Volume 1 - Issue 1. June, 2006

EPI-Net Perspective

www.epi-net.org

# Role of Indicators in Pathogen Detection

M. Carrero-Colón and R. F. Turco

## What are Pathogens?

Pathogens are agents (e.g., bacteria, virus, protozoa) that can cause a disease. While natural environments are filled with millions of different microorganisms, most of these are not pathogens. Most environmental microorganisms are free-living (do not require a host to function), as they are able to utilize the carbon and nitrogen they encounter in soil and water. Pathogens generally originate from an infected host and are transmitted in the environment. For a pathogen to function fully it must encounter a host where it can grow and reproduce. Inside the host the pathogen encounters the appropriate environment (required nutrients, pH, and temperature, to mention a few) that will sustain growth and reproduction. Environmental transmitted pathogens are microorganisms that spend most of their life outside of a host, but when introduced to host can (not always) cause a disease. Primarily while in the environment, pathogens are just able to survive, although, growth and reproduction is possible.

Pathogens can be transmitted in all types of environments including water, soil, air and food. While the direct detection of pathogens in the environment will provide evidence of their presence, this is a difficult task. Unfortunately pathogens are usually dilute (few) and mixed with high number of non-pathogens which makes direct detection difficult (Straub and Chandler, 2003). Many known protocols for sample collection, concentration, and identification are not sensitive enough to detect dilute agents against the large non-pathogenic population. Because of the challenges with direct detection, traditionally scientists have focused on the use of "indicators" that help predict the presence of pathogens in the environment.

## What are Indicators?

Indicators are use to predict the presence of pathogens in the environment (Scott et al., 2002).

Characteristics of a good pathogen indicator

1. Non-pathogenic
2. Rapidly detected and easily enumerated
3. Have survival characteristics similar to the pathogen
4. Can be associated with the presence of pathogenic organism
5. Should not replicate in the environment
6. Should be present in greater numbers than the pathogen

(Scott et al., 2002; Bitton, 2005)

# AEROBIC INDICATORS

## *Coliform bacteria*

©EPI-NET www.epi-net.org

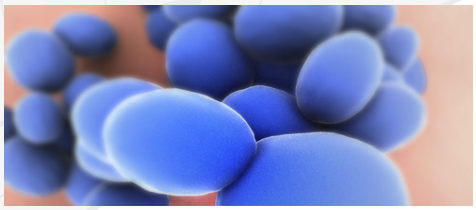
Total coliforms group are comprised of aerobic and facultative anaerobic, gram-negative, non-spore forming, rod shaped bacteria that can ferment lactose to gas at 35ºC (Bitton, 2005). Fecal coliforms are thermotolerant bacteria that can ferment lactose to gas at 44.5ºC and includes *E. coli, K. pneumonae* and *Citrobacter*. The presence of fecal coliforms indicates the occurrence of fecal material which in turns might indicate the presence of pathogens as they are often transmitted from infected hosts in fecal material.

One of the EPA methods for coliform detection is a presence/absence procedure in which 100 mL of water are added to a bottle containing Colilert® or Colisure® which is a patented material containing the required nutrients to support the growth of coliforms.. In addition it has the specific compounds: ONPG (o-nitrophenyl-β-D-galactopyranoside) for coliform bacteria and MUG (4-methylumbelliferyl-β-D-glucuronide) for E. coli; that are enzymatically attacked by the coliforms resulting in a color change reaction. A yellow color in the solution after incubation for 24 hrs at 35ºC represents the presence of total coliforms. A fluoresce blue color under UV light confirms the presence of E. coli. Dim fluorescence or colors is not considered a positive result (Straub and Chandler, 2003).

## *Fecal Streptococci*

This group is comprised of enterococci group D streptococci and are found in the feces of warm blooded animals. While the genus *Streptococci* is comprised of many members i.e., *S. faecalis*, *S. faecium*, *S. avium*, *S gallinarum*, *S. bovis* and *S. equinus* only the *S. bovis* and *equinus* as well as *Entercoccus faecalis* and faecium are considered true *fecal streptococci.*

Fecal streptococci tend to persist longer in the environment than fecal coliforms. Fecal streptococci can be detected in environmental samples in a manner similar to that used for fecal coliform. One of the most common procedures used is the Enterolert™, which uses 4-methylumbellliferone-β-D-glucoside as an indicator. This will emit fluorescence when is metabolized by enterococci (Bundnick et. al., 1996), The technique employs the MPN principles and results are obtained in 24 hrs. Blue fluorescent color in samples indicate positive results (Bitton, 2005).



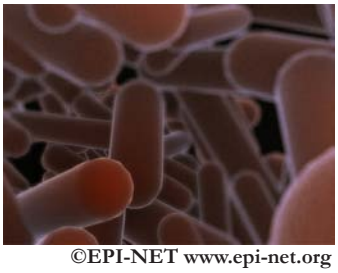
©EPI-NET www.epi-net.org

# ANAEROBIC INDICATORS

## *Bacteroides spp.*

Bacteroides are gram (-) obligate anaerobes that can be found in the intestinal track at higher concentration than fecal coliform bacteria.

Some of the characteristics of this organism that make it suitable as an indicator are: it is present only in human samples which makes this method highly specific, they do not replicate in the environment and its presence significantly correlates to human enteric viruses. One drawback of using this organism is that is not present in highly polluted waters (Scott et al., 2002).

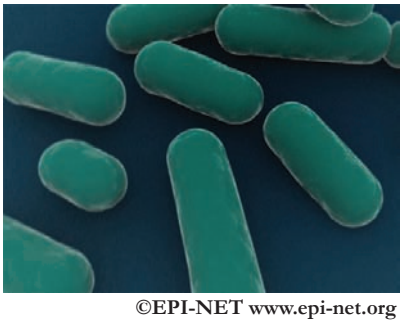


©EPI-NET www.epi-net.org

## *Clostridum perfringes*

Clostridia are anaerobic, gram (+) spore forming, rod shape, pathogenic bacteria found in the colon.

Although it has been proposed as an indicator of fecal pollution, its spore forming capacity is suggested as making it too a strong a survivor in the environment to indicate just recent pollution. It is still recommended as an indicator of remote pollution (Scott et al., 2002)



©EPI-NET www.epi-net.org

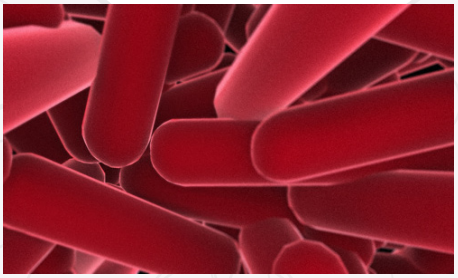
## ANAEROBIC INDICATORS CONT.

### *Bifidobacterium spp.*

Bifidobacteria are obligate anaerobe, non-spore forming, gram (+) bacteria. They are a major component of the microbial population within the human intestine and they are rarely found in animals.

The human isolates have the ability to ferment sorbitol making them potential candidate indicators of human fecal pollution. Another characteristic that hold this organism as a potential good indicator is the fact that can't replicate once deposited in the environment because of its oxygen restrictions. A disadvantage of using this organism as an indicator is that its survival in the environment is highly variable, bacterial numbers can decrease rapidly (Scott et al., 2002).

A simple method for detection requires sample filtration through a membrane. Filters are placed on Human Bifid Sorbitol Agar (HBSA) (Mara & Oragui, 1983) and incubated for 4 to 6 days at 37°C anaerobically. A positive test will display yellow raised colonies.



©EPI-NET www.epi-net.org

## BACTERIOPHAGE

### *F+ coliphage*

Enteric bacteria are not suitable indicators of pathogen viruses and protozoa. Coliphages are viruses that infect E. coli and they appear to be appropriate indicators for viral contamination and F+ (male specific) aid with the distinction between human and non human fecal contamination. Coliphages are non pathogenic to humans, they are persistent in the environment and also are resistant to water treatment process which makes them suitable indicators of fecal contamination. Also detection methods are rapid, easy, inexpensive and reproducible, therefore could be used for MST.

The F+ RNA coliphages are highly correlation with pathogenic viruses in waste water (raw and treated). There are 4 sub-groups, phages belonging to subgroup II and III indicate human feces and the ones from subgroup I and IV indicate animal waste. It does not allow distinguishing between animal sources. The presence of this bacteriophage seemed to be influenced by temperature (Cole et al., 2003).

## REFERENCES


Bitton, G. 2005. *Microbial indicators of fecal contamination: application to microbial source tracking.* Report submitted to the Florida storm water association

Budnick, G.A., R. T. Howard and D.R. Mayo. 1996. *Evaluation of Enterolert for enumeration of enterococci in recreational waters.* Appl. Environ. Microbiol. 62: 3881-3884.

Cole, D., S. C. Long and M. D. Sobsey. 2003. *Evaluation of F+ RNA and DNA coliphages as source-specific indicators of fecal contamination in surface waters.* Appl. Environ. Microbiol. 69: 6507-6514.

Mara, D. D., and J. I. Oragui. 1983. *Sorbitol-fermenting bifidobacteria as specific indicators of human faecal pollution.* J. Appl. Bacteriol. 55: 349-357.

Scott, T. M., F. B. Rose, T. M. Jenkins, S. R. Farrah and J. Lukasik. 2002. *Microbial source tracking: Current methodology and future directions.* Appl. Environ. Microbiol. 68: 5796-5803.

Straub, T. M. and D. P. Chandler. 2003. *Towards a unified system for detecting waterborne pathogens.* J. Microbiol. Methods. 53: 185-197.