from 7.1 x 10(3) to 4.4 x 10(9) copies ml(-1) or 0.002 to 49.7% relative abundance. *T. denitrificans*-like 16S rRNA genes ranged from 9.0 x 10(1) to 2.2 x10(6) copies ml(-1) or 0.01 to 49.7% relative abundance. Detection limits of this real-time-PCR approach were 20 16S rRNA molecules or 0.2 16S rRNA gene ml(-1). The number of ribosomes per *T. denitrificans*-like cell was estimated to range from 20 to 200 in seawater and reached up to 2,000 in the enrichments. The results indicate that our real-time PCR approach can be used to determine cellular and relative abundances of uncultured marine bacterial taxa and to provide information about their levels of activity in their natural environment.

PMID: 15294837 [PubMed - indexed for MEDLINE]

Waage AS, Vardund T, Lund V, Kapperud G. (1999) Detection of small numbers of *Campylobacter jejuni* and *Campylobacter coli* cells in environmental water, sewage, and food samples by a seminested PCR assay. Appl Environ Microbiol. 65(4):1636-43.

A rapid and sensitive assay was developed for detection of small numbers of *Campylobacter jejuni* and *Campylobacter coli* cells in environmental water, sewage, and food samples. Water and sewage samples were filtered, and the filters were enriched overnight in a nonselective medium. The enrichment cultures were prepared for PCR by a rapid and simple procedure consisting of centrifugation, proteinase K treatment, and boiling. A seminested PCR based on specific amplification of the intergenic sequence

between the two *Campylobacter* flagellin genes, flaA and flaB, was performed, and the PCR products were visualized by agarose gel electrophoresis. The assay allowed us to detect 3 to 15 CFU of *C. jejuni* per 100 ml in water samples containing a background flora consisting of up to 8, 700 heterotrophic organisms per ml and 10,000 CFU of coliform bacteria per 100 ml. Dilution of the enriched cultures 1:10 with sterile broth prior to the PCR was sometimes necessary to obtain positive results. The assay was also conducted with food samples analyzed with or without overnight enrichment. As few as </=3 CFU per g of food could be detected with samples subjected to overnight enrichment, while variable results were obtained for samples analyzed without prior enrichment. This rapid and sensitive nested PCR assay provides a useful tool for specific detection of *C. jejuni* or *C. coli* in drinking water, as well as environmental water, sewage, and food samples containing high levels of background organisms.

PMID: 10103261 [PubMed - indexed for MEDLINE]

Lleo MM, Bonato B, Tafi MC, Signoretto C, Pruzzo C, Canepari P. (2005) Molecular vs culture methods for the detection of bacterial faecal indicators in groundwater for human use. Lett Appl Microbiol. 40(4):289-94.

AIMS: The current standard culture methods are unable to detect nongrowing bacteria and, thus, might not be sufficient for precise monitoring of the microbiological quality of waters. The use of a molecular method such as PCR could be a valid alternative to detect bacterial faecal contamination indicators such as *Escherichia coli* and *Enterococcus faecalis* and reveal the presence of culturable and nonculturable bacterial forms. METHODS AND RESULTS: The presence of *E. coli* and *Ent. faecalis* cells in 30 groundwater samples was evaluated with the standard culture method and compared with a specific PCR protocol. A substantial percentage (50%) of the samples not containing culturable cells proved positive in the search for *Ent. faecalis* DNA by PCR. Quantification by competitive PCR (cPCR) of the DNA detected allowed us to calculate the number of nonculturable cells present in water samples: the number varied from 2 to 120 cells ml(-1). Only four samples were positive for *E. coli* DNA and the corresponding nonculturable cells varied from 24 to 70 ml(-1). CONCLUSIONS: This study demonstrates that the standard culture methods in use are unable to detect a substantial proportion of the bacterial population which is nonculturable but, as previously demonstrated, potentially still viable and able to express those pathogenic factors needed for causing infections in humans. SIGNIFICANCE AND IMPACT OF THE STUDY: To protect human health it is necessary to develop and use methods which detect the nonculturable as well as culturable bacteria present in water.

PMID: 15752220 [PubMed - indexed for MEDLINE]

Lazcka O, Del Campo FJ, Munoz FX. (2007) Pathogen detection: a perspective of traditional methods and biosensors. Biosens Bioelectron. 22(7):1205-17.

The detection of pathogenic bacteria is key to the prevention and identification of problems related to health and safety. Legislation is particularly tough in areas such as the food industry, where failure to detect an infection may have terrible consequences. In spite of the real need for obtaining analytical results in the shortest time possible, traditional and standard bacterial detection methods may take up to 7 or 8 days to yield an answer. This is clearly insufficient, and many researchers have recently geared their efforts towards the development of rapid methods. The advent of new technologies, namely biosensors, has brought in new and promising approaches. However, much research and development work is still needed before biosensors become a real and trustworthy alternative.This review not only offers an overview of trends in the area of pathogen detection but it also describes main techniques, traditional methods, and recent developments in the field of pathogen bacteria biosensors.

PMID: 16934970 [PubMed - indexed for MEDLINE]

Rutjes SA, van den Berg HH, Lodder WJ, de Roda Husman AM. (2006) Real-time detection of noroviruses in surface water by use of a broadly reactive nucleic acid sequence-based amplification assay. Appl Environ Microbiol. 72(8):5349-58.

Noroviruses are the most common agents causing outbreaks of viral gastroenteritis. Outbreaks originating from contaminated drinking water and from recreational waters have been described. Due to a lack of cell culture systems, noroviruses are detected mostly by molecular methods. Molecular detection assays for viruses in water are often repressed by inhibitory factors present in the environment, like humic acids and heavy metals. To study the effect of environmental inhibitors on the performance of nucleic acid sequence-based amplification (NASBA), we developed a real-time norovirus NASBA targeting part of the RNA-dependent RNA polymerase (RdRp) gene. Specificity of the assay was studied with 33 divergent clones that contained part of the targeted RdRp gene of noroviruses from 15 different genogroups. Viral RNA originated from commercial oysters, surface waters, and sewage treatment plants in The Netherlands. Ninety-seven percent of the clones derived from human noroviruses were detected by real-time NASBA. Two clones containing animal noroviruses were not detected by NASBA. We compared the norovirus detection by real-time NASBA with that by conventional reverse transcriptase PCR (RT-PCR) with large-volume river water samples and found that inhibitory factors of RT-PCR had little or no effect on the performance of the norovirus NASBA. This consequently resulted in a higher sensitivity of the NASBA assay than of the RT-PCR. We show that by combining an efficient RNA extraction method with real-time NASBA the sensitivity of norovirus detection in water samples increased at least 100 times, which consequently has implications for the outcome of the infectious risk assessment.

PMID: 16885286 [PubMed - indexed for MEDLINE]

Caccio SM. (2003) Molecular techniques to detect and identify protozoan parasites in the environment. Acta Microbiol Pol. 52 Suppl:23-34.

The environmental route of transmission is important for many protozoan and helminth parasites, with water, soil and food being particularly significant. Both the potential for producing large numbers of transmissive stages and their environmental robustness pose persistent threats to public and veterinary health. The introduction of molecular techniques, in particular those based on the amplification of nucleic acids, has provided researchers with highly sensitive and specific assays for the detection and identification of these pathogens. The application of these techniques to clinical, environmental, and food samples is instrumental for a thorough understanding of the epidemiology of the infection and for the implementation of control measures. Here, the advantages and drawbacks of some molecular techniques (Polymerase Chain Reaction-PCR; Reverse-Transcriptase PCR-RT-PCR; Real-time PCR-qPCR; Nucleic Acid Sequence-Based Amplification--NASBA) will be briefly reviewed. Some application of these techniques will be illustrated with reference to two important and widespread human parasites, the apicomplexan *Cryptosporidium* and the flagellate *Giardia*.

PMID: 15058811 [PubMed - indexed for MEDLINE]

Stormer M, Kleesiek K, Dreier J. (2007) High-volume extraction of nucleic acids by magnetic bead technology for ultrasensitive detection of bacteria in blood components. Clin Chem. 53(1):104-10.

BACKGROUND: Nucleic acid isolation, the most technically demanding and laborious procedure performed in molecular diagnostics, harbors the potential for improvements in automation. A recent development is the use of magnetic beads covered with nucleic acid-binding matrices. We adapted this technology with a broad-range 23S rRNA real-time reverse transcription (RT)-PCR assay for fast and sensitive detection of bacterial contamination of blood products. METHODS: We investigated different protocols for an automated high-volume extraction method based on magnetic-separation technology for the extraction of bacterial nucleic acids from platelet concentrates (PCs). We added 2 model bacteria, *Staphylococcus epidermidis* and *Escherichia coli*, to a single pool of apheresis-derived, single-donor platelets and assayed the PCs by real-time RT-PCR analysis with an improved primer-probe system and locked nucleic acid technology. Co-amplification of human beta(2)-microglobulin mRNA served as an internal control (IC). We used probit analysis to calculate the minimum concentration of bacteria that would be detected with 95% confidence. RESULTS: For automated magnetic bead-based extraction technology with the real-time RT-PCR, the 95% detection limit was 29 x 10(3) colony-forming units (CFU)/L for *S. epidermidis* and 22 x 10(3) CFU/L for *E. coli*. No false-positive results occurred, either due to nucleic acid contamination of reagents or externally during testing of 1030 PCs. CONCLUSIONS: High-volume nucleic acid extraction improved the detection limit of the assay. The improvement of the primer-probe system and the integration of an IC make the RT-PCR assay appropriate for bacteria screening of platelets.

PMID: 17110475 [PubMed - indexed for MEDLINE]

Toranzos GA, Alvarez AJ. (1992) Solid-phase polymerase chain reaction: applications for direct detection of enteric pathogens in waters. Can J Microbiol. 38(5):365-9.

The techniques in current use for detection of pathogens in environmental samples are restricted to those organisms whose replication in either culture media or cell culture is feasible. These methods lack the selectivity and sensitivity necessary for their unequivocal detection and identification. We have developed an assay for the detection of bacterial cells in large volumes of water. Low concentrations of cells containing target sequences were concentrated on membrane filters and were subjected to amplification directly using a stepwise polymerase chain reaction. This procedure, together with nucleic acid probes, has enhanced the limit of detection to the level of a single bacterial cell. This technique could be used for the detection of any bacteria or virus in water or air.

PMID: 1643580 [PubMed - indexed for MEDLINE]

Gulliksen A, Solli L, Karlsen F, Rogne H, Hovig E, Nordstrom T, Sirevag R. (2004) Real-time nucleic acid sequence-based amplification in nanoliter volumes. Anal Chem. 76(1):9-14.

Real-time nucleic acid sequence-based amplification (NASBA) is an isothermal method specifically designed for amplification of RNA. Fluorescent molecular beacon probes enable real-time monitoring of the amplification process. Successful identification, utilizing the real-time NASBA technology, was performed on a microchip with oligonucleotides at a concentration of 1.0 and 0.1 microM, in 10- and 50-nL reaction chambers, respectively. The microchip was developed in a silicon-glass structure. An instrument providing thermal control and an optical detection system was built for amplification readout. Experimental results demonstrate distinct amplification processes. Miniaturized real-time NASBA in microchips makes high-throughput diagnostics of bacteria, viruses, and cancer markers possible, at reduced cost and without contamination.

PMID: 14697026 [PubMed - indexed for MEDLINE]

Zhang T, Fang HH. (2006) Applications of real-time polymerase chain reaction for quantification of microorganisms in environmental samples. Appl Microbiol Biotechnol. 70(3):281-9.

Due to the advanced development of fluorogenic chemistry, quantitative real-time polymerase chain reaction (qRT-PCR) has become an emerging technique for the detection and quantification of microorganisms in the environment. Compared with the conventional hybridization- and PCR-based techniques, qRT-PCR not only has better sensitivity and reproducibility, but it is also quicker to perform and has a minimum risk

of amplicon carryover contamination. This article reviews the principle of this emerging technique, its detection reagents, target DNAs, quantification procedures, and affecting factors. The applications of qRT-PCR for the quantification of microorganisms in the environment are also summarized.

PMID: 16470363 [PubMed - indexed for MEDLINE]

Hara-Kudo Y, Yoshino M, Kojima T, Ikedo M. (2005) Loop-mediated isothermal amplification for the rapid detection of *Salmonella*. FEMS Microbiol Lett. 253(1):155-61.

Loop-mediated isothermal amplification (LAMP) assay detected *Salmonella* within 60 min. The 220 strains of 39 serotypes of Salmonella subsp. enterica and 7 strains of *Salmonella enterica* subsp. *arizonae* were amplified, but not 62 strains of 23 bacterial species other than *Salmonella*. The sensitivity of the LAMP assay was found to be >2.2 cfu/test tube using nine serotypes. The specificity was similar to that of a PCR assay, but the sensitivity of LAMP was greater. Both fluorescence and turbidity were able to detect the products in the LAMP assay. *S. enteritidis* in a liquid egg sample artificially inoculated with the organism was detected by the LAMP assay at 2.8 cfu/test tube, although negative by PCR assay. These results indicate that the LAMP assay is a rapid, specific and sensitive detection method for *Salmonella*.

PMID: 16242860 [PubMed - indexed for MEDLINE]

Abd-El-Haleem D, Kheiralla ZH, Zaki S, Rushdy AA, Abd-El-Rahiem W. (2003) Multiplex-PCR and PCR-RFLP assays to monitor water quality against pathogenic bacteria. J Environ Monit. 5(6):865-70.

In this work we developed and optimized two molecular-based approaches to monitor rapidly, sensitively and specifically bacterial pathogens from three different genera, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* spp., directly in waters. To achieve this aim, firstly a multiplex-PCR assay (M-PCR) was optimized using a primer pair specific for each pathogen. Secondly, as a molecular confirmatory test after isolation of the pathogens by classical microbiological methods, PCR-RFLP of their amplified 16S rDNA genes was performed. It was observed from the results that the developed M-PCR assay has significant impact on the ability to detect sensitively, rapidly and specifically the three pathogens directly in water within a short time (5 h from sampling to obtain final results), therefore it represents a considerable advancement over other known more time-consuming and less-sensitive methods for identification and characterization of these kinds of pathogens.

PMID: 14710924 [PubMed - indexed for MEDLINE]

Santo Domingo JW, Siefring SC, Haugland RA. (2003) Real-time PCR method to detect *Enterococcus faecalis* in water. Biotechnol Lett. 25(3):261-5.

A 16S rDNA real-time PCR method was developed to detect *Enterococcus faecalis* in water samples. The dynamic range for cell detection spanned five logs and the detection limit was determined to be 6 cfu/reaction. The assay was capable of detecting *E. faecalis* cells added to biofilms from a simulator of a water distribution system and in freshwater samples. Nucleic acid extraction was not required, permitting the detection of *E. faecalis* cells in less than 3 h.

PMID: 12882582 [PubMed - indexed for MEDLINE]

Deisingh AK, Thompson M. (2004) Biosensors for the detection of bacteria. Can J Microbiol. 50(2):69-77.

This review will consider the role of biosensors towards the detection of infectious bacteria, although non-infectious ones will be considered where necessary. Recently, there has been a heightened interest in developing rapid and reliable methods of detection. This is especially true for detection of organisms involved in bioterrorism, food poisoning, and clinical problems such as antibiotic resistance. Biosensors can assist in achieving these goals, and sensors using several of the different types of transduction modes are discussed: electrochemical, high frequency (surface acoustic wave), and optical. The paper concludes with a discussion of three areas that may make a great impact in the next few years: integrated (lab-on-a-chip) systems, molecular beacons, and aptamers.

PMID: 15052308 [PubMed - indexed for MEDLINE]

Rompre A, Servais P, Baudart J, de-Roubin MR, Laurent P. (2002) Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J Microbiol Methods. 49(1):31-54.

The coliform group has been used extensively as an indicator of water quality and has historically led to the public health protection concept. The aim of this review is to examine methods currently in use or which can be proposed for the monitoring of coliforms in drinking water. Actually, the need for more rapid, sensitive and specific tests is essential in the water industry. Routine and widely accepted techniques are discussed, as are methods which have emerged from recent research developments.Approved traditional methods for coliform detection include the multiple-tube fermentation (MTF) technique and the membrane filter (MF) technique using different specific media and incubation conditions. These methods have limitations, however, such as duration of incubation, antagonistic organism interference, lack of specificity and poor detection of slow-growing or viable but non-culturable (VBNC) microorganisms. Nowadays, the simple and inexpensive membrane filter technique is the most widely used method for routine enumeration of coliforms in drinking water. The detection of coliforms based on specific enzymatic activity has improved the sensitivity of these methods. The enzymes beta-D galactosidase and beta-D glucuronidase are widely used for the detection and enumeration of total coliforms and *Escherichia coli*, respectively. Many chromogenic and fluorogenic substrates exist for the specific detection of these enzymatic activities, and

various commercial tests based on these substrates are available. Numerous comparisons have shown these tests may be a suitable alternative to the classical techniques. They are, however, more expensive, and the incubation time, even though reduced, remains too long for same-day results. More sophisticated analytical tools such as solid phase cytometry can be employed to decrease the time needed for the detection of bacterial enzymatic activities, with a low detection threshold.Detection of coliforms by molecular methods is also proposed, as these methods allow for very specific and rapid detection without the need for a cultivation step. Three molecular-based methods are evaluated here: the immunological, polymerase chain reaction (PCR) and in-situ hybridization (ISH) techniques. In the immunological approach, various antibodies against coliform bacteria have been produced, but the application of this technique often showed low antibody specificity. PCR can be used to detect coliform bacteria by means of signal amplification: DNA sequence coding for the lacZ gene (beta-galactosidase gene) and the uidA gene (beta-D glucuronidase gene) has been used to detect total coliforms and *E. coli*, respectively. However, quantification with PCR is still lacking in precision and necessitates extensive laboratory work. The FISH technique involves the use of oligonucleotide probes to detect complementary sequences inside specific cells. Oligonucleotide probes designed specifically for regions of the 16S RNA molecules of *Enterobacteriaceae* can be used for microbiological quality control of drinking water samples. FISH should be an interesting viable alternative to the conventional culture methods for the detection of coliforms in drinking water, as it provides quantitative data in a fairly short period of time (6 to 8 h), but still requires research effort.This review shows that even though many innovative bacterial detection methods have been developed, few have the potential for becoming a standardized method for the detection of coliforms in drinking water samples.

PMID: 11777581 [PubMed - indexed for MEDLINE]

Gabig-Ciminska M. (2006) Developing nucleic acid-based electrical detection systems. Microb Cell Fact. 5:9.

Development of nucleic acid-based detection systems is the main focus of many research groups and high technology companies. The enormous work done in this field is particularly due to the broad versatility and variety of these sensing devices. From optical to electrical systems, from label-dependent to label-free approaches, from single to multi-analyte and array formats, this wide range of possibilities makes the research field very diversified and competitive. New challenges and requirements for an ideal detector suitable for nucleic acid analysis include high sensitivity and high specificity protocol that can be completed in a relatively short time offering at the same time low detection limit. Morever, systems that can be miniaturized and automated present a significant advantage over conventional technology, especially if detection is needed in the field. Electrical system technology for nucleic acid-based detection is an enabling mode for making miniaturized to micro- and nanometer scale bio-monitoring devices via the fusion of modern micro- and nanofabrication technology and molecular biotechnology. The electrical biosensors that rely on the conversion of the Watson-Crick base-pair recognition event into a useful electrical signal are advancing rapidly, and recently are

receiving much attention as a valuable tool for microbial pathogen detection. Pathogens may pose a serious threat to humans, animal and plants, thus their detection and analysis is a significant element of public health. Although different conventional methods for detection of pathogenic microorganisms and their toxins exist and are currently being applied, improvements of molecular-based detection methodologies have changed these traditional detection techniques and introduced a new era of rapid, miniaturized and automated electrical chip detection technologies into pathogen identification sector. In this review some developments and current directions in nucleic acid-based electrical detection are discussed.

PMID: 16512917 [PubMed]

Fukushima H, Tsunomori Y, Seki R. (2003) Duplex real-time SYBR green PCR assays for detection of 17 species of food- or waterborne pathogens in stools. J Clin Microbiol. 41(11):5134-46.

A duplex real-time SYBR Green LightCycler PCR (LC-PCR) assay with DNA extraction using the QIAamp DNA Stool Mini kit was evaluated with regard to detection of 8 of 17 species of food- or waterborne pathogens in five stool specimens in 2 h or less. The protocol used the same LC-PCR with 20 pairs of specific primers. The products formed were identified based on a melting point temperature (T(m)) curve analysis. The 17 species of food- or waterborne pathogens examined were enteroinvasive Escherichia coli, enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Campylobacter jejuni*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Aeromonas* spp., *Staphylococcus aureus*, *Clostridium perfringens*, and *Bacillus cereus*. No interference with the LC-PCR assay was observed when stool specimens were artificially inoculated with each bacterial species. The detection levels were approximately 10(5) food- or waterborne pathogenic bacteria per g of stool. The protocol for processing stool specimens for less than 10(4) food- or waterborne pathogenic bacteria per g of stool requires an overnight enrichment step to achieve adequate sensitivity. However, the rapid amplification and reliable detection of specific genes of greater than 10(5) food- or waterborne pathogenic bacteria per g in samples should facilitate the diagnosis and management of food- or waterborne outbreaks.

PMID: 14605150 [PubMed - indexed for MEDLINE]

Tantawiwat S, Tansuphasiri U, Wongwit W, Wongchotigul V, Kitayaporn D. (2005) Development of multiplex PCR for the detection of total coliform bacteria for *Escherichia coli* and *Clostridium perfringens* in drinking water. Southeast Asian J Trop Med Public Health. 36(1):162-9.

Multiplex PCR amplification of lacZ, uidA and plc genes was developed for the simultaneous detection of total coliform bacteria for *Escherichia coli* and *Clostridium perfringens*, in drinking water. Detection by agarose gel electrophoresis yielded a band of

876 bp for the lacZ gene of all coliform bacteria; a band of 147 bp for the uidA gene and a band of 876 bp for the lacZ gene of all strains of *E. coli*; a band of 280 bp for the p/c gene for all strains of *C. perfringens*; and a negative result for all three genes when tested with other bacteria. The detection limit was 100 pg for *E. coli* and *C. perfringens*, and 1 ng for coliform bacteria when measured with purified DNA. This assay was applied to the detection of these bacteria in spiked water samples. Spiked water samples with 0-1,000 CFU/ml of coliform bacteria and/or *E. coli* and/or *C. perfringens* were detected by this multiplex PCR after a pre-enrichment step to increase the sensitivity and to ensure that the detection was based on the presence of cultivable bacteria. The result of bacterial detection from the multiplex PCR was comparable with that of a standard plate count on selective medium (p=0.62). When using standard plate counts as a gold standard, the sensitivity for this test was 99.1% (95% CI 95.33, 99.98) and the specificity was 90.9 % (95% CI 75.67, 98.08). Multiplex PCR amplification with a pre-enrichment step was shown to be an effective, sensitive and rapid method for the simultaneous detection of these three microbiological parameters in drinking water.

PMID: 15906661 [PubMed - indexed for MEDLINE]

Li F, Zhao C, Zhang W, Cui S, Meng J, Wu J, Zhang DY. (2005) Use of ramification amplification assay for detection of *Escherichia coli* O157:H7 and other *E. coli* Shiga toxin-producing strains. J Clin Microbiol. 43(12):6086-90.

Escherichia coli O157:H7 and other Shiga toxin-producing *E. coli* (STEC) strains are important human pathogens that are mainly transmitted through the food chain. These pathogens have a low infectious dose and may cause life-threatening illnesses. However, detection of this microorganism in contaminated food or a patient's stool specimens presents a diagnostic challenge because of the low copy number in the sample. Often, a more sensitive nucleic acid amplification method, such as PCR, is required for rapid detection of this microorganism. Ramification amplification (RAM) is a recently introduced isothermal DNA amplification technique that utilizes a circular probe for target detection and achieves exponential amplification through the mechanism of primer extension, strand displacement, and ramification. In this study, we synthesized a circular probe specific for the Shiga toxin 2 gene (stx(2)). Our results showed that as few as 10 copies of stx(2) could be detected, indicating that the RAM assay was as sensitive as conventional PCR. We further tested 33 isolates of *E coli* O157:H7, STEC, *Shigella dysenteriae*, and nonpathogenic *E. coli* by RAM assay. Results showed that all 27 STEC isolates containing the stx(2) gene were identified by RAM assay, while *S. dysenteriae* and nonpathogenic *E. coli* isolates were undetected. The RAM results were 100% in concordance with those of PCR. Because of its simplicity and isothermal amplification, the RAM assay could be a useful method for detecting STEC in food and human specimens.

PMID: 16333102 [PubMed - indexed for MEDLINE]

Bush CE, Vanden Brink KM, Sherman DG, Peterson WR, Beninsig LA, Godsey JH. (1991) Detection of *Escherichia coli* rRNA using target amplification and time-resolved fluorescence detection. Mol Cell Probes. 5(6):467-72.

The development of technology to increase the sensitivity and speed of detection of bacterial pathogens in samples is important for diagnosis and monitoring of illness. We have developed a sensitive and rapid method for the detection of bacteria, using *Escherichia coli* as a model, which combines transcription-based target amplification with a bead-based sandwich hybridization assay using rare earth metal chelate labelled probes and time-resolved fluorescence detection. Using these methods as little as 100 copies (0.00016 attomoles) of purified native *Escherichia coli* rRNA or just one bacterial cell in a spiked sample could be detected. These results demonstrate that amplification of rRNA by transcription-based amplification and detection by time-resolved fluorescence provide a sensitive technology for the direct detection of micro-organisms without the requirement for prior cultivation.

PMID: 1723491 [PubMed - indexed for MEDLINE]

Wharam SD, Marsh P, Lloyd JS, Ray TD, Mock GA, Assenberg R, McPhee JE, Brown P, Weston A, Cardy DL. (2001) Specific detection of DNA and RNA targets using a novel isothermal nucleic acid amplification assay based on the formation of a three-way junction structure. Nucleic Acids Res. 29(11):E54-4.

The formation of DNA three-way junction (3WJ) structures has been utilised to develop a novel isothermal nucleic acid amplification assay (SMART) for the detection of specific DNA or RNA targets. The assay consists of two oligonucleotide probes that hybridise to a specific target sequence and, only then, to each other forming a 3WJ structure. One probe (template for the RNA signal) contains a non-functional single-stranded T7 RNA polymerase promoter sequence. This promoter sequence is made double-stranded (hence functional) by DNA polymerase, allowing T7 RNA polymerase to generate a target-dependent RNA signal which is measured by an enzyme-linked oligosorbent assay (ELOSA). The sequence of the RNA signal is always the same, regardless of the original target sequence. The SMART assay was successfully tested in model systems with several single-stranded synthetic targets, both DNA and RNA. The assay could also detect specific target sequences in both genomic DNA and total RNA from *Escherichia coli*. It was also possible to generate signal from *E.coli* samples without prior extraction of nucleic acid, showing that for some targets, sample purification may not be required. The assay is simple to perform and easily adaptable to different targets.

PMID: 11376166 [PubMed - indexed for MEDLINE]

Wang R, Minunni M, Tombelli S, Mascini M. (2004) A new approach for the detection of DNA sequences in amplified nucleic acids by a surface plasmon resonance biosensor. Biosens Bioelectron. 20(3):598-605.

In this paper, a simple and useful approach for DNA sensing based on surface plasmon resonance (SPR) transduction is reported. A new DNA sample pre-treatment has been optimised to allow fast and simple detection of hybridisation reaction between a target sequence in solution and a probe immobilised on the sensing surface. This pre-treatment consisted in a denaturation procedure of double stranded DNA containing the target sequence and was based on an high temperature treatment (95 degrees C, 5 min) followed by a 1 min incubation with small oligonucleotides. The oligonucleotides are designed to prevent the re-hybridising of the denatured strands, while enabling the target sequence to bind the immobilised probe. The important parameters of the procedure, i.e. incubation time, length and concentration of the oligonucleotides, have been studied in detail. The optimised DNA denaturation procedure has been successfully applied to the detection of amplified DNA with a commercially available SPR biosensor (Biacore X). DNA samples extracted from plant and human blood were tested after amplification by polymerase chain reaction (PCR).

PMID: 15494245 [PubMed - indexed for MEDLINE]

Kurn N, Chen P, Heath JD, Kopf-Sill A, Stephens KM, Wang S. (2005) Novel isothermal, linear nucleic acid amplification systems for highly multiplexed applications. Clin Chem. 51(10):1973-81.

BACKGROUND: Global analysis of the genome, transcriptome, and proteome is facilitated by the recent development of tools for large-scale, highly parallel analysis. We describe a novel nucleic acid amplification system that generates products by several methods. 3'-Ribo-SPIA primes cDNA synthesis at the 3' polyA tail, and whole transcript (WT)-Ribo-SPIA primes cDNA synthesis across the full length of the transcripts and thus provides whole-transcriptome amplification, independent of the 3' polyA tail. METHODS: We developed isothermal linear nucleic acid amplification systems, which use a single chimeric primer, for amplification of DNA (SPIA) and RNA (Ribo-SPIA). The latter allows mRNA amplification from as little as 1 ng of total RNA. Amplification efficiency was calculated based on the delta threshold cycle between nonamplified cDNA targets and amplified cDNA. The amounts and quality of total RNA and amplification products were determined after purification of the amplification products. GeneChip array gene expression profiling and real-time PCR were used to test the accuracy and reproducibility of the method. Quantification of cDNA products (before and after amplification) at the 2 loci along the transcripts was used to assess product length (for evaluation of the 3'-initiated Ribo-SPIA) and equal representation throughout the length of the transcript (for evaluation of the whole transcript amplification system, WT-Ribo-SPIA). RESULTS: Ribo-SPIA-based global RNA amplification exhibited linearity over 6 orders of magnitude of transcript abundance and generated microgram amounts of amplified cDNA from as little as 1 ng of total RNA. CONCLUSIONS: The described methods enable comprehensive gene expression profiling and analysis from limiting biological samples. The WT-Ribo-SPIA procedure, which enables amplification of non-polyA-tailed RNA, is suitable for amplification and gene expression analysis of both eukaryotic and prokaryotic biological samples.

PMID: 16123149 [PubMed - indexed for MEDLINE]

Hafner GJ, Yang IC, Wolter LC, Stafford MR, Giffard PM. (2005) Isothermal amplification and multimerization of DNA by Bst DNA polymerase. Biotechniques. 30(4):852-6, 858, 860 passim.

We have demonstrated the isothermal *in vitro* amplification and multimerization of several different linear DNA targets using only two primers and the strongly strand-displacing exonuclease-negative Bst DNA polymerase. This reaction has been termed linear target isothermal multimerization and amplification (LIMA). LIMA has been compared with cascade rolling-circle amplification and has been found to be less sensitive but to yield similar variable-length multimeric dsDNA molecules. Products from several different LIMA reactions were characterized by restriction analysis and partial sequence determination. They were found to be multimers of subsets of the target sequence and were not purely primer derived. The sensitivities with respect to target concentration of several different LIMA reactions were determined, and they varied from 0.01 amol to 1 fmol. The sensitivity and specificity of LIMA were further tested using *E. coli* genomic DNA, and the selective amplification of a transposon fragment was demonstrated. A successful strategy for reducing LIMA-dependent background DNA synthesis in rolling-circle amplification embodiments was devised. This entailed the affinity purification of circular DNA templates before amplification.

PMID: 11314268 [PubMed - indexed for MEDLINE]

McCarthy EL, Bickerstaff LE, da Cunha MP, Millard PJ. (2007) Nucleic acid sensing by regenerable surface-associated isothermal rolling circle amplification. Biosens Bioelectron. 22(7):1236-44.

A novel method for regenerating biosensors has been developed in which the highly specific detection of nucleic acid sequences is carried out using molecular padlock probe (MPP) technology and surface-associated rolling circle amplification (RCA). This technique has a low occurrence of false positive results when compared to polymerase chain reaction, and is an isothermal reaction, which is advantageous in systems requiring low power consumption such as remote field sensing applications. Gold-sputtered 96-well polystyrene microplates and a fluorescent label were used to explore the detection limits of the surface-associated RCA technique, specificity for different MPP, conditions for regeneration of the biomolecular sensing surface, and reproducibility of measurements on regenerated surfaces. The technique was used to create highly selective biomolecular surfaces capable of discriminating between DNA oligonucleotides with sequences identical to RNA from infectious salmon anemia (ISA) and infectious hematopoietic necrosis (IHN) virus. As little as 0.6 fmol of circularized MPP was detectable with this fluorimetric assay. The sensing layers could be reused for at least four cycles of amplification using thermal denaturation, with less than 33% decrease in RCA response over time. Because the nucleic acid product of the test is attached to a surface during amplification, the technique is directly applicable to a variety of existing sensing platforms, including acoustic wave and optical devices.

PMID: 16797962 [PubMed - indexed for MEDLINE]

Jannes G, De Vos D. (2006) A review of current and future molecular diagnostic tests for use in the microbiology laboratory. Methods Mol Biol. 345:1-21.

Nucleic acid-based diagnostics gradually are replacing or complementing culture-based, biochemical, and immunological assays in routine microbiology laboratories. Similar to conventional tests, the first-generation deoxyribonucleic acid assays determined only a single analyte. Recent improvements in detection technologies have paved the way for the development of multiparameter assays using macroarrays or micro-arrays, while the introduction of closed-tube real-time polymerase chain reaction systems has resulted in the development of rapid microbial diagnostics with a reduced contamination risk. The use of these new molecular technologies is not restricted to detection and identification of microbial pathogens but also can be used for genotyping, allowing one to determine antibiotic resistance or to perform microbial fingerprinting.
PMID: 16957343 [PubMed - indexed for MEDLINE]

Lawson AJ. Discovering new pathogens: culture-resistant bacteria. (2004) Methods Mol Biol. 266:305-22.

Recent advances in gene-amplification technology and molecular phylogenetics have provided the means of detecting and classifying bacteria directly from their natural habitats without the need for culture. These techniques have revolutionized environmental microbiology, and it is now apparent that the global diversity of microorganisms is much greater than previously thought. In the context of clinical microbiology, this molecular-based approach has facilitated the characterization of culture-resistant bacteria associated with human disease. Examples include *Helicobacter heilmannii*, a cause of gastritis, *Tropheryma whippeli* (the agent of Whipple's disease), and the agents of human ehrlichiosis and bacillary angiomatosis. Molecular-based techniques also provide a means of investigating complex bacterial flora within the human ecosystem, such as feces and dental plaque, without the bias of culture-based isolation. This has given a new perspective to the study of polymicrobial infections such as gingivitis, and offers the potential for the detection and identification of novel bacterial pathogens from among complex and numerous endogenous microbial flora.
PMID: 15148425 [PubMed - indexed for MEDLINE]

Guy RA, Payment P, Krull UJ, Horgen PA. (2003) Real-time PCR for quantification of *Giardia* and *Cryptosporidium* in environmental water samples and sewage. Appl Environ Microbiol. 69(9):5178-85.

The protozoan pathogens *Giardia lamblia* and *Cryptosporidium parvum* are major causes of waterborne enteric disease throughout the world. Improved detection methods that are very sensitive and rapid are urgently needed. This is especially the case for analysis of environmental water samples in which the densities of *Giardia* and

Cryptosporidium are very low. Primers and TaqMan probes based on the beta-giardin gene of *G. lamblia* and the COWP gene of *C. parvum* were developed and used to detect DNA concentrations over a range of 7 orders of magnitude. It was possible to detect DNA to the equivalent of a single cyst of *G. lamblia* and one oocyst of *C. parvum*. A multiplex real-time PCR (qPCR) assay for simultaneous detection of *G. lamblia* and *C. parvum* resulted in comparable levels of detection. Comparison of DNA extraction methodologies to maximize DNA yield from cysts and oocysts determined that a combination of freeze-thaw, sonication, and purification using the DNeasy kit (Qiagen) provided a highly efficient method. Sampling of four environmental water bodies revealed variation in qPCR inhibitors in 2-liter concentrates. A methodology for dealing with qPCR inhibitors that involved the use of Chelex 100 and PVP 360 was developed. It was possible to detect and quantify *G. lamblia* in sewage using qPCR when applying the procedure for extraction of DNA from 1-liter sewage samples. Numbers obtained from the qPCR assay were comparable to those obtained with immunofluorescence microscopy. The qPCR analysis revealed both assemblage A and assemblage B genotypes of *G. lamblia* in the sewage. No *Cryptosporidium* was detected in these samples by either method.

PMID: 12957899 [PubMed - indexed for MEDLINE]

Kong RY, Lee SK, Law TW, Law SH, Wu RS. (2002) Rapid detection of six types of bacterial pathogens in marine waters by multiplex PCR. Water Res. 36(11):2802-12.

A rapid multiplex PCR (m-PCR) method that allows the simultaneous detection, in a single tube, of six commonly encountered waterborne pathogens is developed. The target genes used were: the aerolysin (aero) gene of *Aeromonas hydrophila*, the invasion plasmid antigen H (ipaH) gene of *Shigella flexneri*, the attachment invasion locus (ail) gene of *Yersinia enterocolitca*, the invasion plasmid antigen B (ipaB) gene of *Salmonella typhimurium*, the enterotoxin extracellular secretion protein (epsM) gene of *Vibrio cholerae* and a species-specific region of the 16S-23S rDNA (Vpara) gene of *Vibrio parahaemolyticus* were used as the gene targets. Multiplex PCR using the six pairs of primers produced specific amplicons of the expected sizes from mixed populations of reference bacterial strains in seawater and from pure cultures. The m-PCR assay was specific and rapid, with a turnaround time of < 12 h. The detection limit of the assay for the bacterial targets was estimated at 10(0)-10(2) cfu. Multiplex PCR analysis was performed on 19 seawater samples collected around Hong Kong and the results indicated significant levels of four bacterial pathogens at several sites where primary sewage wastes are discharged, and the levels of which showed no correlation with *E. coli* counts. Overall, both laboratory and field validation results demonstrated that the m-PCR assay developed in this study could provide a cost-effective and informative supplement to conventional microbiological methods for routine monitoring and risk assessment of water quality.

PMID: 12146868 [PubMed - indexed for MEDLINE]

Rublee PA, Kempton JW, Schaefer EF, Allen C, Harris J, Oldach DW, Bowers H, Tengs T, Burkholder JM, Glasgow HB (2001) Use of molecular probes to assess geographic distribution of *Pfiesteria* species. Environmental Health Perspectives. 109 Suppl 5:765-7.

Abstract: We have developed multiple polymerase chain reaction (PCR)-based methods for the detection of Pfiesteria sp. in cultures and environmental samples. More than 2,100 water and sediment samples from estuarine sites of the U.S. Atlantic and gulf coasts were assayed for the presence of *Pfiesteria piscicida* and *Pfiesteria shumwayae* by PCR probing of extracted DNA. Positive results were found in about 3% of samples derived from routine monitoring of coastal waters and about 8% of sediments. The geographic range of both species was the same, ranging from New York to Texas. *Pfiesteria* spp. are likely common and generally benign inhabitants of coastal areas, but their presence maintains a potential for fish and human health problems.
PMID: 11677186 [PubMed - indexed for MEDLINE]

Tanriverdi S, Tanyeli A, Başlamişli F, Köksal F, Kilinç Y, Feng X, Batzer G, Tzipori S, Widmer G (2002) Detection and genotyping of oocysts of *Cryptosporidium parvum* by real-time PCR and melting curve analysis. J Clin Microbiol. 40(9):3237-44.

Abstract: Several real-time PCR procedures for the detection and genotyping of oocysts of *Cryptosporidium parvum* were evaluated. A 40-cycle amplification of a 157-bp fragment from the *C. parvum* beta-tubulin gene detected individual oocysts which were introduced into the reaction mixture by micromanipulation. SYBR Green I melting curve analysis was used to confirm the specificity of the method when DNA extracted from fecal samples spiked with oocysts was analyzed. Because *C. parvum* isolates infecting humans comprise two distinct genotypes, designated type 1 and type 2, real-time PCR methods for discriminating *C. parvum* genotypes were developed. The first method used the same beta-tubulin amplification primers and two fluorescently labeled antisense oligonucleotide probes spanning a 49-bp polymorphic sequence diagnostic for *C. parvum* type 1 and type 2. The second genotyping method used SYBR Green I fluorescence and targeted a polymorphic coding region within the GP900/poly(T) gene. Both methods discriminated between type 1 and type 2 *C. parvum* on the basis of melting curve analysis. To our knowledge, this is the first report describing the application of melting curve analysis for genotyping of *C. parvum* oocysts.
PMID: 12202559 [PubMed - indexed for MEDLINE]

Sluter SD, Tzipori S, Widmer G. (1997) Parameters affecting polymerase chain reaction detection of waterborne *Cryptosporidium parvum* oocysts. Appl Microbiol Biotech. 48(3):325-30.

Abstract: *Cryptosporidium parvum* is an enteric protozoan parasite of medical and veterinary importance. Dissemination of environmentally resistant oocysts in surface

water plays an important role in the epidemiology of cryptospridiosis. Although the polymerase chain reaction (PCR) is a well-established technique and is widely used for detecting microorganisms, it is not routinely applied for monitoring waterborne *C. parvum*. In order to facilitate the application of PCR to the detection of waterborne *C. parvum* oocysts, a comparison of published PCR protocols was undertaken and different sample-preparation methods tested. The sensitivity of a one-step PCR method, consisting of 40 temperature cycles, was 10 purified oocysts or fewer than 100 oocysts spiked in raw lake water. The detection limit of two primer pairs, one targeting the ribosomal small subunit and another specific for a *C. parvum* sequence of unknown function, was approximately ten-fold lower than achieved with a primer pair targeting an oocyst shell protein gene. Three cycles of freezing/thawing were sufficient to expose oocyst DNA and resulted in higher sensitivity than proteinase K digestion, sonication or electroporation. Inhibition of PCR by surface water from different local sources was entirely associated with the soluble fraction of lake water. Membrane filtration was evaluated in bench-scale experiments as a means of removing lake water inhibitors and improving the detection limit of PCR. Using gel and membrane filtration, the molecular size of inhibitory solutes from lake water was estimated to less than 27 kDa.

PMID: 9352675 [PubMed - indexed for MEDLINE]

Hwang YC, Leong OM, Chen W, Yates MV (2007) Comparison of a reporter assay and immunomagnetic separation real-time reverse transcription-PCR for the detection of enteroviruses in seeded environmental water samples. Appl Environ Microbiol. 73(7):2338-40.

Abstract: Two newly developed protocols for infective virus detection were compared to the plaque assay. An immunomagnetic separation procedure coupled with real-time reverse transcription-PCR of viral nucleic acids was developed to identify intact enteroviral particles, and a reporter cell system responding to viral replication based on fluorescent resonance energy transfer for detection of infectious enteroviruses was tested. Both new procedures detected infective viruses in environmental samples at the same level as the plaque assay.

PMID: 17277214 [PubMed - indexed for MEDLINE]

Tourlousse DM, Stedtfeld RD, Baushke SW, Wick LM, Hashsham SA. (2007) Virulence factor activity relationships: challenges and development approaches. Water Environ Res. 79(3):246-59.

Abstract: Virulence factor activity relationships (VFAR) is a predictive approach proposed by the National Research Council's Committee on Drinking Water Contaminants (Washington, D.C.) to classify and rank waterborne pathogens. It is based on the presumption that health threats of waterborne pathogens can be predicted from descriptors at different levels of cellular organization. This paper summarizes

challenges that need to be addressed while developing VFAR, with a focus on genomics, such as genomic variability among related pathogens and the need to incorporate genetic descriptors for persistence and host susceptibility. Three key components of VFAR development and validation are also presented, including (1) compilation of a comprehensive VFAR database, (2) development of predictive mathematical models relating descriptors to health effects and other microbial responses, and (3) high-throughput molecular monitoring of drinking water supplies and sources. Bayesian approach and on-chip polymerase chain reaction are discussed as examples of mathematical models and molecular monitoring.

PMID: 17469656 [PubMed - indexed for MEDLINE]

Jenkins TM, Scott TM, Cole JR, Hashsham SA, Rose JB (2004) Assessment of virulence-factor activity relationships (VFARs) for waterborne diseases. Water Sci Technol. 50(1):309-14.

Abstract: Virulence-factor activity relationship (VFAR) is a concept that was developed as a way to relate the architectural and biochemical components of a microorganism to its potential to cause human disease. Development of these relationships requires specialised bioinformatics databases that do not exist at present. A pilot-scale VFAR database was designed for three different waterborne organisms: Escherichia coli, Norovirus and *Cryptosporidium*, to evaluate VFAR relationships. For the web-based database, each organism has separate pages containing virulence genes, occurrence genes, primer sets and probes, taxonomy, outbreaks, and serotype/species/genogroup/genotype. As the database continues to grow, it will be possible to relate the occurrence and prevalence of certain genes in various microorganisms to outbreak data and, subsequently, to establish the utility of using a combination of specific genes as markers of virulence and in establishing virulence-factor activity relationships (VFARs). The database and the VFARs established will be of use to the regulatory community as a way to assist with prioritising those organisms, which need to be regulated.

Stedtfeld RD, Baushke S, Tourlousse D, Chai B, Cole JR, Hashsham SA (2007) Multiplex approach for screening genetic markers of microbial indicators. Water Environ Res. 79(3):260-9.

Abstract: Genetic markers are expected to provide better specificity in epidemiological studies and potentially serve as better indicators of waterborne pathogens. Methods used to assess genetic markers of emerging microbial indicators include pulsed field gel electrophoresis, polymerase chain reaction (PCR), and microarrays. This paper outlines a high-throughput approach to screen for such genetic markers using a set of theoretical and experimental screening tools. The theoretical screening involves evaluating genes related to the ribosomal RNA and specific functions from emerging indicator groups, followed by experimental validation with appropriate sampling schemes and high-throughput and economical screening methods, such as microarrays, real time PCR, and on-chip PCR. Analysis of a wide range of samples

covering temporal variability in location, host, and waterborne disease outbreaks is essential. The proposed approach is expected to shorten the time and cost associated with searching for new genetic markers of emerging indicators by at least 10-fold.

PMID: 17469657 [PubMed - indexed for MEDLINE]

Sen K. (2005) Development of a rapid identification method for *Aeromonas* species by multiplex-PCR. Can J Microbiol. 51(11):957-66.

Abstract: Existing biochemical methods cannot distinguish among some species of Aeromonads, while genetic methods are labor intensive. In this study, primers were developed to three genes of Aeromonas: lipase, elastase, and DNA gyraseB. In addition, six previously described primer sets, five corresponding to species-specific signature regions of the 16S rRNA gene from *A. veronii*, *A. popoffii*, *A. caviae*, *A. jandaei*, and *A. schubertii*, respectively, and one corresponding to *A. hydrophila* specific lipase (hydrolipase), were chosen. The primer sets were combined in a series of multiplex-PCR (mPCR) assays against 38 previously characterized strains. Following PCR, each species was distinguished by the production of a unique combination of amplicons. When the assays were tested using 63 drinking water isolates, there was complete agreement in the species identification (ID) for 59 isolates, with ID established by biochemical assays. Sequencing the gyrB and the 16S rRNA gene from the remaining four strains established that the ID obtained by mPCR was correct for three strains. For only one strain, no consensus ID could be obtained. A rapid and reliable method for identification of different *Aeromonas* species is proposed that does not require restriction enzyme digestions, thus simplifying and speeding up the process.

Sen K, Rodgers M (2004) Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. J Appl Microbiol. 97(5):1077-86.

Abstract: AIMS: To examine whether *Aeromonas* bacteria isolated from municipally treated water had virulence factor genes. METHODS AND RESULTS: A polymerase chain reaction-based genetic characterization determined the presence of six virulence factors genes, elastase (ahyB), lipase (pla/lip/lipH3/alp-1) flagella A and B (flaA and flaB), the enterotoxins, act, alt and ast, in these isolates. New primer sets were designed for all the target genes, except for act. The genes were present in 88% (ahyB), 88% (lip), 59% (fla), 43% (alt), 70% (act) and 30% (ast) of the strains, respectively. Of the 205 isolates tested only one isolate had all the virulence genes. There was a variety of combinations of virulence factors within different strains of the same species. However, a dominant strain having the same set of virulence factors, was usually isolated from any given tap in different rounds of sampling from a single tap. CONCLUSIONS: These results show that *Aeromonas* bacteria found in drinking water possess a wide variety of virulence-related genes and suggest the importance of examining as many isolates as possible in order to better understand the health risk these bacteria may present. SIGNIFICANCE AND IMPACT OF THE STUDY: This study presents a rapid method for characterizing the virulence factors of *Aeromonas* bacteria and suggests that municipally treated drinking water is a source of potentially pathogenic Aeromonas bacteria.

PATHOGEN DETECTION WITH AMPLIFICATION USING MICROARRAYS

Low density microarrays are often used in combination with a multiplexed PCR reaction. Multiplexed PCR reactions frequently results in confounding products that are not resolvable by standard techniques such as gel electrophoresis. The overlapping spectra of chromophores used to detect specific products in multiplexed reactions usually restrict the number of amplified sequences that can be amplified in a single reaction chamber from four to six. Microarrays enable researchers to use the fidelity of DNA hybridization to identify the presence of specific sequences in a complex mixture produced by a highly multiplexed reaction.

The majority of current microarray platforms rely on fluorescent signatures for detection. The highly multiplexed platform is essential for the next generation of detection and characterization, however, the sensitivity and consistency of optical detection using fluors may not be sufficient for medical and environmental applications. Novel methods that rely on electrical signals should provide a significant enhancement over current optical methods. These will improve sensitivity as well as the ability to obtain quantative measurements to determine organism copy number.

Kong F, Gilbert GL. (2006) Multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB)-a practical epidemiological and diagnostic tool. Nat Protoc. 1(6):2668-80.

Combining multiplex PCR, sequentially, with reverse line blot hybridization (mPCR/RLB) is a convenient, objective way to identify up to 43 targets in 43 individual specimens simultaneously (using a 45-lane membrane format). It is more flexible and less expensive than DNA microarray. The number of targets is adequate for epidemiological and most clinical diagnostic applications; based on the same target (43) and specimen numbers (43), it is much more practical than conventional uniplex PCR (uPCR) and mPCR. We have used the protocol to identify and subtype bacteria, viruses and fungi and identify pathogens in clinical specimens; potentially, it could be used for many other applications, such as detection of mutations in, or identification of alleles of, eukaryotic genes. Development of each assay involves (i) careful primer and probe design, based on literature and sequence database searches, which are critical to success of the assay; and (ii) bench-top evaluation, using known samples, controls and dilution series, to confirm sensitivity, specificity and reproducibility. The assay takes about one and half working days to complete; about 4 h for the mPCR and 6 h for the RLB, including a total of 4 h 'hands-on' time.

PMID: 17406523 [PubMed - in process]

Barlaan EA, Furukawa S, Takeuchi K. (2007) Detection of bacteria associated with harmful algal blooms from coastal and microcosm environments using electronic microarrays. Environ Microbiol. 9(3):690-702.

With the global expansion of harmful algal blooms (HABs), several measures, including molecular approaches, have been undertaken to monitor its occurrence. Many reports have indicated the significant roles of bacteria in controlling algal bloom dynamics. Attempts have been made to utilize the bacteria/harmful algae relationship in HAB monitoring. In this study, bacterial assemblages monitored during coastal HABs and bacterial communities in induced microcosm blooms were investigated. Samples were analysed using denaturing gradient gel electrophoresis (DGGE) of the 16S rRNA gene. DGGE bands with peculiar patterns before, during, and after algal blooms were isolated and identified. Probes for six ribotypes representing organisms associated with *Chatonella* spp., *Heterocapsa circularisquama*, or *Heterosigma akashiwo* were used for analysis on NanoChip electronic microarray. In addition, a new approach using cultured bacteria species was developed to detect longer (533 bp) polymerase chain reaction-amplified products on the electronic microarray. The use of fluorescently labelled primers allowed the detection of individual species in single or mixed DNA conditions. The developed approach enabled the detection of the presence or absence and relative abundance of the HAB-related ribotypes in coastal and microcosm blooms. This study indicates the ability of electronic microarray platform to detect or monitor bacteria in natural and induced environments.

PMID: 17298369 [PubMed - indexed for MEDLINE]

Leidreiter M, Kuhne M. (2007) [Determination of the minimum enrichment time for the qualitative detection of *Listeria monocytogenes* in minced pork meat using multiplex-PCR, microarray and ELISA] Berl Munch Tierarztl Wochenschr. 120(1-2):79-85.

[Article in German]

A qualitative detection of *Listeria monocytogenes* was performed in spiked minced pork meat using ELISA, Multiplex-PCR, Microarray and cultural reference method. Minced pork meat in batches of 10 or 25 g was spiked with 25 cfu *Listeria monocytogenes* per gram and incubated in selective enrichment solutions. During enrichment samples were collected continiously and a *Listeria monocytogenes*-ELISA, a Multiplex-PCR with electrophoretical detection (Listeria duplex) and a PCR with detection by Microarray (NUTRI Chip) were performed. The enrichment time after which all sub-samples were positive was defined as minimum enrichment time. With the cultural reference method *Listeria monocytogenes* was detected in 100 % of the samples after a total analysis time of 5 days. With the ELISA kit used in this study positive results were achieved after enrichment for 24 h. Multiplex-PCR with electrophoretical detection was positive after only 16 h of enrichment.The most sensitive detection method was the microarray. Using this technique, an enrichment time of 10 h was sufficient to get positive results in all samples.

PMID: 17290946 [PubMed - indexed for MEDLINE]

Alameda F, Bellosillo B, Fuste P, Musset M, Marinoso ML, Mancebo G, Lopez-Yarto MT, Carreras R, Serrano S. (2007) Human papillomavirus detection in urine samples: an alternative screening method. J Low Genit Tract Dis. 11(1):5-7.

OBJECTIVES: To investigate the usefulness of human papillomavirus detection in the urine of women with poor gynecologic attention. MATERIALS AND METHODS: Fifty urine and 50 cervical samples from 50 women were analyzed. Polymerase chain reaction was performed on these 100 samples using consensus primers and a low-density microarray-based method for human papillomavirus typing. RESULTS: The concordance of the results between both sample groups was 80%. In the urine samples, the sensitivity of polymerase chain reaction for high-grade squamous intraepithelial lesion was 100%, the specificity was 80%, the positive predictive value was 91%, and the negative predictive value was 100%. CONCLUSIONS: Human papillomavirus detection in urine samples may be used as an alternative screening method for women with poor gynecologic attention.

PMID: 17194943 [PubMed - indexed for MEDLINE]

Liu ZL, Slininger PJ. (2007) Universal external RNA controls for microbial gene expression analysis using microarray and qRT-PCR. J Microbiol Methods. 68(3):486-96.

Gene expression analysis provides significant insight to understand regulatory mechanisms of biology, yet acquisition and reproduction of quality data, as well as data confirmation and verification remain challenging due to a lack of proper quality controls across different assay platforms. We present a set of six universal external RNA quality controls for microbial mRNA expression analysis that can be applied to both DNA oligo microarray and real-time qRT-PCR including using SYBR Green and TaqMan probe-based chemistry. This set of controls was applied for *Saccharomyces cerevisiae* and *Pseudomonas fluorescens* Pf-5 microarray assays and qRT-PCR for yeast gene expression analysis. Highly fitted linear relationships between detected signal intensity and mRNA input were described. Valid mRNA detection range, from 10 to 7000 pg and from 100 fg to 1000 pg were defined for microarray and qRT-PCR assay, respectively. Quantitative estimation of mRNA abundance was tested using randomly selected yeast ORF including function unknown genes using the same source of samples by the two assay platforms. Estimates of mRNA abundance by the two methods were similar and highly correlated in an overlapping detection range from 10 to 1000 pg. The universal external RNA controls provide a means to compare microbial gene expression data derived from different experiments and different platforms for verification and confirmation. Such quality controls ensure reliability and reproducibility of gene expression data, and provide unbiased normalization reference for validation, quantification, and estimate of variation of gene expression experiments. Application of these controls also improves efficiency and facilitates high throughput applications of gene expression analysis using the qRT-PCR assay.

PMID: 17173990 [PubMed - indexed for MEDLINE]

Lin B, Blaney KM, Malanoski AP, Ligler AG, Schnur JM, Metzgar D, Russell KL, Stenger DA. (2007) Using a resequencing microarray as a multiple respiratory pathogen detection assay. J Clin Microbiol. 45(2):443-52.

Simultaneous testing for detection of infectious pathogens that cause similar symptoms (e.g., acute respiratory infections) is invaluable for patient treatment, outbreak prevention, and efficient use of antibiotic and antiviral agents. In addition, such testing may provide information regarding possible coinfections or induced secondary infections, such as virally induced bacterial infections. Furthermore, in many cases, detection of a pathogen requires more than genus/species-level resolution, since harmful agents (e.g., avian influenza virus) are grouped with other, relatively benign common agents, and for every pathogen, finer resolution is useful to allow tracking of the location and nature of mutations leading to strain variations. In this study, a previously developed resequencing microarray that has been demonstrated to have these capabilities was further developed to provide individual detection sensitivity ranging from 10(1) to 10(3) genomic copies for more than 26 respiratory pathogens while still retaining the ability to detect and differentiate between close genetic neighbors. In addition, the study demonstrated that this system allows unambiguous and reproducible sequence-based strain identification of the mixed pathogens. Successful proof-of-concept experiments using clinical specimens show that this approach is potentially very useful for both diagnostics and epidemic surveillance.

PMID: 17135438 [PubMed - indexed for MEDLINE]

Kostic T, Weilharter A, Rubino S, Delogu G, Uzzau S, Rudi K, Sessitsch A, Bodrossy L. (2007) A microbial diagnostic microarray technique for the sensitive detection and identification of pathogenic bacteria in a background of nonpathogens. Anal Biochem. 360(2):244-54.

A major challenge in microbial diagnostics is the parallel detection and identification of low-bundance pathogens within a complex microbial community. In addition, a high specificity providing robust, reliable identification at least at the species level is required. A microbial diagnostic microarray approach, using single nucleotide extension labeling with gyrB as the marker gene, was developed. We present a novel concept applying competitive oligonucleotide probes to improve the specificity of the assay. Our approach enabled the sensitive and specific detection of a broad range of pathogenic bacteria. The approach was tested with a set of 35 oligonucleotide probes targeting *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Aeromonas hydrophila*, *Vibrio cholerae*, *Mycobacterium avium*, *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Proteus mirabilis*, *Yersinia enterocolitica*, and *Campylobacter jejuni*. The introduction of competitive oligonucleotides in the labeling reaction successfully suppressed cross-reaction by closely related sequences, significantly improving the performance of the assay. Environmental applicability was tested with environmental and veterinary samples harboring complex microbial communities. Detection sensitivity in the range of 0.1% has

been demonstrated, far below the 5% detection limit of traditional microbial diagnostic microarrays.

PMID: 17123456 [PubMed - indexed for MEDLINE]

Antwerpen MH, Schellhase M, Ehrentreich-Forster E, Bier F, Witte W, Nubel U. (2007) DNA microarray for detection of antibiotic resistance determinants in *Bacillus anthracis* and closely related *Bacillus cereus*. Mol Cell Probes. 21(2):152-60.

We developed a multiplex PCR for amplification of ten genes involved in resistance to ciprofloxacin, doxycycline, rifampin, and vancomycin in *Bacillus anthracis* and closely related *Bacillus cereus*. Enzymatic labelling of PCR products followed by hybridization to oligonucleotide probes on a DNA microarray enabled simultaneous detection of resistance genes tetK, tetL, tetM, tetO, vanA, and vanB and resistance-mediating point mutations in genes gyrA, gyrB, parC, and rpoB. The presented assay allows detection of clinically relevant antibiotic resistance determinants within 4h and can be used as a time-saving tool supporting conventional culture-based diagnostics.

PMID: 17118627 [PubMed - indexed for MEDLINE]

Francois P, Garzoni C, Bento M, Schrenzel J. (2007) Comparison of amplification methods for transcriptomic analyses of low abundance prokaryotic RNA sources. J Microbiol Methods. 68(2):385-91.

Microarrays have established as instrumental for bacterial detection, identification, and genotyping as well as for transcriptomic studies. For gene expression analyses using limited numbers of bacteria (derived from *in vivo* or *ex vivo* origin, for example), RNA amplification is often required prior to labeling and hybridization onto microarrays. Evaluation of the fidelity of the amplification methods is crucial for the robustness and reproducibility of microarray results. We report here the first utilization of random primers and the highly processive Phi29 phage polymerase to amplify material for transcription profiling analyses. We compared two commercial amplification methods (GenomiPhi and MessageAmp kits) with direct reverse-transcription as the reference method, focusing on the robustness of mRNA quantification using either microarrays or quantitative RT-PCR. Both amplification methods using either poly-A tailing followed by in vitro transcription, or direct strand displacement polymerase, showed appreciable linearity. Strand displacement technique was particularly affordable compared to in vitro transcription-based (IVT) amplification methods and consisted in a single tube reaction leading to high amplification yields. Real-time measurements using low-, medium-, and highly expressed genes revealed that this simple method provided linear amplification with equivalent results in terms of relative messenger abundance as those obtained by conventional direct reverse-transcription.

PMID: 17112614 [PubMed - indexed for MEDLINE]

Gao H, Yang ZK, Gentry TJ, Wu L, Schadt CW, Zhou J. (2007) Microarray-based analysis of microbial community RNAs by whole-community RNA amplification. Appl Environ Microbiol. 73(2):563-71.

A new approach, termed whole-community RNA amplification (WCRA), was developed to provide sufficient amounts of mRNAs from environmental samples for microarray analysis. This method employs fusion primers (six to nine random nucleotides with an attached T7 promoter) for the first-strand synthesis. The shortest primer (T7N6S) gave the best results in terms of the yield and representativeness of amplification. About 1,200- to 1,800-fold amplification was obtained with amounts of the RNA templates ranging from 10 to 100 ng, and very representative detection was obtained with 50 to 100 ng total RNA. Evaluation with a *Shewanella oneidensis* Deltafur strain revealed that the amplification method which we developed could preserve the original abundance relationships of mRNAs. In addition, to determine whether representative detection of RNAs can be achieved with mixed community samples, amplification biases were evaluated with a mixture containing equal quantities of RNAs (100 ng each) from four bacterial species, and representative amplification was also obtained. Finally, the method which we developed was applied to the active microbial populations in a denitrifying fluidized bed reactor used for denitrification of contaminated groundwater and ethanol-stimulated groundwater samples for uranium reduction. The genes expressed were consistent with the expected functions of the bioreactor and groundwater system, suggesting that this approach is useful for analyzing the functional activities of microbial communities. This is one of the first demonstrations that microarray-based technology can be used to successfully detect the activities of microbial communities from real environmental samples in a high-throughput fashion.

PMID: 17098911 [PubMed - indexed for MEDLINE]

Sachse K, Hotzel H, Slickers P, Ehricht R. (2006) The use of DNA microarray technology for detection and genetic characterisation of chlamydiae. Dev Biol (Basel). 126:203-10; discussion 326-7.

Due to its highly parallel approach, DNA microarray technology opens up new possibilities that may be particularly beneficial for laboratory diagnosis of infectious diseases. We developed a microarray assay for detection and differentiation of all currently defined chlamydial species belonging to the genera *Chlamydia* and *Chlamydophila* using the ArrayTube system, which we found to be particularly user-friendly and economical. The test includes PCR amplification of a 23S rDNA target region with concurrent biotinylation and subsequent hybridisation in the ArrayTube, a micro-reaction tube carrying the microarray chip on the bottom. In addition to high specificity, the assay was shown to allow detection and genetic characterisation of single PCR-amplifiable target DNA copies.

PMID: 17058496 [PubMed - indexed for MEDLINE]

Li Y, Liu D, Cao B, Han W, Liu Y, Liu F, Guo X, Bastin DA, Feng L, Wang L. (2006) Development of a serotype-specific DNA microarray for identification of some *Shigella* and pathogenic *Escherichia coli* strains. J Clin Microbiol. 44(12):4376-83.

Shigella and pathogenic *Escherichia coli* are major causes of human infectious diseases and are responsible for millions of cases of diarrhea worldwide every year. A convenient and rapid method to identify highly pathogenic serotypes of *Shigella* and *E. coli* is needed for large-scale epidemiologic study, timely clinical diagnosis, and reliable quarantine of the pathogens. In this study, a DNA microarray targeting O-serotype-specific genes was developed to detect 15 serotypes of *Shigella* and *E. coli*, including *Shigella sonnei*; *Shigella flexneri* type 2a; Shigella boydii types 7, 9, 13, 16, and 18; *Shigella dysenteriae* types 4, 8, and 10; and *E. coli* O55, O111, O114, O128, and O157. The microarray was tested against 186 representative strains of all *Shigella* and *E. coli* O serotypes, 38 clinical isolates, and 9 strains of other bacterial species that are commonly present in stool samples and was shown to be specific and reproducible. The detection sensitivity was 50 ng genomic DNA or 10(4) CFU per ml in mock stool specimens. This is the first report of a microarray for serotyping *Shigella* and pathogenic *E. coli*. The method has a number of advantages over traditional bacterial culture and antiserum agglutination methods and is promising for applications in basic microbiological research, clinical diagnosis, food safety, and epidemiological surveillance.

PMID: 17021058 [PubMed - indexed for MEDLINE]

Chiu CY, Rouskin S, Koshy A, Urisman A, Fischer K, Yagi S, Schnurr D, Eckburg PB, Tompkins LS, Blackburn BG, Merker JD, Patterson BK, Ganem D, DeRisi JL. (2006) Microarray detection of human parainfluenzavirus 4 infection associated with respiratory failure in an immunocompetent adult. Clin Infect Dis. 43(8):e71-6.

A pan-viral DNA microarray, the Virochip (University of California, San Francisco), was used to detect human parainfluenzavirus 4 (HPIV-4) infection in an immunocompetent adult presenting with a life-threatening acute respiratory illness. The virus was identified in an endotracheal aspirate specimen, and the microarray results were confirmed by specific polymerase chain reaction and serological analysis for HPIV-4. Conventional clinical laboratory testing using an extensive panel of microbiological tests failed to yield a diagnosis. This case suggests that the potential severity of disease caused by HPIV-4 in adults may be greater than previously appreciated and illustrates the clinical utility of a microarray for broad-based viral pathogen screening.

PMID: 16983602 [PubMed - indexed for MEDLINE]

Burton JE, Oshota OJ, Silman NJ. (2006) Differential identification of *Bacillus anthracis* from environmental *Bacillus* species using microarray analysis. J Appl Microbiol. 101(4):754-63.

AIMS: To determine whether microarray analysis could be employed for the differential identification of a range of environmental *Bacillus* sp. from four strains of *Bacillus anthracis*. METHODS AND RESULTS: Oligonucleotide probes were designed that were specific to virulence factor genes of *B. anthracis* (pag, lef and cap), the variable number tandem repeat region of the *B. anthracis* vrrA gene and to the 16S-23S rRNA intergenic transcribed spacer region (ITS) and pleiotropic regulator (plcR) regions of the *Bacillus cereus* subgroup species. Generic probes were also designed to hybridize with conserved regions of the 16S rRNA genes of *Bacillus* (as a positive control), *Neisseria* sp., *Pseudomonas* sp., *Streptococcus* sp., *Mycobacterium* sp. and to all members of the *Enterobacteriaceae* to allow simultaneous detection of these bacteria. Identification of *B. anthracis* was found to rely entirely on hybridization of DNA specific to regions of the pag, lef and cap genes. Cross-reaction was observed between *B. anthracis* and other *Bacillus* species with all the other *Bacillus* probes tested. Results obtained using microarray hybridizations were confirmed using conventional microbiological techniques and found to have very high comparability. CONCLUSIONS: Microarray-based assays are an effective method for the identification of *B. anthracis* from mixed-culture environmental samples without problems of false-positivity that have been observed with conventional PCR assays. SIGNIFICANCE AND IMPACT OF THE STUDY: Identification of environmental *Bacillus* sp. by conventional PCR is prone to potential for reporting false-positives. This study provides a method for the exclusion of such isolates.

PMID: 16968287 [PubMed - in process]

Mitterer G, Schmidt WM. (2006) Microarray-based detection of bacteria by on-chip PCR. Methods Mol Biol. 345:37-51.

In this chapter, a protocol called on-chip polymerase chain reaction (PCR) is presented for the deoxyribonucleic acid (DNA) microarray-based detection of bacterial target sequences. On-chip PCR combines, in a single step, the conventional amplification of a target with a simultaneous, nested PCR round intended for target detection. While freely diffusible primers are deployed for amplification, the nested PCR is initiated by oligonucleotide primers bound to a solid phase. Thus, on-chip PCR allows the single-step amplification and characterization of a DNA sample as a result of separation in liquid and solid-phase reactions. In contrast to conventional PCR, the reaction is performed directly on the flat surface of a glass slide that holds an array of covalently attached nested primers. The bacterial target DNA is amplified and probed using primers identifying either species-specific sequence regions of ribosomal DNA or unique bacterial target genes, such as virulence or resistance factors. The microarray is produced using standard spotting equipment with an array layout containing a high number of replicates. Fluorescence scanning of on-chip PCR slides allows the rapid detection of the target of interest. The protocol described herein will show how on-chip PCR can be used to detect and precisely identify DNA of bacterial origin.

PMID: 16957345 [PubMed - indexed for MEDLINE]

Brodie EL, Desantis TZ, Joyner DC, Baek SM, Larsen JT, Andersen GL, Hazen TC, Richardson PM, Herman DJ, Tokunaga TK, Wan JM, Firestone MK. (2006) Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. Appl Environ Microbiol. 72(9):6288-98.

Reduction of soluble uranium U(VI) to less-soluble uranium U(IV) is a promising approach to minimize migration from contaminated aquifers. It is generally assumed that, under constant reducing conditions, U(IV) is stable and immobile; however, in a previous study, we documented reoxidation of U(IV) under continuous reducing conditions (Wan et al., Environ. Sci. Technol. 2005, 39:6162-6169). To determine if changes in microbial community composition were a factor in U(IV) reoxidation, we employed a high-density phylogenetic DNA microarray (16S microarray) containing 500,000 probes to monitor changes in bacterial populations during this remediation process. Comparison of the 16S microarray with clone libraries demonstrated successful detection and classification of most clone groups. Analysis of the most dynamic groups of 16S rRNA gene amplicons detected by the 16S microarray identified five clusters of bacterial subfamilies responding in a similar manner. This approach demonstrated that amplicons of known metal-reducing bacteria such as *Geothrix fermentans* (confirmed by quantitative PCR) and those within the *Geobacteraceae* were abundant during U(VI) reduction and did not decline during the U(IV) reoxidation phase. Significantly, it appears that the observed reoxidation of uranium under reducing conditions occurred despite elevated microbial activity and the consistent presence of metal-reducing bacteria. High-density phylogenetic microarrays constitute a powerful tool, enabling the detection and monitoring of a substantial portion of the microbial population in a routine, accurate, and reproducible manner.

PMID: 16957256 [PubMed - indexed for MEDLINE]

Marcelino LA, Backman V, Donaldson A, Steadman C, Thompson JR, Preheim SP, Lien C, Lim E, Veneziano D, Polz MF. (2006) Accurately quantifying low-abundant targets amid similar sequences by revealing hidden correlations in oligonucleotide microarray data. Proc Natl Acad Sci USA. 103(37):13629-34.

Microarrays have enabled the determination of how thousands of genes are expressed to coordinate function within single organisms. Yet applications to natural or engineered communities where different organisms interact to produce complex properties are hampered by theoretical and technological limitations. Here we describe a general method to accurately identify low-abundant targets in systems containing complex mixtures of homologous targets. We combined an analytical predictor of nonspecific probe-target interactions (cross-hybridization) with an optimization algorithm that iteratively deconvolutes true probe-target signal from raw signal affected by spurious contributions (cross-hybridization, noise, background, and unequal specific hybridization response). The method was capable of quantifying, with unprecedented specificity and accuracy, ribosomal RNA (rRNA) sequences in artificial and natural communities. Controlled experiments with spiked rRNA into artificial and natural communities

demonstrated the accuracy of identification and quantitative behavior over different concentration ranges. Finally, we illustrated the power of this methodology for accurate detection of low-abundant targets in natural communities. We accurately identified *Vibrio* taxa in coastal marine samples at their natural concentrations (<0.05% of total bacteria), despite the high potential for cross-hybridization by hundreds of different coexisting rRNAs, suggesting this methodology should be expandable to any microarray platform and system requiring accurate identification of low-abundant targets amid pools of similar sequences.

PMID: 16950880 [PubMed - indexed for MEDLINE]

Bosse JT, Zhou L, Kroll JS, Langford PR. (2006) High-throughput identification of conditionally essential genes in bacteria: from STM to TSM. Infect Disord Drug Targets. 6(3):241-62.

Signature-tagged mutagenesis (STM) provided the first widely applicable high-throughput method for detecting conditionally essential genes in bacteria by using negative selection to screen large pools of transposon (Tn) mutants. STM requires no prior knowledge of the bacterium's genome sequence, and has been used to study a large number of Gram-positive and Gram-negative species, greatly expanding the repertoires of known virulence factors for these organisms. Originally, hybridization of radiolabelled probes to colony or dot blots was used to detect differences in populations of tagged mutants before and after growth under a selective condition. Modifications of the tag detection method involving polymerase chain reaction (PCR) amplification and visualisation by gel electrophoresis have been developed and can be automated through the use of robotics. Genetic footprinting is another negative selection technique that uses PCR amplification to detect loss of mutants from a pool. Unlike PCR-STM, this technique allows direct amplification of Tn-flanking sequences. However, it requires the bacterium's whole genome sequence in order to design specific primers for every gene of interest. More recently, a number of techniques have been described that combine the negative-selection principle of STM and genetic footprinting with the genome-wide screening power of DNA microarrays. These techniques, although also requiring whole genome sequences, use either a form of linker-mediated or semi-random PCR to amplify and label Tn-flanking regions for hybridization to microarrays. The superior sensitivity microarray detection allows greater numbers of mutants to be screened per pool, as well as determination of the coverage/distribution of insertions in the library prior to screening, two significant advantages over STM.

PMID: 16918485 [PubMed - indexed for MEDLINE]

Siripong S, Kelly JJ, Stahl DA, Rittmann BE. (2006) Impact of prehybridization PCR amplification on microarray detection of nitrifying bacteria in wastewater treatment plant samples. Environ Microbiol. 8(9):1564-74.

A gel-based microarray that included a set of 26 oligonucleotide probes targeting all nitrifying bacteria at varying levels of specificity suggested the presence of targeted

microorganisms when hybridized to RNA isolated from a wastewater treatment plant, but could not discriminate between perfectly matched and mismatched sequences due in part to low signal intensity. To enhance sensitivity and improve discrimination, polymerase chain reaction was used to selectively amplify the 16S rRNA genes of specific nitrifier groups. RNA transcribed from these DNA templates was hybridized to the microarray and thermal dissociation analysis was used to characterize the specificity of hybridization. Amplification with *Nitrospira*-specific primers resulted in the selective amplification of this target group, confirmed by both a significant increase in signal intensity and a melting profile identical to the reference RNA. In contrast, *Nitrobacter* was not detected in the environmental samples with probe Nbac1000 despite pre-amplification with *Nitrobacter*-specific primers, indicating the absence of strains containing this *Nitrobacter*-specific sequence. Pre-amplification using primers specific for beta-Proteobacterial ammonia-oxidizing bacteria resulted in a significant increase in signal intensity for probe Nso190, but melting profiles for probe Nso190 showed a slight deviation between amplified RNA and the reference microorganism, suggesting that the amplification products contained some sequences that varied by a single nucleotide difference in the probe target region.

PMID: 16913917 [PubMed - indexed for MEDLINE]

Wang Q, Wang M, Kong F, Gilbert GL, Cao B, Wang L, Feng L. (2007) Development of a DNA microarray to identify the *Streptococcus pneumoniae* serotypes contained in the 23-valent pneumococcal polysaccharide vaccine and closely related serotypes. J Microbiol Methods. 68(1):128-36.

Streptococcus pneumoniae is a major worldwide human pathogen. This investigation has developed a reliable and accurate DNA microarray method for inter-species differentiation of *S. pneumoniae* and intra-species differentiation of the 23 groups of S. pneumoniae including serotypes represented in the 23-valent pneumococcal vaccine and the other 20 closely related serotypes. In addition to 16S rDNA probes, serotype- or serogroup-specific probes targeting the capsular polysaccharide synthesis (cps) genes, wzy or capA were generated. We adopted a two-step multiplex PCR to improve the sensitivity of detection to a level of 10(5) cfu/ml in pure culture or 50 ng DNA. A total of 169 isolates (from China, Australia, Canada and New Zealand) including 147 belonging to 23-valent vaccine and closely related serotypes of *S. pneumoniae*, 11 belonging to other serotypes and 11 of different species commonly isolated from respiratory tract were tested to verify the method. The DNA microarray method developed provides a sensitive means to rapidly identify the members of the most common *S. pneumoniae* serotypes in patients and to monitor their distribution in different patient groups and geographic locations. Such information is needed for disease surveillance and to monitor vaccine efficacy.

PMID: 16904781 [PubMed - indexed for MEDLINE]

Townsend MB, Dawson ED, Mehlmann M, Smagala JA, Dankbar DM, Moore CL, Smith CB, Cox NJ, Kuchta RD, Rowlen KL. (2006) Experimental evaluation of the FluChip diagnostic microarray for influenza virus surveillance. J Clin Microbiol. 44(8):2863-71.

Global surveillance of influenza is critical for improvements in disease management and is especially important for early detection, rapid intervention, and a possible reduction of the impact of an influenza pandemic. Enhanced surveillance requires rapid, robust, and inexpensive analytical techniques capable of providing a detailed analysis of influenza virus strains. Low-density oligonucleotide microarrays with highly multiplexed "signatures" for influenza viruses offer many of the desired characteristics. However, the high mutability of the influenza virus represents a design challenge. In order for an influenza virus microarray to be of utility, it must provide information for a wide range of viral strains and lineages. The design and characterization of an influenza microarray, the FluChip-55 microarray, for the relatively rapid identification of influenza A virus subtypes H1N1, H3N2, and H5N1 are described here. In this work, a small set of sequences was carefully selected to exhibit broad coverage for the influenza A and B viruses currently circulating in the human population as well as the avian A/H5N1 virus that has become enzootic in poultry in Southeast Asia and that has recently spread to Europe. A complete assay involving extraction and amplification of the viral RNA was developed and tested. In a blind study of 72 influenza virus isolates, RNA from a wide range of influenza A and B viruses was amplified, hybridized, labeled with a fluorophore, and imaged. The entire analysis time was less than 12 h. The combined results for two assays provided the absolutely correct types and subtypes for an average of 72% of the isolates, the correct type and partially correct subtype information for 13% of the isolates, the correct type only for 10% of the isolates, false-negative signals for 4% of the isolates, and false-positive signals for 1% of the isolates. In the overwhelming majority of cases in which incomplete subtyping was observed, the failure was due to the nucleic acid amplification step rather than limitations in the microarray.

PMID: 16891504 [PubMed - indexed for MEDLINE]

Albrecht V, Chevallier A, Magnone V, Barbry P, Vandenbos F, Bongain A, Lefebvre JC, Giordanengo V. (2006) Easy and fast detection and genotyping of high-risk human papillomavirus by dedicated DNA microarrays. J Virol Methods. 137(2):236-44.

Persistent cervical high-risk human papillomavirus (HPV) infection is correlated with an increased risk of developing a high-grade cervical intraepithelial lesion. A two-step method was developed for detection and genotyping of high-risk HPV. DNA was firstly amplified by asymmetrical PCR in the presence of Cy3-labelled primers and dUTP. Labelled DNA was then genotyped using DNA microarray hybridization. The current study evaluated the technical efficacy of laboratory-designed HPV DNA microarrays for high-risk HPV genotyping on 57 malignant and non-malignant cervical smears. The approach was evaluated for a broad range of cytological samples: high-grade squamous intraepithelial lesions (HSIL), low-grade squamous intraepithelial lesions (LSIL) and

atypical squamous cells of high-grade (ASC-H). High-risk HPV was also detected in six atypical squamous cells of undetermined significance (ASC-US) samples; among them only one cervical specimen was found uninfected, associated with no histological lesion. The HPV oligonucleotide DNA microarray genotyping detected 36 infections with a single high-risk HPV type and 5 multiple infections with several high-risk types. Taken together, these results demonstrate the sensitivity and specificity of the HPV DNA microarray approach. This approach could improve clinical management of patients with cervical cytological abnormalities.

PMID: 16879879 [PubMed - indexed for MEDLINE]

Bin W, Liu M, Peng J, Sun L, Xu X, Zhang J, Jin Q. (2006) Construction, detection and microarray analysis on *Shigella dysenteriae* a1 IroN, ShuA single, double mutants. Sci China C Life Sci. 49(3):251-8.

In this study, we constructed single mutants MTS-1, MTS-2 of IroN and ShuA gene and double mutant MTS of them in *Shigella dysenteriae* A1 strain 51197 by insert and absence. The functional detection of every mutant was performed at the level of culture medium and cell experiment. The gene expression profiles of the mutants and the wild-type strains under iron-enriched and iron-limited conditions were analyzed by the SD51197 whole genomic microarray. The results showed that all the mutants grew obviously less well than the wild-type strains in L broth appending iron chelator DIP. The addition of iron to the cultures can stimulate the growth of mutants back to wild-type levels. In either the experiments on the ability of intracellular multiplication or the cell-to-cell spread in HeLa and U937 cell lines, mutants showed no obvious change in virulence compared with the parental strain SD51197. However when DIP was added to the cultured HeLa cells, the ability of intracellular multiplication of MTS-1, MTS-2, MTS has reduced about 23.4%, 25.2%, 43.6% respectively. The analysis of expression profiles under the iron-limited condition showed that the mutants were more sensitive for the changes of iron deficiency than the wild-type strains, many genes have been altered. Up-regulated genes mainly involved genes of transcription, coenzyme metabolism, amino acid transport and metabolism, and unknown functional genes, while down-regulated genes mainly involved genes of energy and carbohydrate metabolism and unknown function genes; the expression levels of known iron-transport associated genes generally showed up-regulated. The results demonstrated that iron-transport associated genes IroN, ShuA were likely to have some effects on the virulence and growth of *S. dysenteriae*.

PMID: 16856494 [PubMed - indexed for MEDLINE]

Garaizar J, Rementeria A, Porwollik S. (2006) DNA microarray technology: a new tool for the epidemiological typing of bacterial pathogens? FEMS Immunol Med Microbiol. 47(2):178-89.

Genomic hybridization on whole genome arrays detects the presence or absence of similar DNA regions in sufficiently related microorganisms, allowing genome-wide

comparison of their genetic contents. A whole genome array is based on a sequenced bacterial isolate, and is a collection of DNA probes fixed on a solid support. In a single hybridization experiment, the absence/presence status of all genes of the sequenced microbe in the queried isolate can be examined. The objective of this minireview is to summarize the past usage of DNA microarray technology for microbial strain characterizations, and to estimate its future utilization in epidemiological studies and molecular typing of bacterial pathogens. The studies reviewed here confirm the usefulness of microarray technology for the detection of genetic polymorphisms. However, the construction or purchase of DNA microarrays and the performance of strain to strain hybridization experiments are still prohibitively expensive for routine application. Future use of arrays in epidemiology is likely to depend on the development of more cost-effective protocols, more robust and simplified formats, and the adequate evaluation of their performance (efficacy) and convenience (efficiency) compared with other genotyping methods. It seems more likely that a more focused assay, concentrating on genomic regions of variability previously detected by genome-wide microarrays, will find broad application in routine bacterial epidemiology.

PMID: 16831204 [PubMed - indexed for MEDLINE]

Caoili JC, Mayorova A, Sikes D, Hickman L, Plikaytis BB, Shinnick TM. (2006) Evaluation of the TB-Biochip oligonucleotide microarray system for rapid detection of rifampin resistance in *Mycobacterium tuberculosis*. J Clin Microbiol. 44(7):2378-81.

The TB-Biochip oligonucleotide microarray system is a rapid system to detect mutations associated with rifampin (RIF) resistance in mycobacteria. After optimizing the system with 29 laboratory-generated rifampin-resistant mutants of *Mycobacterium tuberculosis*, we evaluated the performance of this test using 75 clinical isolates of *Mycobacterium tuberculosis*. With this small sample set, the TB-Biochip system displayed a sensitivity of 80% and a specificity of 100% relative to conventional drug susceptibility testing results for RIF resistance. For these samples (approximately 50% tested positive), the positive predictive value was 100% and the negative predictive value was 85%. Four of the seven observed discrepancies were attributed to rare and new mutations not represented in the microarray, while three of the strains with discrepant results did not carry mutations in the RIF resistance-determining region. The results of this study confirm the utility of the system for rapid detection of RIF resistance and suggest approaches to increasing its sensitivity.

PMID: 16825352 [PubMed - indexed for MEDLINE]

Wu L, Liu X, Schadt CW, Zhou J. (2006) Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Appl Environ Microbiol. 72(7):4931-41.

Microarray technology provides the opportunity to identify thousands of microbial genes or populations simultaneously, but low microbial biomass often prevents

application of this technology to many natural microbial communities. We developed a whole-community genome amplification-assisted microarray detection approach based on multiple displacement amplification. The representativeness of amplification was evaluated using several types of microarrays and quantitative indexes. Representative detection of individual genes or genomes was obtained with 1 to 100 ng DNA from individual or mixed genomes, in equal or unequal abundance, and with 1 to 500 ng community DNAs from groundwater. Lower concentrations of DNA (as low as 10 fg) could be detected, but the lower template concentrations affected the representativeness of amplification. Robust quantitative detection was also observed by significant linear relationships between signal intensities and initial DNA concentrations ranging from (i) 0.04 to 125 ng (r2 = 0.65 to 0.99) for DNA from pure cultures as detected by whole-genome open reading frame arrays, (ii) 0.1 to 1,000 ng (r2 = 0.91) for genomic DNA using community genome arrays, and (iii) 0.01 to 250 ng (r2 = 0.96 to 0.98) for community DNAs from ethanol-amended groundwater using 50-mer functional gene arrays. This method allowed us to investigate the oligotrophic microbial communities in groundwater contaminated with uranium and other metals. The results indicated that microorganisms containing genes involved in contaminant degradation and immobilization are present in these communities, that their spatial distribution is heterogeneous, and that microbial diversity is greatly reduced in the highly contaminated environment.

PMID: 16820490 [PubMed - indexed for MEDLINE]

Peplies J, Lachmund C, Glockner FO, Manz W. (2006) A DNA microarray platform based on direct detection of rRNA for characterization of freshwater sediment-related prokaryotic communities. Appl Environ Microbiol. 72(7):4829-38.

A DNA microarray platform for the characterization of bacterial communities in freshwater sediments based on a heterogeneous set of 70 16S rRNA-targeted oligonucleotide probes and directly labeled environmental RNA was developed and evaluated. Application of a simple protocol for the efficient background blocking of aminosilane-coated slides resulted in an improved signal-to-noise ratio and a detection limit of 10 ng for particular 16S rRNA targets. An initial specificity test of the system using RNA from pure cultures of different phylogenetic lineages showed a fraction of false-positive signals of approximately 5% after protocol optimization and a marginal loss of correct positive signals. Subsequent microarray analysis of sediment-related community RNA from four different German river sites suggested low diversity for the groups targeted but indicated distinct differences in community composition. The results were supported by parallel fluorescence in situ hybridization in combination with sensitive catalyzed reporter deposition (CARD-FISH). In comparisons of the data of different sampling sites, specific detection of populations with relative cellular abundances down to 2% as well as a correlation of microarray signal intensities and population size is suggested. Our results demonstrate that DNA microarray technology allows for the fast and efficient precharacterization of complex bacterial communities by the use of standard single-cell hybridization probes and the direct detection of

environmental rRNA, also in methodological challenging habitats such as heterogeneous lotic freshwater sediments.

PMID: 16820477 [PubMed - indexed for MEDLINE]

Kim H, Kane MD, Kim S, Dominguez W, Applegate BM, Savikhin S. (2007) A molecular beacon DNA microarray system for rapid detection of *E. coli* O157:H7 that eliminates the risk of a false negative signal. Biosens Bioelectron. 22(6):1041-7

A DNA hybridization based optical detection platform for the detection of foodborne pathogens has been developed with virtually zero probability of the false negative signal. This portable, low-cost and real-time assaying detection platform utilizes the color changing molecular beacon as a probe for the optical detection of the target sequence. The computer-controlled detection platform exploits the target hybridization induced change of fluorescence color due to the Forster (fluorescence) resonance energy transfer (FRET) between a pair of spectrally shifted fluorophores conjugated to the opposite ends of a beacon (oligonucleotide probe). Unlike the traditional fluorophore-quencher beacon design, the presence of two fluorescence molecules allows to actively visualize both hybridized and unhybridized states of the beacon. This eliminates false negative signal detection characteristic for the fluorophore-quencher beacon where bleaching of the fluorophore or washout of a beacon is indistinguishable from the absence of the target DNA sequence. In perspective, the two-color design allows also to quantify the concentration of the target DNA in a sample down to < =1 ng/microl. The new design is suitable for simultaneous reliable detection of hundreds of DNA target sequences in one test run using a series of beacons immobilized on a single substrate in a spatial format.

PMID: 16815005 [PubMed - indexed for MEDLINE]

Cao X, Wang YF, Zhang CF, Gao WJ. (2006) Visual DNA microarrays for simultaneous detection of *Ureaplasma urealyticum* and *Chlamydia trachomatis* coupled with multiplex asymmetrical PCR. Biosens Bioelectron. 22(3):393-8.

Visual DNA microarrays, based on gold label silver stain (GLSS) and coupled with multiplex asymmetrical PCR, were developed for simultaneous, sensitive and specific detection of *Ureaplasma urealyticum* and *Chlamydia trachomatis*. 5'-end-amino-modified oligonucleotides, which were immobilized on glass surface, acted as capturing probes that were designed to bind complementary biotinylated targets DNA. The gold-conjugated streptavidins were introduced to the microarray for specific binding to biotin. The black image of microarray spots, resulting from the precipitation of silver onto nanogold particles bound to streptavidins, were used to detect biotinylated targets DNA visually or with a visible light scanner. Multiplex asymmetrical PCR of *U. urealyticum*, *C. trachomatis* and *Bacillus* subtilis (used as positive control) was performed to prepare abundant biotinylated single-stranded targets DNA, which affected detection efficiency and sensitivity of hybridization on microarray. Plenty of clinical samples of *U. urealyticum* and *C. trachomatis* from infected patients were tested using home-made DNA microarrays. For its high sensitivity, good specificity, simplicity, cheapness and

speed, the present visual gene-detecting technique has potential applications in clinical fields.

PMID: 16797960 [PubMed - indexed for MEDLINE]

leimanis S, Hernandez M, Fernandez S, Boyer F, Burns M, Bruderer S, Glouden T, Harris N, Kaeppeli O, Philipp P, Pla M, Puigdomenech P, Vaitilingom M, Bertheau Y, Remacle J. (2006) A microarray-based detection system for genetically modified (GM) food ingredients. Plant Mol Biol. 61(1-2):123-39.

A multiplex DNA microarray chip was developed for simultaneous identification of nine genetically modified organisms (GMOs), five plant species and three GMO screening elements, i.e. the 35S promoter, the nos terminator and the nptII gene. The chips also include several controls, such as that for the possible presence of CaMV. The on-chip detection was performed directly with PCR amplified products. Particular emphasis was placed on the reduction of the number of PCR reactions required and on the number of primers present per amplification tube. The targets were biotin labelled and the arrays were detected using a colorimetric methodology. Specificity was provided by specific capture probes designed for each GMO and for the common screening elements. The sensitivity of the assay was tested by experiments carried out in five different laboratories. The limit of detection was lower than 0.3% GMO for all tests and in general around 0.1% for most GMOs. The chip detection system complies with the requirements of current EU regulations and other countries where thresholds are established for the labelling of GMO.

PMID: 16786296 [PubMed - indexed for MEDLINE]

Jaaskelainen AJ, Maunula L. (2006) Applicability of microarray technique for the detection of noro- and astroviruses. J Virol Methods. 136(1-2):210-6.

Noroviruses and astroviruses are widespread viral agents causing gastroenteritis. Noroviruses are actually a diverse group of viruses. A new microarray-based detection method is presented for both noro- and astroviruses. This method enables simultaneous identification of genogroups and types of noro- and astroviruses. For pre-amplification, the monoplex- and multiplex-RT-PCR assays were applied to test sensitivity. The 202 specimens were used to determine the specificity, precision, and accuracy of the microarray. Stool samples representing 13 of norovirus GI and GII genotypes were assayed. The microarray detected all but one genotype. In a panel of 74 stool samples, 45 tested positive for norovirus by microarray, against 35 by a conventional PCR-method. In addition, microarray detected some double infections. In a panel of archival samples, astroviruses of genotype 4 frequently emerged in Finland from 1977 to 1997. This study demonstrates that microarray is useful for simultaneous monitoring of several viruses and their subtypes. Microarray provides a convenient tool for the detection of RNA viruses for which frequent changes in the panel of detection primers may be required.

PMID: 16781784 [PubMed - indexed for MEDLINE]

Chen TC, Chen GW, Hsiung CA, Yang JY, Shih SR, Lai YK, Juang JL. (2006) Combining multiplex reverse transcription-PCR and a diagnostic microarray to detect and differentiate enterovirus 71 and coxsackievirus A16. J Clin Microbiol. 44(6):2212-9.

Cluster A enteroviruses, including enterovirus 71 (EV71) and coxsackievirus A16 (CA16), are known to cause hand-foot-and-mouth disease (HFMD). Despite the close genetic relationship between these two viruses, EV71 is generally known to be a more perpetuating pathogen involved in severe clinical manifestations and deaths. While the serotyping of enteroviruses is mostly done by conventional immunological methods, many clinical isolates remain unclassifiable due to the limited number of antibodies against enterovirus surface proteins. Array-based assays are able to detect several serotypes with high accuracy. We combined an enterovirus microarray with multiplex reverse transcription-PCR to try to develop a method of sensitively and accurately detecting and differentiating EV71 and CA16. In an effort to design serotype-specific probes for detection of the virus, we first did an elaborate bioinformatic analysis of the sequence database derived from different enterovirus serotypes. We then constructed a microarray using 60-mer degenerate oligonucleotide probes covalently bound to array slides. Using this enterovirus microarray to study 144 clinical specimens from patients infected with HFMD or suspected to have HFMD, we found that it had a diagnostic accuracy of 92.0% for EV71 and 95.8% for CA16. Diagnostic accuracy for other enteroviruses (non-EV71 or -CA16) was 92.0%. All specimens were analyzed in parallel by real-time PCR and subsequently confirmed by neutralization tests. This highly sensitive array-based assay may become a useful alternative in clinical diagnostics of EV71 and CA16.

PMID: 16757623 [PubMed - indexed for MEDLINE]

Gheit T, Landi S, Gemignani F, Snijders PJ, Vaccarella S, Franceschi S, Canzian F, Tommasino M. (2006) Development of a sensitive and specific assay combining multiplex PCR and DNA microarray primer extension to detect high-risk mucosal human papillomavirus types. J Clin Microbiol. 44(6):2025-31.

The importance of assays for the detection and typing of human papillomaviruses (HPVs) in clinical and epidemiological studies has been well demonstrated. Several accurate methods for HPV detection and typing have been developed. However, comparative studies showed that several assays have different sensitivities for the detection of specific HPV types, particularly in the case of multiple infections. Here, we describe a novel one-shot method for the detection and typing of 19 mucosal high-risk (HR) HPV types (types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82). This assay combines two different techniques: multiplex PCR with HPV type-specific primers for amplification of viral DNA and array primer extension (APEX) for typing. This novel method has been validated with artificial mixtures of HPV DNAs and clinical samples that were already analyzed for the presence of mucosal HPV types by a different consensus PCR method, i.e., GP5+/GP6+. Our data showed a very good

agreement between the results from the multiplex PCR/APEX assay and those from the GP5+/GP6+ PCR (overall rates of HPV positivity, 63.0 and 60.9%, respectively). Whereas the GP5+/GP6+ PCR was slightly more sensitive for the detection of HPV type 16 (HPV-16), multiplex PCR-APEX found a higher number of infections with HPV-33, HPV-53, and multiple HPV types. These favorable features and the high-throughput potential make our present novel assay ideal for large-scale clinical and epidemiological studies aimed at determining the spectrum of mucosal HR HPV types in cervical specimens.

PMID: 16757593 [PubMed - indexed for MEDLINE]

Tracz DM, Tabor H, Jerome M, Ng LK, Gilmour MW. (2006) Genetic determinants and polymorphisms specific for human-adapted serovars of *Salmonella enterica* that cause enteric fever. J Clin Microbiol. 44(6):2007-18.

Salmonella enterica serovars *Typhi*, *Paratyphi A*, and *Sendai* are human-adapted pathogens that cause typhoid (enteric) fever. The acute prevalence in some global regions and the disease severity of typhoidal *Salmonella* have necessitated the development of rapid and specific detection tests. Most of the methodologies currently used to detect serovar *Typhi* do not identify serovars *Paratyphi A* or *Sendai*. To assist in this aim, comparative sequence analyses were performed at the loci of core bacterial genetic determinants and *Salmonella* pathogenicity island 2 genes encoded by clinically significant *S. enterica* serovars. Genetic polymorphisms specific for serovar Typhi (at trpS), as well as polymorphisms unique to human-adapted typhoidal serovars (at sseC and sseF), were observed. Furthermore, entire coding sequences unique to human-adapted typhoidal *Salmonella* strains (i.e., serovar-specific genetic loci rather than polymorphisms) were observed in publicly available comparative genomic DNA microarray data sets. These polymorphisms and loci were developed into real-time PCR, standard PCR, and liquid microsphere suspension array-based molecular protocols and tested for with a panel of clinical and reference subspecies I *S. enterica* strains. A proportion of the nontyphoidal *Salmonella* strains hybridized with the allele-specific oligonucleotide probes for sseC and sseF; but the trpS allele was unique to serovar Typhi (with a singular serovar Paratyphi B strain as an exception), and the coding sequences STY4220 and STY4221 were unique among serovars *Typhi*, *Paratyphi A*, and *Sendai*. These determinants provided phylogenetic data on the genetic relatedness of serovars *Typhi*, *Paratyphi A*, and *Sendai*; and the protocols developed might allow the rapid identification of these *Salmonella* serovars that cause enteric fever.

PMID: 16757591 [PubMed - indexed for MEDLINE]

Liu HH, Cao X, Yang Y, Liu MG, Wang YF. (2006) Array-based nano-amplification technique was applied in detection of hepatitis E virus. J Biochem Mol Biol. 39(3):247-52.

A rapid method for the detection of Hepatitis E Virus (HEV) was developed by utilizing nano-gold labeled oligonucleotide probes, silver stain enhancement and the

microarray technique. The 5'-end -NH(2) modified oligonucleotide probes were immobilized on the surface of the chip base as the capture probe. The detection probe was made of the 3'-end -SH modified oligonucleotide probe and nano-gold colloid. The optimal concentrations of these two probes were determined. To test the detection sensitivity and specificity of this technique, a conservative fragment of the virus RNA was amplified by the RT-PCR/PCR one step amplification. The cDNA was hybridized with the capture probes and the detection probes on microarray. The detection signal was amplified by silver stain enhancement and could be identified by naked eyes.100 fM of amplicon could be detected out on the microarray. As the results, preparation of nano-gold was improved and faster. Development time also was shortened to 2 min. Thus, considering high efficiency, low cost, good specificity and high sensitivity, this technique is alternative for the detection of HEV.

PMID: 16756752 [PubMed - indexed for MEDLINE]

Sanguin H, Remenant B, Dechesne A, Thioulouse J, Vogel TM, Nesme X, Moenne-Loccoz Y, Grundmann GL. (2006) Potential of a 16S rRNA-based taxonomic microarray for analyzing the rhizosphere effects of maize on *Agrobacterium* spp. and bacterial communities. Appl Environ Microbiol. 72(6):4302-12.

Bacterial diversity is central to ecosystem sustainability and soil biological function, for which the role of roots is important. The high-throughput analysis potential of taxonomic microarray should match the breadth of bacterial diversity. Here, the power of this technology was evidenced through methodological verifications and analysis of maize rhizosphere effect based on a 16S rRNA-based microarray developed from the prototype of H. Sanguin et al. (Environ. Microbiol. 8:289-307, 2006). The current probe set was composed of 170 probes (41 new probes in this work) that targeted essentially the *Proteobacteria*. Cloning and sequencing of 16S rRNA amplicons were carried out on maize rhizosphere and bulk soil DNA. All tested clones that had a perfect match with corresponding probes were positive in the hybridization experiment. The hierarchically nested probes were reliable, but the level of taxonomic identification was variable, depending on the probe set specificity. The comparison of experimental and theoretical hybridizations revealed 0.91% false positives and 0.81% false negatives. The microarray detection threshold was estimated at 0.03% of a given DNA type based on DNA spiking experiments. A comparison of the maize rhizosphere and bulk soil hybridization results showed a significant rhizosphere effect, with a higher predominance of *Agrobacterium* spp. in the rhizosphere, as well as a lower prevalence of *Acidobacteria*, *Bacteroidetes*, *Verrucomicrobia*, and *Planctomycetes*, a new taxon of interest in soil. In addition, well-known taxonomic groups such as *Sphingomonas* spp., *Rhizobiaceae*, and *Actinobacteria* were identified in both microbial habitats with strong hybridization signals. The taxonomic microarray developed in the present study was able to discriminate and characterize bacterial community composition in related biological samples, offering extensive possibilities for systematic exploration of bacterial diversity in ecosystems.

PMID: 16751545 [PubMed - indexed for MEDLINE]

Cassone M, D'Andrea MM, Iannelli F, Oggioni MR, Rossolini GM, Pozzi G. (2006) DNA microarray for detection of macrolide resistance genes. Antimicrob Agents Chemother. 50(6):2038-41.

A DNA microarray was developed to detect bacterial genes conferring resistance to macrolides and related antibiotics. A database containing 65 nonredundant genes selected from publicly available DNA sequences was constructed and used to design 100 oligonucleotide probes that could specifically detect and discriminate all 65 genes. Probes were spotted on a glass slide, and the array was reacted with DNA templates extracted from 20 reference strains of eight different bacterial species (*Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus, Staphylococcus haemolyticus, Escherichia coli,* and *Bacteroides fragilis*) known to harbor 29 different macrolide resistance genes. Hybridization results showed that probes reacted with, and only with, the expected DNA templates and allowed discovery of three unexpected genes, including msr(SA) in *B. fragilis*, an efflux gene that has not yet been described for gram-negative bacteria.

PMID: 16723563 [PubMed - indexed for MEDLINE]

Bruant G, Maynard C, Bekal S, Gaucher I, Masson L, Brousseau R, Harel J. (2006) Development and validation of an oligonucleotide microarray for detection of multiple virulence and antimicrobial resistance genes in *Escherichia coli*. Appl Environ Microbiol. 72(5):3780-4.

An oligonucleotide microarray detecting 189 *Escherichia coli* virulence genes or markers and 30 antimicrobial resistance genes was designed and validated using DNA from known reference strains. This microarray was confirmed to be a powerful diagnostic tool for monitoring emerging *E. coli* pathotypes and antimicrobial resistance, as well as for environmental, epidemiological, and phylogenetic studies including the evaluation of genome plasticity.

PMID: 16672535 [PubMed - indexed for MEDLINE]

Wurdemann C, Peplies J, Schubbe S, Ellrott A, Schuler D, Glockner FO. (2006) Evaluation of gene expression analysis using RNA-targeted partial genome arrays. Syst Appl Microbiol. 29(5):349-57.

Highly parallel cDNA targeting microarrays have been established over the last years as the quasi-standard for genome wide expression profiling in pro- and eukaryotes. Protocols for the direct detection of RNA or aRNA (amplified RNA) are currently emerging. This allows to circumvent the bias introduced by enzymatic target molecule preparation. To systematically evaluate the extent of non-specific target binding on oligonucleotide microarrays designed for total RNA expression profiling, a model system of 70-mer probes targeting genes involved in magnetosome formation (mam genes) of the bacterium *Magnetospirillum gryphiswaldense* was established utilizing wild-type strain

MSR-1 and an isogenic deletion mutant MSR-1B that lacks all mam genes. An optimized protocol for the direct chemical labelling of total cellular RNAs was used. A linear correlation between the amount of applied RNA and the mean global background intensity was found which enables a simple and unbiased way of normalizing the data. The results obtained with the mam deletion mutant MSR-1B revealed a significant number of false positive signals, even under optimal hybridization conditions. This indicates a high degree of non-specific binding in microarray experiments when using longer oligo- or polynucleotides and RNA as target molecule. Comparative microarray analysis of an MSR-1B culture and two MSR-1 wild-type cultures grown under different conditions was done via a three-colour hybridization assay. The additional information provided by the MSR-1B transcriptome revealed differential gene expression in the two MSR-1 cultures, which was otherwise undetectable.

PMID: 16644169 [PubMed - indexed for MEDLINE]

Chou CC, Lee TT, Chen CH, Hsiao HY, Lin YL, Ho MS, Yang PC, Peck K. (2006) Design of microarray probes for virus identification and detection of emerging viruses at the genus level. BMC Bioinformatics. 7:232.

BACKGROUND: Most virus detection methods are geared towards the detection of specific single viruses or just a few known targets, and lack the capability to uncover the novel viruses that cause emerging viral infections. To address this issue, we developed a computational method that identifies the conserved viral sequences at the genus level for all viral genomes available in GenBank, and established a virus probe library. The virus probes are used not only to identify known viruses but also for discerning the genera of emerging or uncharacterized ones. RESULTS: Using the microarray approach, the identity of the virus in a test sample is determined by the signals of both genus and species-specific probes. The genera of emerging and uncharacterized viruses are determined based on hybridization of the viral sequences to the conserved probes for the existing viral genera. A detection and classification procedure to determine the identity of a virus directly from detection signals results in the rapid identification of the virus. CONCLUSION: We have demonstrated the validity and feasibility of the above strategy with a small number of viral samples. The probe design algorithm can be applied to any publicly available viral sequence database. The strategy of using separate genus and species probe sets enables the use of a straightforward virus identity calculation directly based on the hybridization signals. Our virus identification strategy has great potential in the diagnosis of viral infections. The virus genus and specific probe database and the associated summary tables are available at genestamp.sinica.edu.tw/virus/index.htm.

PMID: 16643672 [PubMed - indexed for MEDLINE]

Goldschmidt MC. (2006) The use of biosensor and microarray techniques in the rapid detection and identification of salmonellae. J AOAC Int. 89(2):530-7.

The ever-present need for rapid and sensitive assay methods to detect foodborne pathogens, particularly the salmonellae, has led to increased incorporation of biosensor

technology into microarray and other platforms. The use of mimetics and aptamers has been added to these procedures. Nanoparticles, particularly incorporating fluorophores and quantum dots into various procedures, have decreased the size of instrumentation while increasing automation, sensitivity, and rapidity of results. This article will deal mainly with assays involving the salmonellae.

PMID: 16640303 [PubMed - indexed for MEDLINE]

Skovgaard K, Grell SN, Heegaard PM, Jungersen G, Pudrith CB, Coussens PM. (2006) Differential expression of genes encoding CD30L and P-selectin in cattle with Johne's disease: progress toward a diagnostic gene expression signature. Vet Immunol Immunopathol. 112(3-4):210-24.

Mycobacterium avium subspecies *paratuberculosis* (*Mycobacterium paratuberculosis*), the causative agent of paratuberculosis (paraTB) or Johne's disease in ruminants, is a health problem for the global cattle industry with significant economic losses related to decreased milk production and reduced fertility. Commonly paraTB in cattle is diagnosed by antibody detection by serum enzyme-linked immunosorbent assay (ELISA), by detection of the pathogen by cultivation of individual faecal samples, or by in vitro measurement of cell mediated immune responses using the IFN-gamma test. There is an ongoing need for developing new diagnostic approaches as all currently available diagnostic tests for paraTB may fail to detect sub-clinical infection. We used cDNA microarrays to simultaneously measure expression of over 1300 host genes to help identify a subset of gene expression changes that might provide a unique gene expression signature for paraTB infection. In the present study, non-stimulated leukocytes isolated from 10 sub-clinical paraTB infected cows were examined for genes being expressed at significantly different levels than in similar cells from control cows with the same herd background. We included cattle (Holstein) from two locations (Denmark and USA) for the microarray experiment. Our results indicate that expression profiles of at least 52 genes are different in leukocytes from *M. paratuberculosis* infected cattle compared to control cattle. Gene expression differences were verified by quantitative real-time reverse transcriptase polymerase chain reactions (qRT-PCR) on the same group of cattle (Holstein) used for the microarray experiment. In order to assess the generality of the observed gene expression, a second and different group of cattle (Jersey) was also examined using qRT-PCR. Out of the seven genes selected for qRT-PCR, CD30 ligand (CD30L) and P-selectin were consistently differentially expressed in freshly isolated leukocytes from paraTB infected and control animals of both breeds of cattle. Although further work is clearly needed to develop a more complete gene expression signature specific for paraTB, our results demonstrate that a subset of genes in leukocytes are consistently expressed at different levels, depending upon *M. paratuberculosis* infection status.

PMID: 16621022 [PubMed - indexed for MEDLINE]

Shepard JR. (2006) Polychromatic microarrays: simultaneous multicolor array hybridization of eight samples. Anal Chem. 78(8):2478-86.

High-throughput microscale platforms have transformed modern analytical investigations. Traditional microarray analyses involve a comparative approach, with two samples, a known control and an unknown sample, hybridized side-by-side and then contrasted for genetic differences. The samples are labeled with separate dyes and hybridized together, providing a differential expression pattern based on the reporter intensities. In contrast, the fiber-optic microarray platform described herein is analyzed with a microscope, thereby enabling the use of virtually any reporter, including quantum dots. The instrumentation takes advantage of the narrow emission bands characteristic of quantum dots to perform multiplexed detection of *Bacillus anthracis*. Advancing beyond the standard red/green microarray experiment, a panel of eight reporters were linked to eight *B. anthracis* samples and simultaneously analyzed in a microarray format. The ability to employ an assortment of reporters, along with the capacity to simultaneously hybridize eight samples confers an unprecedented flexibility to array-based analyses, providing a 4-fold increase in throughput over standard two-color assays.

PMID: 16615753 [PubMed - indexed for MEDLINE]

Aragon LM, Navarro F, Heiser V, Garrigo M, Espanol M, Coll P. (2006) Rapid detection of specific gene mutations associated with isoniazid or rifampicin resistance in *Mycobacterium tuberculosis* clinical isolates using non-fluorescent low-density DNA microarrays. J Antimicrob Chemother. 57(5):825-31.

BACKGROUND: A new, fast 'low cost and density' DNA microarray (LCD array), designed for the detection of mutations that confer isoniazid or rifampicin resistance in Mycobacterium tuberculosis isolates, has been developed and was evaluated using 46 resistant clinical isolates from Barcelona. METHODS: LCD chips are pre-structured polymer supports using a non-fluorescent detection principle based on the precipitation of a clearly visible dark substrate. One LCD chip consists of eight identical microarrays, designed to detect mutations within the 90 bp rpoB region, codon 315 in the katG gene and the mabA-inhA regulatory region. A total of 22 strains with a katG 315 mutation, 19 strains with alterations in the mabA-inhA regulatory region and 16 strains with mutations in the rpoB region, characterized previously, were studied. RESULTS: The identification of S315T and S315N mutations using the LCD was 100% concordant with the sequencing data. A strain with the S315R mutation, which is not tiled on the LCD array, was detected by the absence of hybridization using the wild-type probe. Of 19 strains with low-level isoniazid resistance related to the mabA-inhA regulatory region, 18 were identified correctly. The detection of mutations in the rpoB region was 93.8% concordant with the sequencing data. One mabA-inhA and rpoB mutated strain showed a cross-hybridization. CONCLUSIONS: The LCD array protocol takes 45 min (15 min 'hands-on' time) after prior PCR amplification. Only minimal laboratory equipment is required. LCD arrays provide a rapid and economical method to characterize mutations in codon 315 of the katG gene, in the mabA-inhA regulatory region and in the rpoB gene.

PMID: 16547071 [PubMed - indexed for MEDLINE]

Ryu M, Kim JD, Min BG, Kim J, Kim YY. (2006) Probe classification of on-off type DNA microarray images with a nonlinear matching measure. J Biomed Opt. 11(1):014027.

We propose a nonlinear matching measure, called counting measure, as a signal detection measure that is defined as the number of on pixels in the spot area. It is applied to classify probes for an on-off type DNA microarray, where each probe spot is classified as hybridized or not. The counting measure also incorporates the maximum response search method, where the expected signal is obtained by taking the maximum among the measured responses of the various positions and sizes of the spot template. The counting measure was compared to existing signal detection measures such as the normalized covariance and the median for 2390 patient samples tested on the human papillomavirus (HPV) DNA chip. The counting measure performed the best regardless of whether or not the maximum response search method was used. The experimental results showed that the counting measure combined with the positional search was the most preferable.
PMID: 16526904 [PubMed - indexed for MEDLINE]

Frye JG, Jesse T, Long F, Rondeau G, Porwollik S, McClelland M, Jackson CR, Englen M, Fedorka-Cray PJ. (2006) DNA microarray detection of antimicrobial resistance genes in diverse bacteria. Int J Antimicrob Agents. 27(2):138-51.

High throughput genotyping is essential for studying the spread of multiple antimicrobial resistance. A test oligonucleotide microarray designed to detect 94 antimicrobial resistance genes was constructed and successfully used to identify antimicrobial resistance genes in control strains. The microarray was then used to assay 51 distantly related bacteria, including Gram-negative and Gram-positive isolates, resulting in the identification of 61 different antimicrobial resistance genes in these bacteria. These results were consistent with their known gene content and resistance phenotypes. Microarray results were confirmed by polymerase chain reaction and Southern blot analysis. These results demonstrate that this approach could be used to construct a microarray to detect all sequenced antimicrobial resistance genes in nearly all bacteria.
PMID: 16427254 [PubMed - indexed for MEDLINE]

Sanguin H, Herrera A, Oger-Desfeux C, Dechesne A, Simonet P, Navarro E, Vogel TM, Moenne-Loccoz Y, Nesme X, Grundmann GL. (2006) Development and validation of a prototype 16S rRNA-based taxonomic microarray for *Alphaproteobacteria*. Environ Microbiol. 8(2):289-307.

The microarray approach has been proposed for high throughput analysis of the microbial community by providing snapshots of the microbial diversity under different environmental conditions. For this purpose, a prototype of a 16S rRNA-based taxonomic microarray was developed and evaluated for assessing bacterial community diversity. The prototype microarray is composed of 122 probes that target bacteria at various

taxonomic levels from phyla to species (mostly *Alphaproteobacteria*). The prototype microarray was first validated using bacteria in pure culture. Differences in the sequences of probes and potential target DNAs were quantified as weighted mismatches (WMM) in order to evaluate hybridization reliability. As a general feature, probes having a WMM > 2 with target DNA displayed only 2.8% false positives. The prototype microarray was subsequently tested with an environmental sample, which consisted of an *Agrobacterium*-related polymerase chain reaction amplicon from a maize rhizosphere bacterial community. Microarray results were compared to results obtained by cloning-sequencing with the same DNA. Microarray analysis enabled the detection of all 16S rRNA gene sequences found by cloning-sequencing. Sequences representing only 1.7% of the clone library were detected. In conclusion, this prototype 16S rRNA-based taxonomic microarray appears to be a promising tool for the analysis of *Alphaproteobacteria* in complex ecosystems.

PMID: 16423016 [PubMed - indexed for MEDLINE]

Palmer C, Bik EM, Eisen MB, Eckburg PB, Sana TR, Wolber PK, Relman DA, Brown PO. (2006) Rapid quantitative profiling of complex microbial populations. Nucleic Acids Res. 34(1):e5.

Diverse and complex microbial ecosystems are found in virtually every environment on earth, yet we know very little about their composition and ecology. Comprehensive identification and quantification of the constituents of these microbial communities--a 'census'--is an essential foundation for understanding their biology. To address this problem, we developed, tested and optimized a DNA oligonucleotide microarray composed of 10,462 small subunit (SSU) ribosomal DNA (rDNA) probes (7167 unique sequences) selected to provide quantitative information on the taxonomic composition of diverse microbial populations. Using our optimized experimental approach, this microarray enabled detection and quantification of individual bacterial species present at fractional abundances of <0.1% in complex synthetic mixtures. The estimates of bacterial species abundance obtained using this microarray are similar to those obtained by phylogenetic analysis of SSU rDNA sequences from the same samples--the current 'gold standard' method for profiling microbial communities. Furthermore, probes designed to represent higher order taxonomic groups of bacterial species reliably detected microbes for which there were no species-specific probes. This simple, rapid microarray procedure can be used to explore and systematically characterize complex microbial communities, such as those found within the human body.

PMID: 16407321 [PubMed - indexed for MEDLINE]

Garrido P, Blanco M, Moreno-Paz M, Briones C, Dahbi G, Blanco J, Blanco J, Parro V. (2006) STEC-EPEC oligonucleotide microarray: a new tool for typing genetic variants of the LEE pathogenicity island of human and animal Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains. Clin Chem. 52(2):192-201.

BACKGROUND: Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) are important emerging pathogens that can cause a severe and sometimes fatal illness. Differentiation of eae, tir, espA, espD, and espB gene variants of the locus of enterocyte effacement (LEE) pathogenicity island represents an important tool for typing in routine diagnostics as well as in pathogenesis, epidemiologic, clonal, and immunologic studies. METHODS: Type-specific oligonucleotide microarrays and a PCR scheme were designed and constructed for the detection and typing of genetic variants of the LEE genes. Oligonucleotide probes were tested for their specificity against the corresponding type strain by microarray hybridization using fluorescent DNA, either PCR-amplified (single, multiplex, long-range), chromosomal, or amplified chromosomal DNA. RESULTS: The PCR scheme and the oligonucleotide microarray allowed us to distinguish 16 variants (alpha1, alpha2, beta1, beta2, gamma1, gamma2/theta, delta/kappa, epsilon, zeta, eta, iota, lambda, mu, nu, xi, omicron) of the eae gene, 4 variants (alpha1, beta1, gamma1, gamma2/theta) of the tir gene, 4 variants (alpha1, beta1, beta2, gamma1) of the espA gene, 3 variants (alpha1, beta1, gamma1) of the espB gene, and 3 variants (alpha1, beta1, gamma1) of the espD gene. We found a total of 12 different combinations of tir, espA, espB, and espD genes among the 25 typed strains. CONCLUSIONS: The PCR scheme and the oligonucleotide microarray described are effective tools to rapidly screen multiple virulence genes and their variants in *E. coli* strains isolated from human and animal infections. The results demonstrate the great genetic diversity among LEE genes of human and animal STEC and EPEC strains.

PMID: 16384888 [PubMed - indexed for MEDLINE]

Li C, Chen RS, Hung SK, Lee YT, Yen CY, Lai YW, Teng RH, Huang JY, Tang YC, Tung CP, Wei TT, Shieh B, Liu ST. (2006) Detection of Epstein-Barr virus infection and gene expression in human tumors by microarray analysis. J Virol Methods. 133(2):158-66.

Epstein-Barr virus (EBV) genome-chips are employed to determine the EBV infection rate and to reveal the gene expression patterns of EBV in tumor biopsies. These chips are produced with 71 consecutive PCR-amplified EBV DNA fragments of 1-3 kbp covering the entire EBV genome. The specificity of the EBV-chips is determined by hybridizing the DNA on the chips with biotin-labeled cDNA probes reverse transcribed from the mRNA of P3HR1 cells, which were B-cell infected latently by EBV. Hybridization results revealed only the expression of EBNA1, EBNA2, EBER1 and EBER2 in these cells. On the other hand, EBV lytic genes are expressed after the cells are treated with 12-O-tetradecanoylphorbol-13-acetate and sodium butyrate to induce the EBV lytic cycle. Fourty-four tumor biopsies from different organs are assayed with these chips, which showed many defined and interesting EBV gene expression patterns. This study demonstrates that the EBV-chip is useful for screening infection with EBV in tumors, which may lead to insights into tumorigenesis associated with this virus.

PMID: 16384612 [PubMed - indexed for MEDLINE]

Seo SS, Song YS, Kim JW, Park NH, Kang SB, Lee HP. (2006) Good correlation of HPV DNA test between self-collected vaginal and clinician-collected cervical samples by the oligonucleotide microarray. Gynecol Oncol. 102(1):67-73.

OBJECTIVES: To evaluate the efficacy of self-collected vaginal samples for high-risk HPV detection by the HPV oligonucleotide microarray method (HPVDNAChip). METHODS: One hundred and eighteen patients with abnormal Pap smears were included. Self-collected vaginal and clinician-collected cervical samples for HPV testing were obtained. The result of the HPV DNA test was compared with the histopathological diagnosis or colposcopic finding. RESULTS: Of the 118 patients, 42 (35.6%) had >or= cervical intraepithelial neoplasia (CIN) III lesions. Using the HPVDNAChip, high-risk types of HPV were detected in 38 of these 42 patients (90.5%) with the self-collected vaginal samples and in 37 of 42 (88.1%) with the clinician-collected cervical samples. The agreement of HPVDNAchip results between self- and clinician-collected samples was very good (kappa = 0.81) with a 93.2% concordance rate. Multiple HPV infections were found in 17 of 88 (19.3%) HPV-positive clinician-collected cervical samples. The rate of multiple HPV infection tended to decrease as the degree of pathologic classification increased. CONCLUSION: Using the HPVDNAchip to assay for HPV infection, results from self-collected vaginal samples were compatible with those from clinician-collected cervical samples.

PMID: 16375952 [PubMed - indexed for MEDLINE]

Tobler NE, Pfunder M, Herzog K, Frey JE, Altwegg M. (2006) Rapid detection and species identification of *Mycobacterium* spp. using real-time PCR and DNA-microarray. J Microbiol Methods. 66(1):116-24.

Infections with mycobacteria are an important issue in public health care. Here we present a "proof-of-principle" concept for the identification of 37 different *Mycobacterium* species using 5' exonuclease real-time PCR and DNA microarray based on the region upstream of the 65 kDa heat shock protein. With our two PCR probes, one complementary to all mycobacteria species, the other specific for the M. tbc-complex, 34 species were properly classified by real-time PCR. After reamplification and hybridization to a DNA microarray, all species showed a specific pattern. All 10 blindly tested positive cultures revealed a positive real-time PCR signal with the genus probe. After reamplification and hybridization, six samples could unambiguously be identified. One sample showed a mixture of presumably three species-specific patterns and sequencing the 16S rRNA confirmed the presence of a mixture. The hybridization results of three specimens could not be interpreted because the signal to background ratio was not sufficient. Two samples considered as negative controls (LAL Reagent Water (Cambrex) and DNA of *Candida albicans*) gave neither a genus nor a M. tbc-complex positive PCR signal. Based on these results we consider our method to be a promising tool for the rapid identification of different mycobacteria species, with the advantage of possible identification of mixed infections or contaminations.

PMID: 16360893 [PubMed - indexed for MEDLINE]

Vora GJ, Meador CE, Bird MM, Bopp CA, Andreadis JD, Stenger DA. (2005) Microarray-based detection of genetic heterogeneity, antimicrobial resistance, and the viable but nonculturable state in human pathogenic Vibrio spp. Proc Natl Acad Sci USA. 102(52):19109-14.

The morbidity and mortality associated with *Vibrio*-mediated waterborne diseases necessitates the development of sensitive detection technologies that are able to elucidate the identity, potential pathogenicity, susceptibility, and viability of contaminating bacteria in a timely manner. For this purpose, we have designed a single multiplex PCR assay to simultaneously amplify 95 diagnostic regions (encompassing species/serogroup-specific, antimicrobial resistance, and known toxin markers) and combined it with a long oligonucleotide microarray to create a platform capable of rapidly detecting and discriminating the major human pathogenic species from the genus *Vibrio*: *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. mimicus*. We were able to validate this strategy by testing 100 geographically and temporally distributed isolates and observed an excellent concordance between species- and serotype-level microarray-based identification and traditional typing methods. In addition to accurate identification, the microarray simultaneously provided evidence of antibiotic resistance genes and mobile genetic elements, such as sulfamethoxazole-trimethoprim constins and class I integrons, and common toxin (ctxAB, rtxA, hap, hlyA, tl, tdh, trh, vvhA, vlly, and vmhA) and pathogenicity (tcpA, type III secretion system) genes that are associated with pathogenic *Vibrio*. The versatility of this method was further underscored by its ability to detect the expression of known toxin and virulence genes from potentially harmful viable but nonculturable organisms. The results suggest that this molecular identification method provides rapid and definitive information that would be of value in epidemiological, environmental, and health risk assessment surveillance.

PMID: 16354840 [PubMed - indexed for MEDLINE]

Maynard C, Berthiaume F, Lemarchand K, Harel J, Payment P, Bayardelle P, Masson L, Brousseau R. (2005) Waterborne pathogen detection by use of oligonucleotide-based microarrays. Appl Environ Microbiol. 71(12):8548-57.

A small-oligonucleotide microarray prototype was designed with probes specific for the universal 16S rRNA and cpn60 genes of several pathogens that are usually encountered in wastewaters. In addition to these two targets, wecE-specific oligonucleotide probes were included in the microarray to enhance its discriminating power within the *Enterobacteriaceae* family. Universal PCR primers were used to amplify variable regions of 16S rRNA, cpn60, and wecE genes directly in *Escherichia coli* and *Salmonella enterica* serovar *Typhimurium* genomic DNA mixtures (binary); *E. coli*, *S. enterica* serovar Typhimurium, and *Yersinia enterocolitica* genomic DNA mixtures (ternary); or wastewater total DNA. Amplified products were fluorescently labeled and hybridized on the prototype chip. The detection sensitivity for *S. enterica* serovar Typhimurium was estimated to be on the order of 0.1% (10(4) *S. enterica* genomes) of the total DNA for the combination of PCR followed by microarray

hybridization. The sensitivity of the prototype could be increased by hybridizing amplicons generated by PCR targeting genes specific for a bacterial subgroup, such as wecE genes, instead of universal taxonomic amplicons. However, there was evidence of PCR bias affecting the detection limits of a given pathogen as increasing amounts of a different pathogen were spiked into the test samples. These results demonstrate the feasibility of using DNA microarrays in the detection of waterborne pathogens within mixed populations but also raise the problem of PCR bias in such experiments.

PMID: 16332846 [PubMed - indexed for MEDLINE]

Ehricht R, Slickers P, Goellner S, Hotzel H, Sachse K. (2006) Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol Cell Probes. 20(1):60-3.

This study was conducted to determine the detection limit of an optimized DNA microarray assay for detection and species identification of chlamydiae. Examination of dilution series of a plasmid standard carrying the target sequence from *Chlamydia trachomatis* and genomic DNA of this organism revealed that a single PCR-amplifiable target copy was sufficient to obtain a specific hybridization pattern. This performance renders the test suitable for routine testing of clinical samples.

PMID: 16330186 [PubMed - indexed for MEDLINE]

Myers KM, Gaba J, Al-Khaldi SF. (2006) Molecular identification of *Yersinia enterocolitica* isolated from pasteurized whole milk using DNA microarray chip hybridization. Mol Cell Probes. 20(2):71-80.

A DNA microarray chip of four virulence genes and 16S ribosomal DNA gene conserved region among all Gram negative species, including *Yersinia*, as a positive control was developed and evaluated using 22 *Yersinia enterocolitica* isolates. Eight different oligonucleotide probes (oligoprobes) with an average size of 22 bp, complementary to the unique sequences of each gene, were designed and immobilized on the surface of chemically modified slides. Multiplex PCR was used to simultaneously amplify DNA target regions of all five genes, and single stranded DNA (ssDNA) samples for microarray analysis were prepared by using a primer extension of amplicons in the presence of one primer of all genes. The presence of genes in *Y. enterocolitica* was established by hybridization of the fluorescently labeled ssDNA representing different samples of the microarray gene-specific oligoprobes and confirmed by PCR. Results of the study showed specificity of genotyping *Y. enterocolitica* using multiple microarray-based assays. Final validation of the chip's ability to identify *Y. enterocolitica* genes from adulterated pasteurized whole milk was confirmed and successful. The limit of chip detection of virulence genes in pasteurized whole milk was found to be 1000 CFU per hybridization.

PMID: 16330182 [PubMed - indexed for MEDLINE]

Jin HY, Tao KH, Li YX, Li FQ, Li SQ. (2005) Microarray analysis of *Escherichia coli* O157:H7. World J Gastroenterol. 11(37):5811-5.

AIM: To establish the rapid, specific, and sensitive method for detecting O157:H7 with DNA microchips. METHODS: Specific oligonucleotide probes (26-28 nt) of bacterial antigenic and virulent genes of *E. coli* O157:H7 and other related pathogen genes were pre-synthesized and immobilized on a solid support to make microchips. The four genes encoding O157 somatic antigen (rfbE), H7 flagellar antigen (fliC) and toxins (SLT1, SLT2) were monitored by multiplex PCR with four pairs of specific primers. Fluorescence-Cy3 labeled samples for hybridization were generated by PCR with Cy3-labeled single prime. Hybridization was performed for 60 min at 45 degrees. Microchip images were taken using a confocal fluorescent scanner. RESULTS: Twelve different bacterial strains were detected with various combinations of four virulent genes. All the O157:H7 strains yielded positive results by multiplex PCR. The size of the PCR products generated with these primers varied from 210 to 678 bp. All the rfbE/fliC/SLT1/SLT2 probes specifically recognized Cy3-labeled fluorescent samples from O157:H7 strains, or strains containing O157 and H7 genes. No cross hybridization of O157:H7 fluorescent samples occurred in other probes. Non-O157:H7 pathogens failed to yield any signal under comparable conditions. If the Cy3-labeled fluorescent product of O157 single PCR was diluted 50-fold, no signal was found in agarose gel electrophoresis, but a positive signal was found in microarray hybridization. CONCLUSION: Microarray analysis of O157:H7 is a rapid, specific, and efficient method for identification and detection of bacterial pathogens.

PMID: 16270390 [PubMed - indexed for MEDLINE]

Lee DY, Shannon K, Beaudette LA. (2006) Detection of bacterial pathogens in municipal wastewater using an oligonucleotide microarray and real-time quantitative PCR. J Microbiol Methods. 65(3):453-67.

As a first step toward building a comprehensive microarray, two low density DNA microarrays were constructed and evaluated for the accurate detection of wastewater pathogens. The first one involved the direct hybridization of wastewater microbial genomic DNA to the functional gene probes while the second involved PCR amplification of 23S ribosomal DNA. The genomic DNA microarray employed 10 functional genes as detection targets. Sensitivity of the microarray was determined to be approximately 1.0 microg of *Esherichia coli* genomic DNA, or 2 x 10(8) copies of the target gene, and only *E. coli* DNA was detected with the microarray assay using municipal raw sewage. Sensitivity of the microarray was enhanced approximately by 6 orders of magnitude when the target 23S rRNA gene sequences were PCR amplified with a novel universal primer set and allowed hybridization to 24 species-specific oligonucleotide probes. The minimum detection limit was estimated to be about 100 fg of *E. coli* genomic DNA or 1.4 x 10(2) copies of the 23S rRNA gene. The PCR amplified DNA microarray successfully detected multiple bacterial pathogens in wastewater. As a parallel study to verify efficiency of the DNA microarray, a real-time quantitative PCR assay was also developed based on the fluorescent TaqMan probes (Applied Biosystems).

PMID: 16239042 [PubMed - indexed for MEDLINE]

Francois P, Charbonnier Y, Jacquet J, Utinger D, Bento M, Lew D, Kresbach GM, Ehrat M, Schlegel W, Schrenzel J. (2006) Rapid bacterial identification using evanescent-waveguide oligonucleotide microarray classification. J Microbiol Methods. 65(3):390-403.

Bacterial identification relies primarily on culture-based methodologies and requires 48-72 h to deliver results. We developed and used i) a bioinformatics strategy to select oligonucleotide signature probes, ii) a rapid procedure for RNA labelling and hybridization, iii) an evanescent-waveguide oligoarray with exquisite signal/noise performance, and iv) informatics methods for microarray data analysis. Unique 19-mer signature oligonucleotides were selected in the 5'-end of 16s rDNA genes of human pathogenic bacteria. Oligonucleotides spotted onto a Ta(2)O(5)-coated microarray surface were incubated with chemically labelled total bacterial RNA. Rapid hybridization and stringent washings were performed before scanning and analyzing the slide. In the present paper, the eight most abundant bacterial pathogens representing >54% of positive blood cultures were selected. Hierarchical clustering analysis of hybridization data revealed characteristic patterns, even for closely related species. We then evaluated artificial intelligence-based approaches that outperformed conventional threshold-based identification schemes on cognate probes. At this stage, the complete procedure applied to spiked blood cultures was completed in less than 6 h. In conclusion, when coupled to optimal signal detection strategy, microarrays provide bacterial identification within a few hours post-sampling, allowing targeted antimicrobial prescription.

PMID: 16216356 [PubMed - indexed for MEDLINE]

Nagaoka T, Horii T, Satoh T, Ito T, Monji A, Takeshita A, Maekawa M. (2005) Use of a three-dimensional microarray system for detection of levofloxacin resistance and the mec A gene in *Staphylococcus aureus*. J Clin Microbiol. 43(10):5187-94.

We evaluated a novel three-dimensional microarray (Pam Chip microarray) system to detect the presence of levofloxacin-related resistance mutations and the mecA gene. The results were compared to those obtained for 27 *Staphylococcus aureus* isolates by conventional DNA sequencing or PCR methods. Hybridization and fluorescence detection were performed using an FD 10 system designed for Pam Chip microarray under conditions optimized for each target/probe on the array. In dilution series analysis using multiplex PCR samples, the sensitivity of the microarray was about 10 times greater than that of conventional PCR methods. A high level of data reproducibility was also confirmed in those analyses. Various point mutations in quinolone resistance-determining regions detected by our system corresponded perfectly to the results obtained by conventional DNA sequencing. The results of the mec A gene detection using our system also corresponded to the PCR method; that is, signal/band was detected in all

isolates of methicillin-resistant *S. aureus*, and no signal/band was detected in any isolate of methicillin-susceptible *S. aureus*. In conclusion, our novel three-dimensional microarray system provided rapid, specific, easy, and reproducible results for the simultaneous detection of levofloxacin resistance and the mec A gene in *S. aureus*.

PMID: 16207982 [PubMed - indexed for MEDLINE]

Zhu P, Shelton DR, Karns JS, Sundaram A, Li S, Amstutz P, Tang CM. (2005) Detection of water-borne *E. coli* O157 using the integrating waveguide biosensor. Biosens Bioelectron. 21(4):678-83.

Escherichia coli O157:H7, the most common serotype of enterohemorrhagic *E. coli* (EHEC), is responsible for numerous food-borne and water-borne infections worldwide. An integrating waveguide biosensor is described for the detection of water-borne *E. coli* O157, based on a fluorescent sandwich immunoassay performed inside a glass capillary waveguide. The genomic DNA of captured *E. coli* O157 cells was extracted and quantitative real-time PCR subsequently performed to assess biosensor-capture efficiency. In vitro microbial growth in capillary waveguide is also documented. The biosensor allows for quantitative detection of as few as 10 cells per capillary (0.075 ml volume) and can be used in conjunction with cell amplification, PCR and microarray technologies to positively identify a pathogen.

PMID: 16202883 [PubMed - indexed for MEDLINE]

Borden JR, Paredes CJ, Papoutsakis ET. (2005) Diffusion, mixing, and associated dye effects in DNA-microarray hybridizations. Biophys J. 89(5):3277-84.

Typical DNA microarrays utilize diffusion of dye-labeled cDNA probes followed by sequence-specific hybridization to immobilized targets. Here we experimentally estimated the distance typical probes travel during static 16-h hybridizations. Probes labeled with Cy3 and Cy5 were individually introduced to opposite sides of a microarray with minimal convective mixing. Oppositely labeled probes diffused across the initial front separating the two solutions, generating a zone with both dyes present. Diffusion-distance estimates for Cy3- and Cy5-labeled cDNAs were 3.8 mm and 2.6 mm, respectively, despite having almost identical molecular masses. In separate 16-h hybridization experiments with oppositely labeled probes premixed, arrays that were continuously mixed had 15-20% higher signal intensities than arrays hybridized statically. However, no change was observed in the Cy3/Cy5 signal intensity ratio between continuously mixed and static hybridizations. This suggests that the observed dye bias in diffusion-distance estimates results from differences in the detection limits of Cy3 and Cy5-labeled cDNA, a potential concern for array data on low-abundance transcripts. Our conservative diffusion-distance estimates indicate that replicate targets >7.6 mm apart will not compete for scarce probes. Also, raising the microarray gap height would delay the onset of diffusion-limited hybridization by increasing the amount of available probe.

PMID: 16100268 [PubMed - indexed for MEDLINE]

Liu M, Liu H, Sun L, Dong J, Xue Y, Chen S, Jin Q. (2005) Construction, detection and microarray analysis on the *Shigella flexneri* 2a sitC mutant. Sci China C Life Sci. 48(3):228-40.

In order to overcome the defects of difficult gene operations in low-copy suicide plasmid pCVD442, Gateway technology was applied in the construction process of recombinant plasmid for gene knockout in this study. With this improved knockout system, we inactivated sitC gene, which is associated with iron transport in *Shigella flexneri* 2a strain 301, to yield the mutant, MTS. The functional detection of the mutant was performed at the level of culture medium, cell and animal experiment, respectively. The gene expression profiles were compared with DNA microarray between the mutant and the wild type under iron-restricted conditions. The results showed that MTS grew obviously less well than the wild-type strains in L broth containing 150 micromol/L iron chelator DIP (2,2'-dipyridyl). Addition of iron or manganese to the cultures stimulated the growth of MTS to wild-type levels in rich culture medium. In either the experiment on the ability of intracellular multiplication and cell-to-cell spread in HeLa and U937 cell lines, or the experiment on keratoconjunctivitis in guinea pigs, MTS showed no obvious changes in virulence compared with the parental strain Sf301. When 65 micromol/L DIP was added to the cultured HeLa cells, the ability of intracellular multiplication of MTS reduced about 51.6% as compared with that of Sf301. The analysis of expression profiles under iron-limited condition showed that MTS was more sensitive for the change of iron deficiency than Sf301. There are 106 more up-regulated genes in MTS than in wild-type strains, which are involved in membrane transportation, amino acid metabolism and uncategorized function genes, while down-regulated genes are mainly involved in energy and carbohydrate metabolism. Under low iron conditions, the expression levels of known iron-transport associated genes generally increased. Additionally, the number of these genes and their increase amplitude in MTS are more than those in Sf301. Together, these results confirmed that Sit iron-transport system is important for the growth of *Shigella*.

PMID: 16092755 [PubMed - indexed for MEDLINE]

Franke-Whittle IH, Klammer SH, Mayrhofer S, Insam H. (2006) Comparison of different labeling methods for the production of labeled target DNA for microarray hybridization. J Microbiol Methods. 65(1):117-26.

Different labeling methods were studied to compare various approaches to the preparation of labeled target DNA for microarray experiments. The methods under investigation included a post-PCR labeling method using the Klenow fragment and a DecaLabel DNA labeling kit, the use of a Cy3-labeled forward primer in the PCR, generating either double-stranded or single-stranded PCR products, and the incorporation of Cy3-labeled dCTPs in the PCR. A microarray that had already been designed and used for the detection of microorganisms in compost was used in the study. PCR products from the organisms *Burkholderia cepacia* and *Staphylococcus aureus* were used in the comparison study, and the signals from the probes for these organisms analyzed. The

highest signals were obtained when using the post-PCR labeling method, although with this method, more non-specific hybridizations were found. Single-stranded PCR products that had been labeled by the incorporation of a Cy3-labeled forward primer in the PCR were found to give the next highest signals upon hybridization for a majority of the tested probes, with less non-specific hybridizations. Hybridization with double-stranded PCR product labeled with a Cy3-labeled forward primer, or labeled by the incorporation of Cy3-labeled dCTPs resulted in acceptable signal to noise ratios for all probes except the UNIV 1389a and *Burkholderia* genus probes, both located toward the 3' end of the 16S rRNA gene. The comparison of the different DNA labeling methods revealed that labeling via the Cy3-forward primer approach is the most appropriate of the studied methods for the preparation of labeled target DNA for our purposes.

PMID: 16043246 [PubMed - indexed for MEDLINE]

Call DR. (2005) Challenges and opportunities for pathogen detection using DNA microarrays. Crit Rev Microbiol. 31(2):91-9.

DNA microarrays offer the potential for simultaneous detection of many pathogens that are of interest to homeland security, public health, medicine, and veterinary diagnostics. These tools are best suited for detecting the presence or absence of genetic sequences characteristic of specific pathogens, but microarrays are poorly suited for determining pathogen viability, and current methods provide only limited potential for pathogen enumeration. Two basic strategies have been described for pathogen detection: using enzymatic amplification to generate targets for interrogation with a microarray, or using direct interrogation of DNA or RNA without pre-amplification. Multiplex PCR has the advantage of a high degree of sensitivity and specificity, but associated microarrays are necessarily limited in scope. PCR-independent, whole-genome amplification eliminates biases inherent in PCR amplification and can accommodate more extensive microarrays, but assay sensitivity is compromised and these methods are probably of limited use when testing tissue samples. Direct hybridization of DNA or RNA provides the least bias in gene detection, but also the lowest level of analytic sensitivity. Ultimately, cost and limited sample throughput make it unlikely that planar microarrays will play a significant role in future pathogen detection schemes. Alternative microarray formats such as bead arrays, however, may circumvent the cost and throughput limitations and permit us to apply what we have learned from planar microarrays to develop robust pathogen detection systems. Assay validation and sample preparation will continue to be significant challenges for these detection systems.

PMID: 15988839 [PubMed - indexed for MEDLINE]

Li Y, Cu YT, Luo D. (2005) Multiplexed detection of pathogen DNA with DNA-based fluorescence nanobarcodes. Nat Biotechnol. 23(7):885-9.

Rapid, multiplexed, sensitive and specific molecular detection is of great demand in gene profiling, drug screening, clinical diagnostics and environmental analysis. One of the major challenges in multiplexed analysis is to identify each specific reaction with a

distinct label or 'code'. Two encoding strategies are currently used: positional encoding, in which every potential reaction is preassigned a particular position on a solid-phase support such as a DNA microarray, and reaction encoding, where every possible reaction is uniquely tagged with a code that is most often optical or particle based. The micrometer size, polydispersity, complex fabrication process and nonbiocompatibility of current codes limit their usability. Here we demonstrate the synthesis of dendrimer-like DNA-based, fluorescence-intensity-coded nanobarcodes, which contain a built-in code and a probe for molecular recognition. Their application to multiplexed detection of the DNA of several pathogens is first shown using fluorescence microscopy and dot blotting, and further demonstrated using flow cytometry that resulted in detection that was sensitive (attomole) and rapid.

PMID: 15951805 [PubMed - indexed for MEDLINE]

Perreten V, Vorlet-Fawer L, Slickers P, Ehricht R, Kuhnert P, Frey J. (2005) Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. J Clin Microbiol. 43(5):2291-302.

A disposable microarray was developed for detection of up to 90 antibiotic resistance genes in gram-positive bacteria by hybridization. Each antibiotic resistance gene is represented by two specific oligonucleotides chosen from consensus sequences of gene families, except for nine genes for which only one specific oligonucleotide could be developed. A total of 137 oligonucleotides (26 to 33 nucleotides in length with similar physicochemical parameters) were spotted onto the microarray. The microarrays (ArrayTubes) were hybridized with 36 strains carrying specific antibiotic resistance genes that allowed testing of the sensitivity and specificity of 125 oligonucleotides. Among these were well-characterized multidrug-resistant strains of *Enterococcus faecalis*, *Enterococcus faecium*, and *Lactococcus lactis* and an avirulent strain of *Bacillus anthracis* harboring the broad-host-range resistance plasmid pRE25. Analysis of two multidrug-resistant field strains allowed the detection of 12 different antibiotic resistance genes in a *Staphylococcus haemolyticus* strain isolated from mastitis milk and 6 resistance genes in a *Clostridium perfringens* strain isolated from a calf. In both cases, the microarray genotyping corresponded to the phenotype of the strains. The ArrayTube platform presents the advantage of rapidly screening bacteria for the presence of antibiotic resistance genes known in gram-positive bacteria. This technology has a large potential for applications in basic research, food safety, and surveillance programs for antimicrobial resistance.

PMID: 15872258 [PubMed - indexed for MEDLINE]

Kim BC, Park JH, Gu MB. (2005) Multiple and simultaneous detection of specific bacteria in enriched bacterial communities using a DNA microarray chip with randomly generated genomic DNA probes. Anal Chem. 77(8):2311-7.

A DNA microarray chip for detecting the presence of specific bacterial strains was developed using random genomic probes derived from genomic DNA, i.e., without any

sequence information. Thirteen bacteria from different genuses were selected as targets. For the fabrication of the random genomic probes, genomic DNA from pure cultures of each bacterium was fractionated using several pairs of restriction endonucleases. After size fractionation of the genomic DNA fragments, random genomic libraries for each bacterium were constructed. From the library, specific probes were amplified by PCR and the probes were affixed to a slide glass to fabricate the DNA microarray chip. The results from tests with pure and mixed cultures of the bacteria used in the fabrication of the chips showed specific responses and only a small portion of cross-hybridization. This DNA microarray chip was also tested to detect the presence of specific bacteria in mixed populations. In these tests, it was demonstrated that this system provided a fast and specific response to the presence of bacterial species in mixed samples, even in activated sludge samples. This indicates that any DNA microarray chip for the detection of specific bacteria can be fabricated using the same protocols as presented in this study without requiring any genus level sequence information from pure isolates.

PMID: 15828762 [PubMed - indexed for MEDLINE]

van Hoek AH, Scholtens IM, Cloeckaert A, Aarts HJ. (2005) Detection of antibiotic resistance genes in different *Salmonella* serovars by oligonucleotide microarray analysis. J Microbiol Methods. 62(1):13-23.

In this study the feasibility of 50- and 60-mer oligonucleotides in microarray analysis for the detection and identification of antibiotic resistance genes in various *Salmonella* strains was assessed. The specificity of the designed oligonucleotides was evaluated, furthermore the optimal spotting concentration was determined. The oligonucleotide microarray was used to screen two sets of *Salmonella* strains for the presence of several antibiotic resistance genes. Set 1 consisted of strains with variant Salmonella Genomic Island 1 (SGI1) multidrug resistance (MDR) regions of which the antibiotic resistance profiles and genotypes were known. The second set contained strains of which initially only phenotypic data were available. The microarray results of the first set of *Salmonella* strains perfectly matched with the phenotypic and genotypic information. The microarray data of the second set were almost completely in concordance with the available phenotypic data. It was concluded that the microarray technique in combination with random primed genomic labeling and 50- or 60-mer oligonucleotides is a powerful tool for the detection of antibiotic resistance genes in bacteria.

PMID: 15823391 [PubMed - indexed for MEDLINE]

Abdullahi I, Koerbler M, Stachewicz H, Winter S. (2005) The 18S rDNA sequence of *Synchytrium endobioticum* and its utility in microarrays for the simultaneous detection of fungal and viral pathogens of potato. Appl Microbiol Biotechnol. 68(3):368-75.

Resting spores extracted from wart (*Synchytrium endobioticum*)-infected potato tubers were used for DNA extraction and amplification of 18S rDNA. Analysis of the cloned, sequenced fragment revealed high similarity to members of the *Chytridiomycota*.

Using this information, specific oligonucleotide probes were designed and arrayed onto glass slides for detection of the pathogen. Viral sequence information available in the databank was retrieved, or new viral sequences were generated, and used to design probes for specific detection of important quarantine viruses of potato. To determine the sensitivity and specificity of the oligonucleotide probes, total RNA from infected plants was reverse transcribed, labelled with Cyanine 5, and hybridised with the microarray. A significant number of the oligonucleotide probes exhibited high specificity to *S. endobioticum*, Andean potato latent virus, Andean potato mottle virus, Potato black ringspot virus, and Potato spindle tuber viroid. Hybridisation signals of sub-arrays within slides were reproducible (r = 0.79) with a high correlation coefficient of hybridisation repetitions (0.73). Our results demonstrate the potential of microarray-based hybridisation for identification of multiple pathogen targets, which will find application in quarantine laboratories, where parallel testing for diverse pathogens is essential.

PMID: 15800764 [PubMed - indexed for MEDLINE]

Choi JW, Park KW, Lee DB, Lee W, Lee WH. (2005) Cell immobilization using self-assembled synthetic oligopeptide and its application to biological toxicity detection using surface plasmon resonance. Biosens Bioelectron. 20(11):2300-5.

The immobilized cell using self-assembled synthetic oligopeptide was applied to the biological toxicity detection of environmental pollutant. Thin films based on cysteine-terminated synthetic oligopeptides were fabricated for the immobilization of *Escherichia coli* O157:H7 on gold (Au) substrate. Layer formation and immobilization of *E. coli* O157:H7 were investigated with surface plasmon resonance (SPR) and atomic force microscopy (AFM). Experimental results showed that the thin film of cysteine-terminated synthetic oligopeptide was successfully fabricated and it could be applied for the immobilization of *E. coli* O157:H7. The attached living cell was exposed to toxic chemical such as phenol, which induced the change of SPR angle. As the exposed concentration of phenol was increased, the change of plasmon resonance angle was increased, which indicates the decrease of cell viability. The detection limit based on SPR was determined as 5 ppm. The proposed cell immobilization method using self-assembly technique can be applied to construct the cell microarray for the diagnosis, drug detection, and on-site monitoring.

PMID: 15797329 [PubMed - indexed for MEDLINE]

Wu L, Thompson DK, Liu X, Fields MW, Bagwell CE, Tiedje JM, Zhou J. (2004) Development and evaluation of microarray-based whole-genome hybridization for detection of microorganisms within the context of environmental applications. Environ Sci Technol. 38(24):6775-82.

The detection and identification of microorganisms in natural communities is a great challenge to biologists. Microarray-based genomic technology provides a promising high-throughput alternative to traditional microbial characterization. A novel prototype microarray containing whole genomic DNA, termed community genome array (CGA),

was constructed and evaluated. Microarray hybridizations at 55 degrees C using 50% formamide permitted the examined bacteria to be distinguished at the species level, while strain-level differentiation was obtained at hybridization temperatures of 65 or 75 degrees C. The detection limit was estimated to be approximately 0.2 ng with genomic DNA from a single pure culture using a reduced hybridization volume (3 microL). Using mixtures of known amounts of DNA or a known number of cells from 14 or 16 different species, respectively, about 5 ng of genomic DNA or 2.5 x 10(5) cells were detected under the hybridization conditions used. In addition, strong linear relationships were observed between hybridization signal intensity and target DNA concentrations for pure cultures, a mixture of DNA templates, and a population of mixed cells ($r2 = 0.95$-0.98, $P < 0.01$). Finally, the prototype CGA revealed differences in microbial community composition in soil, river, and marine sediments. The results suggest that CGA hybridization has potential as a specific, sensitive, and quantitative tool for detection and identification of microorganisms in environmental samples.

PMID: 15669338 [PubMed - indexed for MEDLINE]

Matsubara Y, Kerman K, Kobayashi M, Yamamura S, Morita Y, Tamiya E. (2005) Microchamber array based DNA quantification and specific sequence detection from a single copy via PCR in nanoliter volumes. Biosens Bioelectron. 20(8):1482-90.

A novel method for DNA quantification and specific sequence detection in a highly integrated silicon microchamber array is described. Polymerase chain reaction (PCR) mixture of only 40 nL volume could be introduced precisely into each chamber of the mineral oil layer coated microarray by using a nanoliter dispensing system. The elimination of carry-over and cross-contamination between microchambers, and multiple DNA amplification and detection by TaqMan chemistry were demonstrated, for the first time, by using our system. Five different gene targets, related to *Escherichia coli* were amplified and detected simultaneously on the same chip by using DNA from three different serotypes as the templates. The conventional method of DNA quantification, which depends on the real-time monitoring of variations in fluorescence intensity, was not applied to our system, instead a simple method was established. Counting the number of the microchambers with a high fluorescence signal as a consequence of TaqMan PCR provid ed the precise quantification of trace amounts of DNA. The initial DNA concentration for Rhesus D (RhD) gene in each microchamber was ranged from 0.4 to 12 copies, and quantification was achieved by observing the changes in the released fluorescence signals of the microchambers on the chip. DNA target could be detected as small as 0.4 copies. The amplified DNA was detected with a CCD camera built-in to a fluorescence microscope, and also evaluated by a DNA microarray scanner with associated software. This simple method of counting the high fluorescence signal released in microchambers as a consequence of TaqMan PCR was further integrated with a portable miniaturized thermal cycler unit. Such a small device is surely a strong candidate for low-cost DNA amplification, and detected as little as 0.4 copies of target DNA.

PMID: 15626601 [PubMed - indexed for MEDLINE]

Barlaan EA, Sugimori M, Furukawa S, Takeuchi K. (2005) Electronic microarray analysis of 16S rDNA amplicons for bacterial detection. J Biotechnol. 115(1):11-21.

Electronic microarray technology is a potential alternative in bacterial detection and identification. However, conditions for bacterial detection by electronic microarray need optimization. Using the NanoChip electronic microarray, we investigated eight marine bacterial species. Based on the 16S rDNA sequences of these species, we constructed primers, reporter probes, and species-specific capture probes. We carried out two separate analyses for longer (533 bp) and shorter (350 and 200 bp) amplified products (amplicons). To detect simultaneously the hybridization signals for the 350- and 200-bp amplicons, we designed a common reporter probe from an overlapping sequence within both fragments. We developed methods to optimize detection of hybridization signals for processing the DNA chips. A matrix analysis was performed for different bacterial species and complementary capture probes on electronic microarrays. Results showed that, when using the longer amplicon, not all bacterial targets hybridized with the complementary capture probes, which was characterized by the presence of false-positive signals. However, with the shorter amplicons, all bacterial species were correctly and completely detected using the constructed complementary capture probes.

PMID: 15607221 [PubMed - indexed for MEDLINE]

Castiglioni B, Rizzi E, Frosini A, Sivonen K, Rajaniemi P, Rantala A, Mugnai MA, Ventura S, Wilmotte A, Boutte C, Grubisic S, Balthasart P, Consolandi C, Bordoni R, Mezzelani A, Battaglia C, De Bellis G. (2004) Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. Appl Environ Microbiol. 70(12):7161-72.

The cyanobacteria are photosynthetic prokaryotes of significant ecological and biotechnological interest, since they strongly contribute to primary production and are a rich source of bioactive compounds. In eutrophic fresh and brackish waters, their mass occurrences (water blooms) are often toxic and constitute a high potential risk for human health. Therefore, rapid and reliable identification of cyanobacterial species in complex environmental samples is important. Here we describe the development and validation of a microarray for the identification of cyanobacteria in aquatic environments. Our approach is based on the use of a ligation detection reaction coupled to a universal array. Probes were designed for detecting 19 cyanobacterial groups including *Anabaena/Aphanizomenon, Calothrix, Cylindrospermopsis, Cylindrospermum, Gloeothece, halotolerants, Leptolyngbya, Palau Lyngbya, Microcystis, Nodularia, Nostoc, Planktothrix, Antarctic Phormidium, Prochlorococcus, Spirulina, Synechococcus, Synechocystis, Trichodesmium,* and *Woronichinia*. These groups were identified based on an alignment of over 300 cyanobacterial 16S rRNA sequences. For validation of the microarrays, 95 samples (24 axenic strains from culture collections, 27

isolated strains, and 44 cloned fragments recovered from environmental samples) were tested. The results demonstrated a high discriminative power and sensitivity to 1 fmol of the PCR-amplified 16S rRNA gene. Accurate identification of target strains was also achieved with unbalanced mixes of PCR amplicons from different cyanobacteria and an environmental sample. Our universal array method shows great potential for rapid and reliable identification of cyanobacteria. It can be easily adapted to future development and could thus be applied both in research and environmental monitoring.

PMID: 15574913 [PubMed - indexed for MEDLINE]

Pannucci J, Cai H, Pardington PE, Williams E, Okinaka RT, Kuske CR, Cary RB. (2004) Virulence signatures: microarray-based approaches to discovery and analysis. Biosens Bioelectron. 20(4):706-18.

Rapid, accurate, and sensitive detection of biothreat agents requires a broad-spectrum assay capable of discriminating between closely related microbial or viral pathogens. Moreover, in cases where a biological agent release has been identified, forensic analysis demands detailed genetic signature data for accurate strain identification and attribution. To date, nucleic acid sequences have provided the most robust and phylogentically illuminating signature information. Nucleic acid signature sequences are not often linked to genomic or extrachromosomal determinants of virulence, a link that would further facilitate discrimination between pathogens and closely related species. Inextricably coupling genetic determinants of virulence with highly informative nucleic acid signatures would provide a robust means of identifying human, livestock, and agricultural pathogens. By means of example, we present here an overview of two general applications of microarray-based methods for: (1) the identification of candidate virulence factors; and (2) the analysis of genetic polymorphisms that are coupled to *Bacillus anthracis* virulence factors using an accurate, low cost solid-phase mini-sequencing assay. We show that microarray-based analysis of gene expression can identify potential virulence associated genes for use as candidate signature targets, and, further, that microarray-based single nucleotide polymorphism assays provide a robust platform for the detection and identification of signature sequences in a manner independent of the genetic background in which the signature is embedded. We discuss the strategy as a general approach or pipeline for the discovery of virulence-linked nucleic acid signatures for biothreat agents.

PMID: 15522585 [PubMed - indexed for MEDLINE]

Sergeev N, Distler M, Courtney S, Al-Khaldi SF, Volokhov D, Chizhikov V, Rasooly A. (2004) Multipathogen oligonucleotide microarray for environmental and biodefense applications. Biosens Bioelectron. 20(4):684-98

Food-borne pathogens are a major health problem. The large and diverse number of microbial pathogens and their virulence factors has fueled interest in technologies capable of detecting multiple pathogens and multiple virulence factors simultaneously. Some of these pathogens and their toxins have potential use as bioweapons. DNA

microarray technology allows the simultaneous analysis of thousands of sequences of DNA in a relatively short time, making it appropriate for biodefense and for public health uses. This paper describes methods for using DNA microarrays to detect and analyze microbial pathogens. The FDA-1 microarray was developed for the simultaneous detection of several food-borne pathogens and their virulence factors including *Listeria* spp., *Campylobacter* spp., *Staphylococcus aureus* enterotoxin genes and *Clostridium perfringens* toxin genes. Three elements were incorporated to increase confidence in the microarray detection system: redundancy of genes, redundancy of oligonucleotide probes (oligoprobes) for a specific gene, and quality control oligoprobes to monitor array spotting and target DNA hybridization. These elements enhance the reliability of detection and reduce the chance of erroneous results due to the genetic variability of microbes or technical problems with the microarray. The results presented demonstrate the potential of oligonucleotide microarrays for detection of environmental and biodefense relevant microbial pathogens.

PMID: 15522583 [PubMed - indexed for MEDLINE]

Lemarchand K, Masson L, Brousseau R. (2004) Molecular biology and DNA microarray technology for microbial quality monitoring of water. Crit Rev Microbiol. 30(3):145-72.

Public concern over polluted water is a major environmental issue worldwide. Microbial contamination of water arguably represents the most significant risk to human health on a global scale. An important challenge in modern water microbial quality monitoring is the rapid, specific, and sensitive detection of microbial indicators and waterborne pathogens. Presently, microbial tests are based essentially on time-consuming culture methods. Rapid microbiological analyses and detection of rare events in water systems are important challenges in water safety assessment since culture methods present serious limitations from both quantitative and qualitative points of view. To circumvent lengthy culture methods, newer enzymatic, immunological, and genetic methods are being developed as an alternative. DNA microarray technology is a new and promising tool that allows the detection of several hundred or even thousands DNA sequences simultaneously. Recent advances in sample processing and DNA microarray technologies provide new perspectives to assess microbial water quality. The aims of this review are to (1) summarize what is currently known about microbial indicators, (2) describe the most important waterborne pathogens, (3) present molecular methods used to monitor the presence of pathogens in water, and (4) show the potential of DNA microarrays in water quality monitoring.

PMID: 15490968 [PubMed - indexed for MEDLINE]

Striebel HM, Birch-Hirschfeld E, Egerer R, Foldes-Papp Z, Tilz GP, Stelzner A. (2004) Enhancing sensitivity of human herpes virus diagnosis with DNA microarrays using dendrimers. Exp Mol Pathol. 77(2):89-97.

DNA microarray technology has become a promising new tool for the detection and identification of viral pathogens in human plasma and cell cultures. For exploration of this technology, we have developed DNA microarrays that encode capture oligonucleotide probes for different human herpes viruses: herpes simplex virus (HSV) HSV-1, HSV-2, varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and HHV-6. The on-chip hybridization is accomplished with the PCR amplicons of the respective human herpes virus types. In this original article, we attached multiple Cy3-fluorophores to the branched 5' ends of the labeling oligonucleotide primers. For the first time, we experimentally demonstrated that the self-designed, knowledge-based, and focused microarrays specifically hybridized to fluorophore-labeled pathogenic DNAs using dendrimer technology. The fluorescence signal enhancement via the dendrimers was up to 30 times compared with the quenched single Cy3-fluorophore-labeled HSV-1 DNA. The on-chip signal-amplifying effect depended upon the number of branches and the concentration of fluorophore-labeled pathogenic DNAs. Treblers were superior to doublers, as trebler-labeled nucleic acids had fluorescence-signal-enhancing effects over a broad range of labeled DNA concentrations exemplified for the quenched single Cy3-fluorophore-labeled HSV-1 and non-quenched single Cy3-fluorophore-labeled CMV DNAs.

PMID: 15351231 [PubMed - indexed for MEDLINE]

Chou CC, Chen CH, Lee TT, Peck K. (2004) Optimization of probe length and the number of probes per gene for optimal microarray analysis of gene expression. Nucleic Acids Res. 32(12):e99.

Gene-specific oligonucleotide probes are currently used in microarrays to avoid cross-hybridization of highly similar sequences. We developed an approach to determine the optimal number and length of gene-specific probes for accurate transcriptional profiling studies. The study surveyed probe lengths from 25 to 1000 nt. Long probes yield better signal intensity than short probes. The signal intensity of short probes can be improved by addition of spacers or using higher probe concentration for spotting. We also found that accurate gene expression measurement can be achieved with multiple probes per gene and fewer probes are needed if longer probes rather than shorter probes are used. Based on theoretical considerations that were confirmed experimentally, our results showed that 150mer is the optimal probe length for expression measurement. Gene-specific probes can be identified using a computational approach for 150mer probes and they can be treated like long cDNA probes in terms of the hybridization reaction for high sensitivity detection. Our experimental data also show that probes which do not generate good signal intensity give erroneous expression ratio measurement results. To use microarray probes without experimental validation, gene-specific probes approximately 150mer in length are necessary. However, shorter oligonucleotide probes also work well in gene expression analysis if the probes are validated by experimental selection or if multiple probes per gene are used for expression measurement.

PMID: 15243142 [PubMed - indexed for MEDLINE]

Vora GJ, Meador CE, Stenger DA, Andreadis JD. (2004) Nucleic acid amplification strategies for DNA microarray-based pathogen detection. Appl Environ Microbiol. 70(5):3047-54.

DNA microarray-based screening and diagnostic technologies have long promised comprehensive testing capabilities. However, the potential of these powerful tools has been limited by front-end target-specific nucleic acid amplification. Despite the sensitivity and specificity associated with PCR amplification, the inherent bias and limited throughput of this approach constrain the principal benefits of downstream microarray-based applications, especially for pathogen detection. To begin addressing alternative approaches, we investigated four front-end amplification strategies: random primed, isothermal Klenow fragment-based, phi29 DNA polymerase-based, and multiplex PCR. The utility of each amplification strategy was assessed by hybridizing amplicons to microarrays consisting of 70-mer oligonucleotide probes specific for enterohemorrhagic *Escherichia coli* O157:H7 and by quantitating their sensitivities for the detection of O157:H7 in laboratory and environmental samples. Although nearly identical levels of hybridization specificity were achieved for each method, multiplex PCR was at least 3 orders of magnitude more sensitive than any individual random amplification approach. However, the use of Klenow-plus-Klenow and phi29 polymerase-plus-Klenow tandem random amplification strategies provided better sensitivities than multiplex PCR. In addition, amplification biases among the five genetic loci tested were 2- to 20-fold for the random approaches, in contrast to >4 orders of magnitude for multiplex PCR. The same random amplification strategies were also able to detect all five diagnostic targets in a spiked environmental water sample that contained a 63-fold excess of contaminating DNA. The results presented here underscore the feasibility of using random amplification approaches and begin to systematically address the versatility of these approaches for unbiased pathogen detection from environmental sources.

PMID: 15128566 [PubMed - indexed for MEDLINE]

Mitterer G, Huber M, Leidinger E, Kirisits C, Lubitz W, Mueller MW, Schmidt WM. (2004) Microarray-based identification of bacteria in clinical samples by solid-phase PCR amplification of 23S ribosomal DNA sequences. J Clin Microbiol. 42(3):1048-57

The rapid identification of the bacteria in clinical samples is important for patient management and antimicrobial therapy. We describe a DNA microarray-based PCR approach for the quick detection and identification of bacteria from cervical swab specimens from mares. This on-chip PCR method combines the amplification of a variable region of bacterial 23S ribosomal DNA and the simultaneous sequence-specific detection on a solid phase. The solid phase contains bacterial species-specific primers covalently bound to a glass support. During the solid-phase amplification reaction the polymerase elongates perfectly matched primers and incorporates biotin-labeled nucleotides. The reaction products are visualized by streptavidin-cyanine 5 staining,

followed by fluorescence scanning. This procedure successfully identified from pure cultures 22 bacteria that are common causes of abortion and sterility in mares. Using the on-chip PCR method, we also tested 21 cervical swab specimens from mares for the presence of pathogenic bacteria and compared the results with those of conventional bacteriological culture methods. Our method correctly identified the bacteria in 12 cervical swab samples, 8 of which contained more than one bacterial species. Due to the higher sensitivity of the on-chip PCR, this method identified bacteria in five cervical swab samples which were not detected by the conventional identification procedure. Our results show that this method will have great potential to be incorporated into the routine microbiology laboratory.

PMID: 15004052 [PubMed - indexed for MEDLINE]

Francois P, Bento M, Vaudaux P, Schrenzel J. (2003) Comparison of fluorescence and resonance light scattering for highly sensitive microarray detection of bacterial pathogens. J Microbiol Methods. 55(3):755-62

Microarrays have emerged as potential tools for bacterial detection and identification. Given their high parallelism, they might represent a breakthrough in current diagnostic methods, provided they can be coupled to simplified labeling protocols and detected with adequate sensitivities. We describe here a technique to directly label total bacterial RNA, thus avoiding the multiple steps and possible biases associated with enzymatic amplification (e.g. PCR). We have then compared the performances of one white-light source and two laser-based fluorescence scanners for detection reliability and sensitivity. Our study reveals that nanoparticle-labeled bacterial RNA generates reproducible resonance light scattering signals that are at least 50 times more intense than state-of-the-art confocal-based fluorescence signals.

PMID: 14607418 [PubMed - indexed for MEDLINE]

Perrin A, Duracher D, Perret M, Cleuziat P, Mandrand B. (2003) A combined oligonucleotide and protein microarray for the codetection of nucleic acids and antibodies associated with human immunodeficiency virus, hepatitis B virus, and hepatitis C virus infections. Anal Biochem. 322(2):148-55

A multiplexed assay based on the codetection of nucleic acids and antibodies in human serum infected by human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus was proposed. The combined immuno- and oligosorbent array (CombOLISA) microarray is prepared in 96-well standard microplates by spotting (1). nucleic probes specific for a virus genome, (2). viral proteins for the capture of serum antibodies, and (3). nonspecific proteins for verifying specificity. Experimental assay conditions were optimized so that both DNA hybridization and immunological reactions can be achieved simultaneously in the same well and buffer and all at the same temperature. A generic detection system based on the precipitation of an insoluble colorimetric substrate in the presence of enzyme-labeled antibodies or streptavidin was proposed. The optical density of each spot was correlated to the corresponding analyte

concentration. The influence of critical parameters on CombOLISA performance such as serum concentration was studied. Calibration curves and sensitivity thresholds were established for each parameter. Serial dilutions of serum were correlated to results obtained with validated immunoassay platforms such as a microplate enzyme-linked immunosorbent assay or the VIDAS automat. Also, several HIV- and HBV-infected serum samples were tested independently by CombOLISA and VIDAS. Coefficients of variation for genomic and proteomic parameters vs spot density were below 15%.

PMID: 14596821 [PubMed - indexed for MEDLINE]

Keramas G, Bang DD, Lund M, Madsen M, Rasmussen SE, Bunkenborg H, Telleman P, Christensen CB. (2003) Development of a sensitive DNA microarray suitable for rapid detection of *Campylobacter* spp. Mol Cell Probes. 17(4):187-96.

Campylobacter is the most common cause of human acute bacterial gastroenteritis worldwide, widely distributed and isolated from human clinical samples as well as from many other different sources. To comply with the demands of consumers for food safety, there is a need for development of a rapid, sensitive and specific detection method for *Campylobacter*. In this study, we present the development of a novel sensitive DNA-microarray based detection method, evaluated on *Campylobacter* and non-*Campylobacter* reference strains, to detect *Campylobacter* directly from the faecal cloacal swabs. The DNA-microarray method consists of two steps: first, both universal bacterial sequences and specific *Campylobacter* sequences (size range: 149-307 bp) are amplified and fluorescently labeled using multiplex-PCR, targeting the 16S rRNA, the 16S-23S rRNA intergenic region and specific *Campylobacter* genes. Secondly, the Cy5 labeled PCR-amplicons are hybridised to immobilised capture probes on the microarray. The method allows detection of three to thirty genome equivalents (6-60 fg DNA) of Campylobacter within 3 h, with a hands on time of only 15 min. Using the DNA-microarrays, two closely related *Campylobacter* species, *Campylobacter jejuni* and *Campylobacter coli* could be detected and differentiated directly from chicken faeces. The DNA-microarray method has a high potential for automation and incorporation into a dedicated mass screening microsystem.

PMID: 12944122 [PubMed - indexed for MEDLINE]

Springer AL, Booth LR, Braid MD, Houde CM, Hughes KA, Kaiser RJ, Pedrak C, Spicer DA, Stolyar S. (2003) A rapid method for manual or automated purification of fluorescently labeled nucleic acids for sequencing, genotyping, and microarrays. J Biomol Tech. 14(1):17-32

Fluorescent dyes provide specific, sensitive, and multiplexed detection of nucleic acids. To maximize sensitivity, fluorescently labeled reaction products (e.g., cycle sequencing or primer extension products) must be purified away from residual dye-labeled precursors. Successful high-throughput analyses require that this purification be reliable, rapid, and amenable to automation. Common methods for purifying reaction products involve several steps and require processes that are not easily automated.

Prolinx, Inc. has devel oped RapXtract superparamagnetic separation technology affording rapid and easy-to-perform methods that yield high-quality product and are easily automated. The technology uses superparamagnetic particles that specifically remove unincorporated dye-labeled precursors. These particles are efficiently pelleted in the presence of a magnetic field, making them ideal for purification because of the rapid separations that they allow. RapXtract-purified sequencing reactions yield data with good signal and high Phred quality scores, and they work with various sequencing dye chemistries, including BigDye and near-infrared fluorescence IRDyes. RapXtract technology can also be used to purify dye primer sequencing reactions, primer extension reactions for genotyping analysis, and nucleic acid labeling reactions for microarray hybridization. The ease of use and versatility of RapXtract technology makes it a good choice for manual or automated purification of fluorescently labeled nucleic acids.

PMID: 12901608 [PubMed - indexed for MEDLINE]

Kakinuma K, Fukushima M, Kawaguchi R. (2003) Detection and identification of *Escherichia coli*, *Shigella*, and *Salmonella* by microarrays using the gyrB gene. Biotechnol Bioeng. 83(6):721-8.

Commonly, 16S ribosome RNA (16S rRNA) sequence analysis has been used for identifying enteric bacteria. However, it may not always be applicable for distinguishing closely related bacteria. Therefore, we selected gyrB genes that encode the subunit B protein of DNA gyrase (a topoisomerase type II protein) as target genes. The molecular evolution rate of gyrB genes is higher than that of 16S rRNA, and gyrB genes are distributed universally among bacterial species. Microarray technology includes the methods of arraying cDNA or oligonucleotides on substrates such as glass slides while acquiring a lot of information simultaneously. Thus, it is possible to identify the enteric bacteria easily using microarray technology. We devised a simple method of rapidly identifying bacterial species through the combined use of gyrB genes and microarrays. Closely related bacteria were not identified at the species level using 16S rRNA sequence analysis, whereas they were identified at the species level based on the reaction patterns of oligonucleotides on our microarrays using gyrB genes.

PMID: 12889036 [PubMed - indexed for MEDLINE]

Bekal S, Brousseau R, Masson L, Prefontaine G, Fairbrother J, Harel J. (2003) Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. J Clin Microbiol. 41(5):2113-25.

One approach to the accurate determination of the pathogenic potential (pathotype) of isolated *Escherichia coli* strains would be through a complete assessment of each strain for the presence of all known *E. coli* virulence factors. To accomplish this, an *E. coli* virulence factor DNA microarray composed of 105 DNA PCR amplicons printed on glass slides and arranged in eight subarrays corresponding to different *E. coli* pathotypes was developed. Fluorescently labeled genomic DNAs from *E. coli* strains representing known pathotypes were initially hybridized to the virulence gene microarrays for both chip

optimization and validation. Hybridization pattern analysis with clinical isolates permitted a rapid assessment of their virulence attributes and determination of the pathogenic group to which they belonged. Virulence factors belonging to two different pathotypes were detected in one human *E. coli* isolate (strain H87-5406). The microarray was also tested for its ability to distinguish among phylogenetic groups of genes by using gene probes derived from the attaching-and-effacing locus (espA, espB, tir). After hybridization with these probes, we were able to distinguish *E. coli* strains harboring espA, espB, and tir sequences closely related to the gene sequences of an enterohemorrhagic strain (EDL933), a human enteropathogenic strain (E2348/69), or an animal enteropathogenic strain (RDEC-1). Our results show that the virulence factor microarray is a powerful tool for diagnosis-based studies and that the concept is useful for both gene quantitation and subtyping. Additionally, the multitude of virulence genes present on the microarray should greatly facilitate the detection of virulence genes acquired by horizontal transfer and the identification of emerging pathotypes.

PMID: 12734257 [PubMed - indexed for MEDLINE]

Wagner VE, Bushnell D, Passador L, Brooks AI, Iglewski BH. (2003) Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. J Bacteriol. 185(7):2080-95.

Comment in: J Bacteriol. 185(7):2061-5.

Bacterial communication via quorum sensing (QS) has been reported to be important in the production of virulence factors, antibiotic sensitivity, and biofilm development. Two QS systems, known as the las and rhl systems, have been identified previously in the opportunistic pathogen *Pseudomonas aeruginosa*. High-density oligonucleotide microarrays for the *P. aeruginosa* PAO1 genome were used to investigate global gene expression patterns modulated by QS regulons. In the initial experiments we focused on identifying las and/or rhl QS-regulated genes using a QS signal generation-deficient mutant (PAO-JP2) that was cultured with and without added exogenous autoinducers [N-(3-oxododecanoyl) homoserine lactone and N-butyryl homoserine lactone]. Conservatively, 616 genes showed statistically significant differential expression (P </= 0.05) in response to the exogenous autoinducers and were classified as QS regulated. A total of 244 genes were identified as being QS regulated at the mid-logarithmic phase, and 450 genes were identified as being QS regulated at the early stationary phase. Most of the previously reported QS-promoted genes were confirmed, and a large number of additional QS-promoted genes were identified. Importantly, 222 genes were identified as being QS repressed. Environmental factors, such as medium composition and oxygen availability, eliminated detection of transcripts of many genes that were identified as being QS regulated.

PMID: 12644477 [PubMed - indexed for MEDLINE]

Call DR, Borucki MK, Besser TE. (2003) Mixed-genome microarrays reveal multiple serotype and lineage-specific differences among strains of *Listeria monocytogenes*. J Clin Microbiol. 41(2):632-9.

Epidemiological studies and analysis of putative virulence genes have shown that *Listeria monocytogenes* has diverged into several phylogenetic divisions. We hypothesize that similar divergence has occurred for many genes that influence niche-specific fitness and virulence and that identifying these differences may offer new opportunities for the detection, treatment, and control of this important pathogen. To explore this issue further, we developed a microarray composed of fragmented DNA taken from 10 strains of *L. monocytogenes*. We then hybridized genomic DNA from 50 different strains to replicate arrays and analyzed the resulting hybridization patterns. A simple Euclidean distance metric permitted the reconstruction of previously described genetic relationships between serotypes, and only four microarray probes were needed to discriminate between the most important serotypes (1/2a, 1/2b, 1/2c, and 4). We calculated an index of linkage equilibrium from the microarray data and confirmed that *L. monocytogenes* has a strongly clonal population structure (I(A) = 3.85). Twenty-nine informative probes were retrieved from the library and sequenced. These included genes associated with repairing UV-damaged DNA, salt tolerance, biofilm formation, heavy metal transport, ferrous iron transport, and teichoic acid synthesis. Several membrane-bound lipoproteins and one internalin were identified, plus three phage sequences and six sequences with unknown function. Collectively, these data confirm that many genes have diverged between lineages of *L. monocytogenes*. Furthermore, these results demonstrate the value of mixed-genome microarrays as a tool for deriving biologically useful information and for identifying and screening genetic markers for clinically important microbes.

PMID: 12574259 [PubMed - indexed for MEDLINE]

Volokhov D, Rasooly A, Chumakov K, Chizhikov V. (2002) Identification of *Listeria* species by microarray-based assay. J Clin Microbiol. 40(12):4720-8.

We have developed a rapid microarray-based assay for the reliable detection and discrimination of six species of the Listeria genus: *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri,* and *L. grayi*. The approach used in this study involves one-tube multiplex PCR amplification of six target bacterial virulence factor genes (iap, hly, inlB, plcA, plcB, and clpE), synthesis of fluorescently labeled single-stranded DNA, and hybridization to the multiple individual oligonucleotide probes specific for each *Listeria* species and immobilized on a glass surface. Results of the microarray analysis of 53 reference and clinical isolates of *Listeria* spp. demonstrated that this method allowed unambiguous identification of all six *Listeria* species based on sequence differences in the iap gene. Another virulence factor gene, hly, was used for detection and genotyping all *L. monocytogenes*, all *L. ivanovii*, and 8 of 11 *L. seeligeri* isolates. Other members of the genus *Listeria* and three *L. seeligeri* isolates did not contain the hly gene. There was complete agreement between the results of genotyping based on the hly and iap gene sequences. All *L. monocytogenes* isolates were found to be positive for the inlB, plcA, plcB, and clpE virulence genes specific only to this species.

Our data on *Listeria* species analysis demonstrated that this microarray technique is a simple, rapid, and robust genotyping method that is also a potentially valuable tool for identification and characterization of bacterial pathogens in general.
PMID: 12454178 [PubMed - indexed for MEDLINE]

Busti E, Bordoni R, Castiglioni B, Monciardini P, Sosio M, Donadio S, Consolandi C, Rossi Bernardi L, Battaglia C, De Bellis G. (2002) Bacterial discrimination by means of a universal array approach mediated by LDR (ligase detection reaction). BMC Microbiol. 2:27.

BACKGROUND: PCR amplification of bacterial 16S rRNA genes provides the most comprehensive and flexible means of sampling bacterial communities. Sequence analysis of these cloned fragments can provide a qualitative and quantitative insight of the microbial population under scrutiny although this approach is not suited to large-scale screenings. Other methods, such as denaturing gradient gel electrophoresis, heteroduplex or terminal restriction fragment analysis are rapid and therefore amenable to field-scale experiments. A very recent addition to these analytical tools is represented by microarray technology. RESULTS: Here we present our results using a Universal DNA Microarray approach as an analytical tool for bacterial discrimination. The proposed procedure is based on the properties of the DNA ligation reaction and requires the design of two probes specific for each target sequence. One oligo carries a fluorescent label and the other a unique sequence (cZipCode or complementary ZipCode) which identifies a ligation product. Ligated fragments, obtained in presence of a proper template (a PCR amplified fragment of the 16s rRNA gene) contain either the fluorescent label or the unique sequence and therefore are addressed to the location on the microarray where the ZipCode sequence has been spotted. Such an array is therefore "Universal" being unrelated to a specific molecular analysis. Here we present the design of probes specific for some groups of bacteria and their application to bacterial diagnostics. CONCLUSIONS: The combined use of selective probes, ligation reaction and the Universal Array approach yielded an analytical procedure with a good power of discrimination among bacteria.
PMID: 12243651 [PubMed - indexed for MEDLINE]

Alexandre I, Houbion Y, Collet J, Hamels S, Demarteau J, Gala JL, Remacle J. (2002) Compact disc with both numeric and genomic information as DNA microarray platform. Biotechniques. 33(2):435-6, 438-9.

The compact disc (CD) is an ideal toolfor reading, writing, and storing numeric information. It was used in this work as a support for constructing DNA microarrays suited for genomic analysis. The CD was divided into two functional areas: the external ring of the CD was used for multiparametric DNA analysis on arrays, and the inner portion was usedfor storing numeric information. Because polycarbonate and CD resins autofluoresce, a colorimetric method for DNA microarray detection was used that is well adaptedfor the fast detection necessary when using a CD reader. A double-sided CD

reader was developed for the simultaneous analysis of both array and numeric data. The numeric data are engraved as pits in the CD tracks and result in the succession of 0/1, which results from the modulation of the laser reflection when one reads the edges of the pit. Another diffraction-based laser was placed above the CD for the detection of the DNA targets on the microarrays. Both readersfit easily in a PC tower. Both numeric and genomic information data were simultaneously acquired, and each array was reconstituted, analyzed, and processed for quantification by the appropriate software.

PMID: 12188198 [PubMed - indexed for MEDLINE]

Wilson WJ, Strout CL, DeSantis TZ, Stilwell JL, Carrano AV, Andersen GL. (2002) Sequence-specific identification of 18 pathogenic microorganisms using microarray technology. Mol Cell Probes. 16(2):119-27.

We have developed a Multi-Pathogen Identification (MPID) microarray for high confidence identification of eighteen pathogenic prokaryotes, eukaryotes and viruses. Analysis of amplified products from pathogen genomic DNA using microarray hybridization allows for highly specific and sensitive detection, and allows the discrimination between true amplification products and false positive amplification products that might be derived from primers annealing tonon-target sequences. Species-specific primer sets were used to amplify multiple diagnostic regions unique to each individual pathogen. Amplified products were washed over the surface of the microarray, and labelled with phycoerythrin-streptavidin for fluorescence detection. A series of overlapping 20-mer oligonucleotide probes hybridize to the entire diagnostic region, while parallel hybridizations on the same surface allow simultaneous screening for all organisms. Comparison to probes that differ by a single mismatch at the central position reduced the contribution of non-specific hybridization. Samples containing individual pathogens were analyzed in separate experiments and the corresponding species-specific diagnostic regions were identified by fluorescence among their highly redundant probe sets. On average, 91% of the 53,660 pathogen probes on the MPID microarray performed as predicted. The limit of detection was found to be as little as 10 fg of *B. anthracis* DNA in samples that were amplified with six diagnostic primer-pairs. In contrast, PCR products were not observed at this concentration when identical samples were prepared and visualized by agarose gel electrophoresis.

PMID: 12030762 [PubMed - indexed for MEDLINE]

Chizhikov V, Rasooly A, Chumakov K, Levy DD. (2001) Microarray analysis of microbial virulence factors. Appl Environ Microbiol. 67(7):3258-63.

Hybridization with oligonucleotide microchips (microarrays) was used for discrimination among strains of *Escherichia coli* and other pathogenic enteric bacteria harboring various virulence factors. Oligonucleotide microchips are miniature arrays of gene-specific oligonucleotide probes immobilized on a glass surface. The combination of this technique with the amplification of genetic material by PCR is a powerful tool for the detection of and simultaneous discrimination among food-borne human pathogens. The presence of six genes (eaeA, slt-I, slt-II, fliC, rfbE, and ipaH) encoding bacterial

antigenic determinants and virulence factors of bacterial strains was monitored by multiplex PCR followed by hybridization of the denatured PCR product to the gene-specific oligonucleotides on the microchip. The assay was able to detect these virulence factors in 15 *Salmonella*, *Shigella*, and *E. coli* strains. The results of the chip analysis were confirmed by hybridization of radiolabeled gene-specific probes to genomic DNA from bacterial colonies. In contrast, gel electrophoretic analysis of the multiplex PCR products used for the microarray analysis produced ambiguous results due to the presence of unexpected and uncharacterized bands. Our results suggest that microarray analysis of microbial virulence factors might be very useful for automated identification and characterization of bacterial pathogens.

PMID: 11425749 [PubMed - indexed for MEDLINE]

Carter DJ, Cary RB. (2007) Lateral flow microarrays: a novel platform for rapid nucleic acid detection based on miniaturized lateral flow chromatography. Nucleic Acids Res. 2007 May 3; [Epub ahead of print]

Widely used nucleic acid assays are poorly suited for field deployment where access to laboratory instrumentation is limited or unavailable. The need for field deployable nucleic acid detection demands inexpensive, facile systems without sacrificing information capacity or sensitivity. Here we describe a novel microarray platform capable of rapid, sensitive nucleic acid detection without specialized instrumentation. The approach is based on a miniaturized lateral flow device that makes use of hybridization-mediated target capture. The miniaturization of lateral flow nucleic acid detection provides multiple advantages over traditional lateral flow devices. Ten-microliter sample volumes reduce reagent consumption and yield analyte detection times, excluding sample preparation and amplification, of <120 s while providing sub-femtomole sensitivity. Moreover, the use of microarray technology increases the potential information capacity of lateral flow. Coupled with a hybridization-based detection scheme, the lateral flow microarray (LFM) enables sequence-specific detection, opening the door to highly multiplexed implementations for broad-range assays well suited for point-of-care and other field applications. The LFM system is demonstrated using an isothermal amplification strategy for detection of *Bacillus anthracis*, the etiologic agent of anthrax. RNA from as few as two *B. anthracis* cells was detected without thermocycling hardware or fluorescence detection systems.

PMID: 17478499 [PubMed - as supplied by publisher]

Heinemann JA, Rosen H, Savill M, Burgos-Caraballo S, Toranzos GA. (2006) Environment arrays: a possible approach for predicting changes in waterborne bacterial disease potential. Environ Sci Technol. 40(23):7150-6.

Current molecular techniques for identifying bacteria in water have proven useful, but they are not reliably predictive of impending disease outbreaks. Genomics-based approaches will help to detect the presence of pathogens quickly and well before they grow into a population that poses a risk to public health. We suggest that genomics is only one component of the toolbox that will be needed to identify emerging waterborne

threats. We propose a methodology beyond genomics, based on activity in the mobile genome. This approach makes use of a new device called an environment array. The array will depend upon the same research necessary for genomics-based detection, but will not require an a priori knowledge of virulence genes. Environment arrays are assembled from molecular profiles of the infectious elements that transfer between bacteria. The advantage of the array is that it monitors the activity of the mobile genome, rather than the presence of particular DNA sequences. Environmental arrays should thus be many times more sensitive than traditional hybridization or PCR-based techniques that target already-known DNA sequences. Mobile elements are known to respond to new environmental conditions that may correlate with a chemical contamination or the bloom of bacterial pathogens, potentially allowing for a much broader application in detecting unknown or unanticipated biological and chemical contaminants.

PMID: 17180961 [PubMed - indexed for MEDLINE]

WHOLE SYSTEMS WITHOUT AMPLIFICATION

The transition of a laboratory-based protocol into an operational system requires a detailed and complex series of tasks. Systems developers use a variety of methods to characterize the technical maturity of a system. For example, NASA developed Technology Readiness Levels (TRL) for the characterization of technical maturity that are now routinely used by the US Department of Defense and other technology developers. Additional information and definitions on TRLs can be found at Wikopedia http://en.wikipedia.org/wiki/Technology_Readiness_Level

At a minimum, a complete "system" for identification of water borne pathogens must have the following elements: sample collection, sample preparation, sample analysis, data analysis and results readout. A myriad of technical approaches are available for each step in such a system. The abstracts presented in this section describe a variety of technical approaches that incorporate multiple steps applicable to preparation and analysis of samples containing water-borne pathogens. The systems in this section do not rely on nucleic acid amplification for the detection event to occur.

Bowden M, Song L, Walt DR. (2005) Development of a microfluidic platform with an optical imaging microarray capable of attomolar target DNA detection. Anal Chem. 77(17):5583-8.

In this paper, DNA hybridization in a microfluidic manifold is performed using fluorescence detection on a fiber-optic microarray. The microfluidic device integrates optics, sample transport, and fluidic interconnects on a single platform. A high-density optical imaging fiber array containing oligonucleotide-labeled microspheres was developed. DNA hybridization was observed at concentrations as low as 10 aM with response times of less than 15 min at a flow rate of 1 microL/min using 50 microL of target DNA samples. The fast response times coupled with the low sample volumes and the use of a high-density, fiber-optic microarray format make this method highly advantageous. This paper describes the initial development, optimization, and integration of the microfluidic platform with imaging fiber arrays.

PMID: 16131069 [PubMed - indexed for MEDLINE]

Straub TM, Dockendorff BP, Quinonez-Diaz MD, Valdez CO, Shutthanandan JI, Tarasevich BJ, Grate JW, Bruckner-Lea CJ. (2005) Automated methods for multiplexed pathogen detection. J Microbiol Methods. 62(3):303-16.

Detection of pathogenic microorganisms in environmental samples is a difficult process. Concentration of the organisms of interest also co-concentrates inhibitors of many end-point detection methods, notably, nucleic acid methods. In addition, sensitive, highly multiplexed pathogen detection continues to be problematic. The primary function of the BEADS (Biodetection Enabling Analyte Delivery System) platform is the automated concentration and purification of target analytes from interfering substances,

often present in these samples, via a renewable surface column. In one version of BEADS, automated immunomagnetic separation (IMS) is used to separate cells from their samples. Captured cells are transferred to a flow-through thermal cycler where PCR, using labeled primers, is performed. PCR products are then detected by hybridization to a DNA suspension array. In another version of BEADS, cell lysis is performed, and community RNA is purified and directly labeled. Multiplexed detection is accomplished by direct hybridization of the RNA to a planar microarray. The integrated IMS/PCR version of BEADS can successfully purify and amplify 10 *E. coli* O157:H7 cells from river water samples. Multiplexed PCR assays for the simultaneous detection of *E. coli* O157:H7, *Salmonella*, and *Shigella* on bead suspension arrays was demonstrated for the detection of as few as 100 cells for each organism. Results for the RNA version of BEADS are also showing promising results. Automation yields highly purified RNA, suitable for multiplexed detection on microarrays, with microarray detection specificity equivalent to PCR. Both versions of the BEADS platform show great promise for automated pathogen detection from environmental samples. Highly multiplexed pathogen detection using PCR continues to be problematic, but may be required for trace detection in large volume samples. The RNA approach solves the issues of highly multiplexed PCR and provides "live vs. dead" capabilities. However, sensitivity of the method will need to be improved for RNA analysis to replace PCR.

PMID: 15979746 [PubMed - indexed for MEDLINE]

Chandler DP, Brown J, Call DR, Wunschel S, Grate JW, Holman DA, Olson L, Stottlemyre MS, Bruckner-Lea CJ. (2001) Automated immunomagnetic separation and microarray detection of *E. coli* O157:H7 from poultry carcass rinse. Int J Food Microbiol. 70(1-2):143-54.

We describe the development and application of an electromagnetic flow cell and fluidics system for automated immunomagnetic separation (IMS) of *Escherichia coli* O157:H7 directly from poultry carcass rinse. We further describe the biochemical coupling of automated sample preparation with nucleic acid microarrays. Both the cell concentration system and microarray detection method did not require cell growth or enrichment from the poultry carcass rinse prior to IMS. Highly porous Ni foam was used to enhance the magnetic field gradient within the flow path, providing a mechanism for immobilizing immunomagnetic particles throughout the fluid rather than the tubing wall. A maximum of 32% recovery efficiency of non-pathogenic *E. coli* was achieved within the automated system with 6 s cell contact times using commercially available antibodies targeted against the O and K antigens. A 15-min protocol (from sample injection though elution) provided a cell recovery efficiency that was statistically similar to > I h batch captures. O157:H7 cells were reproducibly isolated directly from poultry carcass rinse with 39% recovery efficiency at 10(3) CFU ml(-1) inoculum. Direct plating of washed beads showed positive recovery of O157:H7 directly from poultry carcass rinse at an inoculum of 10 CFU ml(-1). Recovered beads were used for direct polymerase chain reaction (PCR) amplification and microarray detection, with a process-level detection limit (automated cell concentration though microarray detection) of < 10(3)CFU ml(-1) in poultry carcass rinse.

PMID: 11759752 [PubMed - indexed for MEDLINE]

Ligler FS, Sapsford KE, Golden JP, Shriver-Lake LC, Taitt CR, Dyer MA, Barone S, Myatt CJ. (2007) The array biosensor: portable, automated systems. Anal Sci. 23(1):5-10.

With recent advances in surface chemistry, microfluidics, and data analysis, there are ever increasing reports of array-based methods for detecting and quantifying multiple targets. However, only a few systems have been described that require minimal preparation of complex samples and possess a means of quantitatively assessing matrix effects. The NRL Array Biosensor has been developed with the goal of rapid and sensitive detection of multiple targets from multiple samples analyzed simultaneously. A key characteristic of this system is its two-dimensional configuration, which allows controls and standards to be analyzed in parallel with unknowns. Although the majority of our work has focused on instrument automation and immunoassay development, we have recently initiated efforts to utilize alternative recognition molecules, such as peptides and sugars, for detection of a wider variety of targets. The array biosensor has demonstrated utility for a variety of applications, including food safety, disease diagnosis, monitoring immune response, and homeland security, and is presently being transitioned to the commercial sector for manufacturing.
PMID: 17213615 [PubMed - indexed for MEDLINE]

Kramer MF, Lim DV. (2004) A rapid and automated fiber optic-based biosensor assay for the detection of *Salmonella* in spent irrigation water used in the sprouting of sprout seeds. J Food Prot. 67(1):46-52.

Recent outbreaks of foodborne illness have been linked to the consumption of contaminated sprouts. The spent irrigation water used to irrigate sprouts can carry many microorganisms, including pathogenic strains of *Escherichia coli* and *Salmonella enterica*. These pathogens are believed to originate from the seeds. The U.S. Food and Drug Administration recommends that sprout producers conduct microbiological testing of spent irrigation water from each production lot at least 48 h after seeds have germinated. Microbial analysis for the detection of *Salmonella* is labor-intensive and takes days to complete. A rapid and automated fiber-optic biosensor assay for the detection of *Salmonella* in sprout rinse water was developed in this study. Alfalfa seeds contaminated with various concentrations of *Salmonella Typhimurium* were sprouted. The spent irrigation water was assayed 67 h after alfalfa seed germination with the RAPTOR (Research International, Monroe, Wash.), an automated fiber optic-based detector. *Salmonella Typhimurium* could be positively identified in spent irrigation water when seeds were contaminated with 50 CFU/g. Viable *Salmonella Typhimurium* cells were also recovered from the waveguides after the assay. This biosensor assay system has the potential to be directly connected to water lines within the sprout-processing facility and to operate automatically, requiring manual labor only for preventative maintenance. Therefore, the presence of *Salmonella Typhimurium* in spent irrigation water could be

continuously and rapidly detected 3 to 5 days before the completion of the sprouting process.

PMID: 14717350 [PubMed - indexed for MEDLINE]

Dunbar SA, Vander Zee CA, Oliver KG, Karem KL, Jacobson JW. (2003) Quantitative, multiplexed detection of bacterial pathogens: DNA and protein applications of the Luminex LabMAP system. J Microbiol Methods. 53(2):245-52.

Escherichia coli, *Salmonella*, *Listeria monocytogenes* and *Campylobacter jejuni* are bacterial pathogens commonly implicated in foodborne illnesses. Generally used detection methods (i.e., culture, biochemical testing, ELISA and nucleic acid amplification) can be laborious, time-consuming and require multiple tests to detect all of the pathogens. Our objective was to develop rapid assays to simultaneously detect these four organisms through the presence of antigen or DNA using the Luminex LabMAP system. For nucleic acid detection, organism-specific capture probes corresponding to the 23S ribosomal RNA gene (rrl) were coupled covalently to LabMAP microspheres. Target molecules included synthetic complementary oligonucleotides and genomic DNA isolated from ATCC type strains or other well-characterized strains of each organism. Universal PCR primers were designed to amplify variable regions of bacterial 23S ribosomal DNA, yielding biotinylated amplicons of 86 to 109 bp in length. Varying quantities of targets were hybridized to the combined microsphere sets, labeled with streptavidin-R-phycoerythrin and analyzed on the Luminex(100) system. Results of nucleic acid detection assays, obtained in 30 to 40 min following amplification, correctly and specifically identified each bacterial species with a detection sensitivity of 10(3) to 10(5) genome copies. Capture-sandwich immunoassays were developed with organism-specific antibodies coupled to different microsphere sets. Microspheres were incubated with organism-specific standards and reactivity was assessed with biotinylated detection antibodies and streptavidin-R-phycoerythrin. In the immunoassays, microsphere-associated fluorescence was organism concentration dependent with detectable response at < or = 1000 organisms/ml and with no apparent cross-reactivity. We have demonstrated that the Luminex LabMAP system is a rapid, flexible platform capable of simultaneous, sensitive and specific detection of pathogens. The practical significance of this multiplexing approach would be to provide more timely, economical and comprehensive information than is available with conventional isolation and identification methodologies.

PMID: 12654495 [PubMed - indexed for MEDLINE]

Tschmelak J, Proll G, Riedt J, Kaiser J, Kraemmer P, Barzaga L, Wilkinson JS, Hua P, Hole JP, Nudd R, Jackson M, Abuknesha R, Barcelo D, Rodriguez-Mozaz S, de Alda MJ, Sacher F, Stien J, Slobodnik J, Oswald P, Kozmenko H, Korenkova E, Tothova L, Krascsenits Z, Gauglitz G. (2005) Automated Water Analyser Computer Supported System (AWACSS) Part II: Intelligent, remote-controlled, cost-effective, on-line, water-monitoring measurement system. Biosens Bioelectron. 20(8):1509-19.

A novel analytical system AWACSS (Automated Water Analyser Computer Supported System) based on immunochemical technology has been evaluated that can measure several organic pollutants at low nanogram per litre level in a single few-minutes analysis without any prior sample pre-concentration or pre-treatment steps. Having in mind actual needs of water-sector managers related to the implementation of the Drinking Water Directive (DWD) [98/83/EC, 1998. Council Directive (98/83/EC) of 3 November 1998 relating to the quality of water intended for human consumption. Off. J. Eur. Commun. L330, 32-54] and Water Framework Directive (WFD) [2000/60/EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off. J. Eur. Commun. L327, 1-72], drinking, ground, surface, and waste waters were major media used for the evaluation of the system performance. The first part article gave the reader an overview of the aims and scope of the AWACSS project as well as details about basic technology, immunoassays, software, and networking developed and utilised within the research project. The second part reports on the system performance, first real sample measurements, and an international collaborative trial (inter-laboratory tests) to compare the biosensor with conventional anayltical methods. The systems' capability for analysing a wide range of environmental organic micro-pollutants, such as modern pesticides, endocrine disrupting compounds and pharmaceuticals in surface, ground, drinking and waste water is shown. In addition, a protocol using reconstitution of extracts of solid samples, developed and applied for analysis of river sediments and food samples, is presented. Finally, the overall performance of the AWACSS system in comparison to the conventional analytical techniques, which included liquid and gas chromatographic systems with diode-array UV and mass spectrometric detectors, was successfully tested in an inter-laboratory collaborative trial among six project partners.

PMID: 15626604 [PubMed - indexed for MEDLINE]

Rasooly A, Herold KE. (2006) Biosensors for the analysis of food- and waterborne pathogens and their toxins. J AOAC Int. 89(3):873-83.

Biosensors are devices which combine a biochemical recognition element with a physical transducer. There are various types of biosensors, including electrochemical, acoustical, and optical sensors. Biosensors are used for medical applications and for environmental testing. Although biosensors are not commonly used for food microbial analysis, they have great potential for the detection of microbial pathogens and their toxins in food. They enable fast or real-time detection, portability, and multipathogen detection for both field and laboratory analysis. Several applications have been developed for microbial analysis of food pathogens, including *E. coli* O157:H7, *Staphylococcus aureus, Salmonella*, and *Listeria monocytogenes*, as well as various microbial toxins such as staphylococcal enterotoxins and mycotoxins. Biosensors have several potential advantages over other methods of analysis, including sensitivity in the range of ng/mL for microbial toxins and <100 colony-forming units/mL for bacteria. Fast or real-time detection can provide almost immediate interactive information about the sample tested, enabling users to take corrective measures before consumption or further contamination can occur. Miniaturization of biosensors enables biosensor integration into

various food production equipment and machinery. Potential uses of biosensors for food microbiology include online process microbial monitoring to provide real-time information in food production and analysis of microbial pathogens and their toxins in finished food. Biosensors can also be integrated into Hazard Analysis and Critical Control Point programs, enabling critical microbial analysis of the entire food manufacturing process. In this review, the main biosensor approaches, technologies, instrumentation, and applications for food microbial analysis are described.

PMID: 16792089 [PubMed - indexed for MEDLINE]

WHOLE SYSTEMS WITH AMPLIFICATION

The systems in this section rely on nucleic acid amplification for the detection event to occur.

Liu RH, Yang J, Lenigk R, Bonanno J, Grodzinski P. (2004) Self-contained, fully integrated biochip for sample preparation, polymerase chain reaction amplification, and DNA microarray detection. Anal Chem. 76(7):1824-31.

A fully integrated biochip device that consists of microfluidic mixers, valves, pumps, channels, chambers, heaters, and DNA microarray sensors was developed to perform DNA analysis of complex biological sample solutions. Sample preparation (including magnetic bead-based cell capture, cell preconcentration and purification, and cell lysis), polymerase chain reaction, DNA hybridization, and electrochemical detection were performed in this fully automated and miniature device. Cavitation microstreaming was implemented to enhance target cell capture from whole blood samples using immunomagnetic beads and accelerate DNA hybridization reaction. Thermally actuated paraffin-based microvalves were developed to regulate flows. Electrochemical pumps and thermopneumatic pumps were integrated on the chip to provide pumping of liquid solutions. The device is completely self-contained: no external pressure sources, fluid storage, mechanical pumps, or valves are necessary for fluid manipulation, thus eliminating possible sample contamination and simplifying device operation. Pathogenic bacteria detection from approximately milliliters of whole blood samples and single-nucleotide polymorphism analysis directly from diluted blood were demonstrated. The device provides a cost-effective solution to direct sample-to-answer genetic analysis and thus has a potential impact in the fields of point-of-care genetic analysis, environmental testing, and biological warfare agent detection.

PMID: 15053639 [PubMed - indexed for MEDLINE]

Zaytseva NV, Goral VN, Montagna RA, Baeumner AJ. (2005) Development of a microfluidic biosensor module for pathogen detection. Lab Chip. 5(8):805-11.

The development of a microfluidic biosensor module with fluorescence detection for the identification of pathogenic organisms and viruses is presented in this article. The microfluidic biosensor consists of a network of microchannels fabricated in polydimethylsiloxane (PDMS) substrate. The microchannels are sealed with a glass substrate and packed in a Plexiglas housing to provide connection to the macro-world and ensure leakage-free flow operation. Reversible sealing permits easy disassembly for

cleaning and replacing the microfluidic channels. The fluidic flow is generated by an applied positive pressure gradient, and the module can be operated under continuous solution flow of up to 80 microL min(-1). The biosensor recognition principle is based on DNA/RNA hybridization and liposome signal amplification. Superparamagnetic beads are incorporated into the system as a mobile solid support and are an essential part of the analysis scheme. In this study, the design, fabrication and the optimization of concentrations and amounts of the different biosensor components are carried out. The total time required for an assay is only 15 min including sample incubation time. The biosensor module is designed so that it can be easily integrated with a micro total analysis system, which will combine sample preparation and detection steps onto a single chip.

PMID: 16027930 [PubMed - indexed for MEDLINE]

REVIEWS

Previous sections of this report contain review articles associated with specific technical approaches. This section contains reviews that are relevant to the detection of water borne pathogens but do not fit into any of the specific categories covered int his report.

Leclerc H, Schwartzbrod L, Dei-Cas E. (2002) Microbial agents associated with waterborne diseases. Crit Rev Microbiol. 28(4):371-409.

Many classes of pathogens excreted in feces are able to initiate waterborne infections. There are bacterial pathogens, including enteric and aquatic bacteria, enteric viruses, and enteric protozoa, which are strongly resistant in the water environment and to most disinfectants. The infection dose of viral and protozoan agents is lower than bacteria, in the range of one to ten infectious units or oocysts. Waterborne outbreaks of bacterial origin (particularly typhoid fever) in the developing countries have declined dramatically from 1900s. Therefore, some early bacterial agents such as *Shigella* sonnei remains prevalent and new pathogens of fecal origin such as zoonotic *C. jejuni* and *E. coli* O157:H7 may contaminate pristine waters through wildlife or domestic animal feces. The common feature of these bacteria is the low inoculum (a few hundred cells) that may trigger disease. The emergence in early 1992 of serotype O139 of *V. cholerae* with epidemic potential in Southeast Asia suggests that other serotypes than *V. cholerae* O1 could also getting on epidemic. Some new pathogens include environmental bacteria that are capable of surviving and proliferating in water distribution systems. Other than specific hosts at risk, the general population is refractory to infection with ingested P. aeruginosa. The significance of *Aeromonas* spp. in drinking water to the occurrence of acute gastroenteritis remains a debatable point and has to be evaluated in further epidemiological studies. *Legionella* and *Mycobacterium avium* complex (MAC) are environmental pathogens that have found an ecologic niche in drinking and hot water supplies. Numerous studies have reported Legionnaires' disease caused by *L. pneumophila* occurring in residential and hospital water supplies. M. avium complex frequently causes disseminated infections in AIDS patients and drinking water has been suggested as a source of infection; in some cases the relationship has been proven. More and more numerous reports show that *Helicobacter pylori* DNA can be amplified from feces samples of infected patients, which strongly suggests fecal-to-oral transmission. Therefore, it is possible that *H. pylori* infection is waterbome, but these assumptions need to be substantiated. Giardiasis has become the most common cause of human waterborne disease in the U.S. over the last 30 years. However, as a result of the massive outbreak of waterborne cryptosporidiosis in Milwaukee, Wisconsin, affecting an estimated 403,000 persons, there is increasing interest in the epidemiology and prevention of new infection disease caused by *Cryptosporidium* spp. as well as monitoring water quality. The transmission of *Cryptosporidium* and *Giardia* through treated water supplies that meet water quality standards demonstrates that water treatment technologies have become inadequate, and that a negative coliform no longer guarantees that water is free from all pathogens, especially from protozoan agents. Substantial concern persists that low levels of pathogen occurrence may be responsible for the endemic transmission of enteric

disease. In addition to *Giardia* and *Cryptosporidium*, some species of genera *Cyclospora*, Isospora, and of family *Microsporidia* are emerging as opportunistic pathogens and may have waterborne routes of transmission. More than 15 different groups of viruses, encompassing more than 140 distinct types can be found in the human gut. Some cause illness unrelated with the gut epithelium, such as Hepatitis A virus (HAV) and Hepatitis E virus (HEV). Numerous large outbreaks have been documented in the U.S. between 1950 and 1970, and the incidence rate has strongly declined in developing countries since the 1970s. Hepatitis E is mostly confined to tropical and subtropical areas, but recent reports indicate that it can occur at a low level in Europe. A relatively small group of viruses have been incriminated as causes of acute gastroenteritis in humans and fewer have proven to be true etiologic agents, including rotavirus, calicivirus, astrovirus, and some enteric adenovirus. These enteric viruses have infrequently been identified as the etiologic agents of waterborne disease outbreaks, because of inadequate diagnostic technology, but many outbreaks of unknown etiology currently reported are likely due to viral agents. Actually, Norwalk virus and Norwalk-like viruses are recognized as the major causes of waterborne illnesses world-wide. The global burden of infectious waterborne disease is considerable. Reported numbers highly underestimate the real incidence of waterborne diseases. The most striking concern is that enteric viruses such as caliciviruses and some protozoan agents, such as *Cryptosporidium*, are the best candidates to reach the highest levels of endemic transmission, because they are ubiquitous in water intended for drinking, being highly resistant to relevant environmental factors, including chemical disinfecting procedures. Other concluding concerns are the enhanced risks for the classic group of debilitated subjects (very young, old, pregnant, and immunocompromised individuals) and the basic requirement of to take specific measures aimed at reducing the risk of waterborne infection diseases in this growing, weaker population.

PMID: 12546197 [PubMed - indexed for MEDLINE]

Dufva M, Christensen CB. (2005) Diagnostic and analytical applications of protein microarrays. Expert Rev Proteomics. 2(1):41-8.

DNA microarrays have changed the field of biomedical sciences over the past 10 years. For several reasons, antibody and other protein microarrays have not developed at the same rate. However, protein and antibody arrays have emerged as a powerful tool to complement DNA microarrays during the past 5 years. A genome-scale protein microarray has been demonstrated for identifying protein-protein interactions as well as for rapid identification of protein binding to a particular drug. Furthermore, protein microarrays have been shown as an efficient tool in cancer profiling, detection of bacteria and toxins, identification of allergen reactivity and autoantibodies. They have also demonstrated the ability to measure the absolute concentration of small molecules. Besides their capacity for parallel diagnostics, microarrays can be more sensitive than traditional methods such as enzyme-linked immunosorbent assay, mass spectrometry or high-performance liquid chromatography-based assays. However, for protein and antibody arrays to be successfully introduced into diagnostics, the biochemistry of immunomicroarrays must be better characterized and simplified, they must be validated

in a clinical setting and be amenable to automation or integrated into easy-to-use systems, such as micrototal analysis systems or point-of-care devices.
PMID: 15966851 [PubMed - indexed for MEDLINE]

Keer JT, Birch L. (2003) Molecular methods for the assessment of bacterial viability. J Microbiol Methods. 53(2):175-83.

A significant number of pathogenic microorganisms can be found in environmental reservoirs (air, water, soil). It is important to assess the viability status of these organisms to determine whether they pose a threat to public health. Classical methods for determining viability are time consuming. Hence, molecular methods have been developed to address this problem. Molecular methods offer speed, sensitivity and specificity. Both DNA and RNA have been analysed using molecular amplification methods such as polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR) and nucleic acid sequence-based amplification (NASBA). However, due to the variable persistence of nucleic acids in cells post-death, the correlation between presence of DNA and RNA and viability is not clear-cut. Similarly, the choice of target and sensitivity of the method can significantly affect the validity of the viability assay. This review assesses the molecular methods currently available and evaluates their ability to assess cell viability with emphasis on environmental pathogens.
PMID: 12654489 [PubMed - indexed for MEDLINE]

Rudi K, Jakobsen KS. (2006) Overview of DNA purification for nucleic acid-based diagnostics from environmental and clinical samples. Methods Mol Biol. 345:23-35.

Direct deoxyribonucleic acid (DNA)-based detection methods are crucial for future environmental monitoring and clinical diagnosis. In this chapter, we provide an overview of the various sample preparation approaches for bacteria for direct analyses (i.e., without culturing) in environmental and clinical samples. The issues of sampling, sample preservation, separation of the microorganisms from the environmental or clinical matrix, and DNA purification are covered. This chapter will focus on the advantages and the disadvantages of the methods available.
PMID: 16957344 [PubMed - indexed for MEDLINE]

Straub TM, Chandler DP. (2003) Towards a unified system for detecting waterborne pathogens. J Microbiol Methods. 53(2):185-97.

Currently, there is no single method to collect, process, and analyze a water sample for all pathogenic microorganisms of interest. Some of the difficulties in developing a universal method include the physical differences between the major pathogen groups (viruses, bacteria, protozoa), efficiently concentrating large volume water samples to detect low target concentrations of certain pathogen groups, removing co-concentrated

inhibitors from the sample, and standardizing a culture-independent endpoint detection method. Integrating the disparate technologies into a single, universal, simple method and detection system would represent a significant advance in public health and microbioligical water quality analysis. Recent advances in sample collection, on-line sample processing and purification, and DNA microarray technologies may form the basis of a universal method to detect known and emerging waterborne pathogens. This review discusses some of the challenges in developing a universal pathogen detection method, current technology that may be employed to overcome these challenges, and the remaining needs for developing an integrated pathogen detection and monitoring system for source or finished water.

PMID: 12654490 [PubMed - indexed for MEDLINE]

Ligler FS, Sapsford KE, Golden JP, Shriver-Lake LC, Taitt CR, Dyer MA, Barone S, Myatt CJ. (2007) The array biosensor: portable, automated systems. Anal Sci. 23(1):5-10.

With recent advances in surface chemistry, microfluidics, and data analysis, there are ever increasing reports of array-based methods for detecting and quantifying multiple targets. However, only a few systems have been described that require minimal preparation of complex samples and possess a means of quantitatively assessing matrix effects. The NRL Array Biosensor has been developed with the goal of rapid and sensitive detection of multiple targets from multiple samples analyzed simultaneously. A key characteristic of this system is its two-dimensional configuration, which allows controls and standards to be analyzed in parallel with unknowns. Although the majority of our work has focused on instrument automation and immunoassay development, we have recently initiated efforts to utilize alternative recognition molecules, such as peptides and sugars, for detection of a wider variety of targets. The array biosensor has demonstrated utility for a variety of applications, including food safety, disease diagnosis, monitoring immune response, and homeland security, and is presently being transitioned to the commercial sector for manufacturing.

PMID: 17213615 [PubMed - indexed for MEDLINE]

Theron J, Cloete TE. (2002) Emerging waterborne infections: contributing factors, agents, and detection tools. Crit Rev Microbiol. 28(1):1-26.

Because microorganisms are easily dispersed, display physiological diversity, and tolerate extreme conditions, they are ubiquitous and may contaminate and grow in water. The presence of waterborne enteric pathogens (bacteria, viruses, and protozoa) in domestic water supplies represents a potentially significant human health risk. Even though major outbreaks of waterborne disease are comparatively rare, there is substantial evidence that human enteric pathogens that are frequently present in domestic water supplies are responsible for low-level incidence of waterborne microbial disease. Although these diseases are rarely debilitating to healthy adults for more than a few hours

to a few days, enteric pathogens can cause severe illness, even death, for young children, the elderly, or those with compromised immune systems. As the epidemiology of waterborne diseases is changing, there is a growing global public health concern about new and reemerging infectious diseases that are occurring through a complex interaction of social, economic, evolutionary, and ecological factors. New microbial pathogens have emerged, and some have spread worldwide. Alternative testing strategies for waterborne diseases should significantly improve the ability to detect and control the causative pathogenic agents. In this article, we provide an overview of the current state of knowledge of waterborne microbial pathogens, their detection, and the future of new methods in controlling these infectious agents.

PMID: 12003038 [PubMed - indexed for MEDLINE]

Marshall MM, Naumovitz D, Ortega Y, Sterling CR. (1997) Waterborne protozoan pathogens. Clin Microbiol Rev. 10(1):67-85.

Erratum in: Clin Microbiol Rev 11(2):404.

Protozoan parasites were the most frequently identified etiologic agents in waterborne disease outbreak from 1991 to 1994. The waterborne parasites *Giardia lamblia, Naegleria fowleri, Acanthamoeba spp., Entamoeba histolytica, Cryptosporidium parvum, Cyclospora cayetanesis, Isospora belli,* and the microsporidia are reviewed. For each parasite, the review includes history, life cycle, incidence, symptoms, and therapy. Clinical detection methods are compared, and emerging technologies are discussed. Information on the association of these parasites with waterborne outbreaks is reviewed. Current information on protozoan parasites identified as etiological agents in waterborne outbreaks is discussed. Water industry issues related to recent disease outbreaks are examined in the context of water quality testing regulations for *G. lamblia* and those proposed for *C. parvum*. The review identifies the limitations of the American Society of Testing and Materials water-testing method for these parasites. An overview of federal regulations affecting the water industry and laboratories that test for water quality is also provided. The article highlights the importance of the clinical laboratory as a frontline defense for the detection of infectious organisms. The review points to the need for clinical laboratories, physicians, and public health personnel to cooperatively plan and assess the challenge of meeting this potential public health threat.

PMID: 8993859 [PubMed - indexed for MEDLINE]

Pozio E. (2003) Foodborne and waterborne parasites. Acta Microbiol Pol. 52 Suppl:83-96.

More than 72 species of protozoan and helminth parasites can reach humans by food and water, and most of these infections are zoonoses. Some parasites show a cosmopolitan distribution, others a more restricted distribution due to their complex life cycles, which need the presence of one or more intermediate hosts. Of this large number of pathogens, only Toxoplasma gondii can be transmitted to humans by two different

ways, i.e., by cysts present in infected meat and by oocysts contaminating food and water. Eleven helminthic species (*Taenia saginata, Taenia solium, Taenia asiatica, Trichinella spiralis, Tr. nativa, Tr. britovi, Tr. pseudospiralis, Tr. murrelli, Tr nelsoni, Tr. papuae* and *Tr. zimbabwensis*) can grow in meat of different animal species and can be transmitted to humans by the consumption of raw meat or meat products. Twenty trematode species, four cestode species and seven nematode species can infect humans through the consumption of raw sea- and/or fresh-water food (fishes, molluscs, frogs, tadpoles, camarons, crayfishes). Six species of *Cryptosporidium, Isospora belli, Cyclospora cayetanensis, Giardia duodenalis* and Entamoeba histolytica/*E. dispar* can contaminate food and water. Among the helminths, seven trematode species, seven cestode species and five species of nematodes can reach humans by contaminated food and water. Diagnostic and detection methods that can be carried out routinely on food and water samples are available only for few parasites (*Cryptosporidium* sp., *Giardia* sp., *Anisakidae, Trichinella* sp., *Taenia* sp.), i.e., for parasites which represent a risk to human populations living in industrialised countries. The majority of food and waterborne infections of parasitic origin are related to poverty, low sanitation, and old food habits.

PMID: 15058817 [PubMed - indexed for MEDLINE]

Slifko TR, Smith HV, Rose JB. (2000) Emerging parasite zoonoses associated with water and food. Int J Parasitol. 30(12-13):1379-93.

The environmental route of transmission is important for many protozoan and helminth parasites, with water, soil and food being particularly significant. Both the potential for producing large numbers of transmissive stages and their environmental robustness, being able to survive in moist microclimates for prolonged periods of time, pose a persistent threat to public and veterinary health. The increased demands on natural resources increase the likelihood of encountering environments and produce contaminated with parasites. For waterborne diseases, the protozoa, *Cryptosporidium, Giardia* and *Toxoplasma*, are the most significant causes, yet, with the exception of *Toxoplasma*, the contribution of zoonotic transmission remains unclear due to the absence of 'standardised' methods. The microsporidia have been documented in one waterborne outbreak, but the role of animals as the cause of contamination was not elucidated. In foods, surface contamination is associated with the faecal-oral pathogens, and some data are available to indicate that animal wastes remain an important source of contamination (e.g. cattle faeces and apple cider outbreaks), however, further work should focus on examining the source of contamination on fruit and vegetables. Increasing recognition of the burden of human fascioliasis has occurred; it is now recognised as an emerging zoonosis by the WHO. *Toxoplasma*, *Trichinella* and *Taenia* spp. remain important meatborne parasites, however, others, including Pleistophora-like microsporidians may beacquired from raw or lightly cooked fish or crustaceans. With increased international travel, the public health importance of the foodborne trematodiases must also be realised. Global sourcing of food, coupled with changing consumer vogues, including the consumption of raw vegetables and undercooking to retain the natural taste and preserve heat-labile nutrients, can increase the risk of foodborne transmission. A greater awareness of parasite contamination of our

environment and its impact on health has precipitated the development of better detection methods. Robust, efficient detection, viability and typing methods are required to assess risks and to further epidemiological understanding.

PMID: 11113263 [PubMed - indexed for MEDLINE]