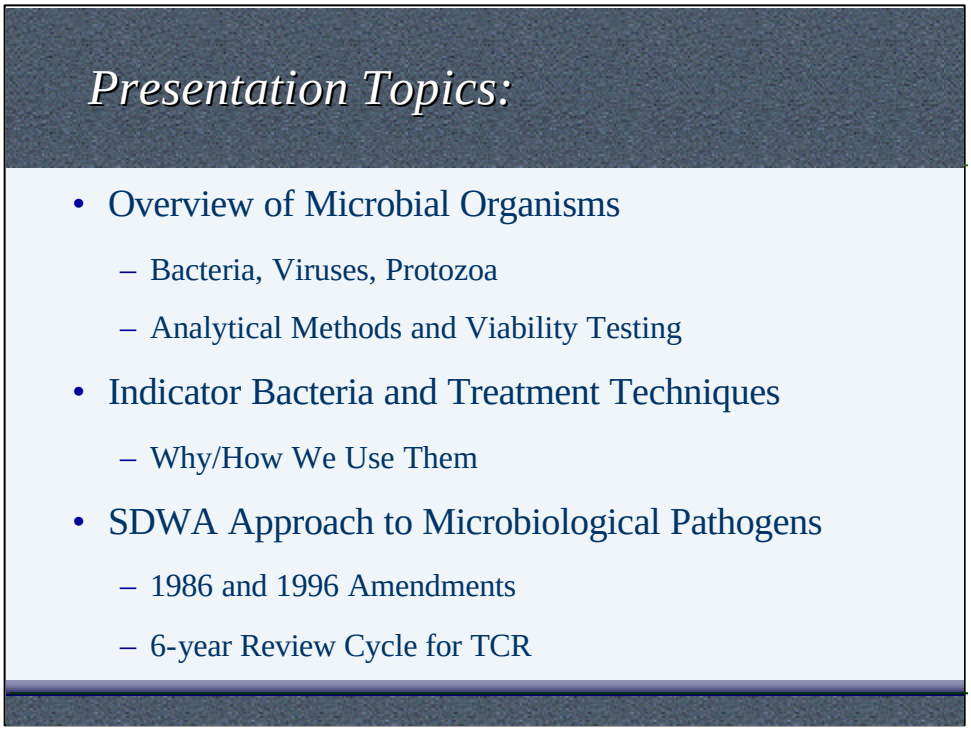
The following slides provide an introduction to the protection of public health from the most common types of microbial pathogens found in drinking water. Pathogens are defined as organisms that cause disease. This introductory session was designed to provide basic microbial background for subsequent sessions on the Surface Water Treatment Rule and Total Coliform Rule.



A brief overview of three common types of pathogens is provided (bacteria, viruses and protozoa), as well as the limitations of currently-available (as of Summer, 2001) methods for microbial monitoring and viability assessment for each type. Analytical and viability testing methods undergo nearly continuous improvement so the methods described herein should be considered examples and not state-of-the art techniques.

In light of the limitations of currently available analytical methods, desirable characteristics of indicator organisms are also reviewed and compared to currently used indicators (total coliforms, fecal coliforms and turbidity). The establishment of treatment techniques in lieu of pathogen monitoring is discussed as an introduction to the application of treatment technique requirements in the Surface Water Treatment Rule.

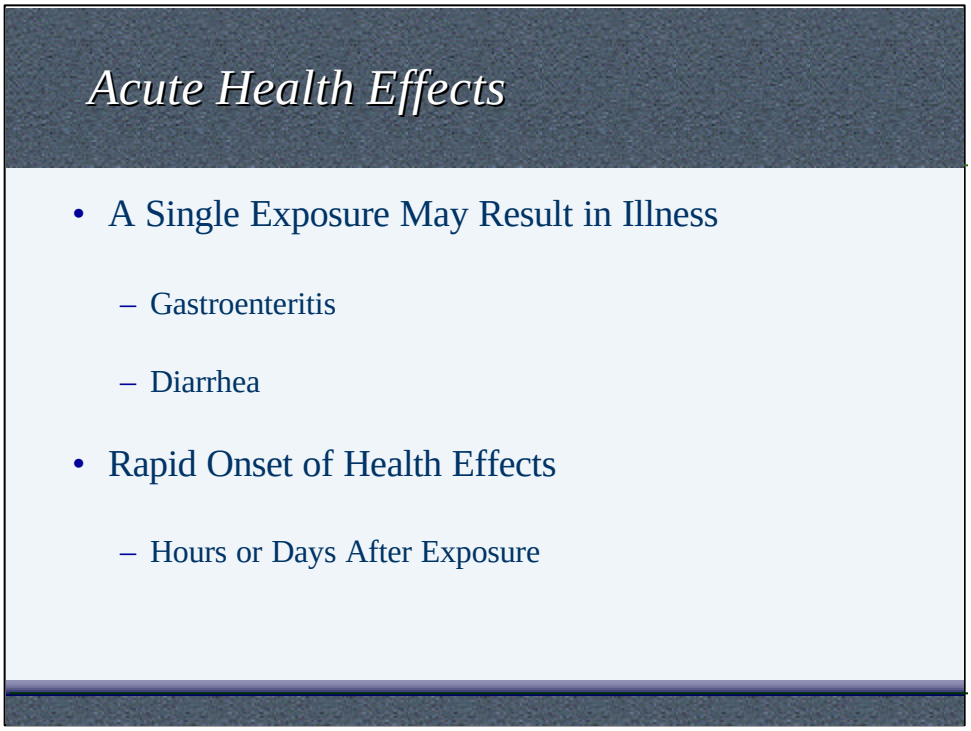
Because this session was designed as an introduction to the regulation of microbial pathogens in drinking water, pertinent provisions of the 1986 and 1996 SDWA Amendments as well as the review of the current Total Coliform Rule are summarized.

The Challenge of Microbial Organisms

- What They Do Was Known Before What They Are
  - Hippocrates: Boil and Strain Water ~300BC
  - Chlorination to Prevent 'Child Bed Fever' - 1846
  - John Snow Closes the Broad Street Well, London, 1854 - Terminates Cholera Epidemic
- What They Do Was Known Before How To Detect Them In Drinking Water

The challenge of addressing microbial organisms in drinking water is unique. Historically, we have been able to determine the health effects of microbial pathogens long before we have had reliable methods to identify them and recover them from contaminated water. Contrast this pattern to that for chemical contaminants – where the chemicals are relatively easily identified down to minute concentrations in water, yet their health effects may remain more elusive.

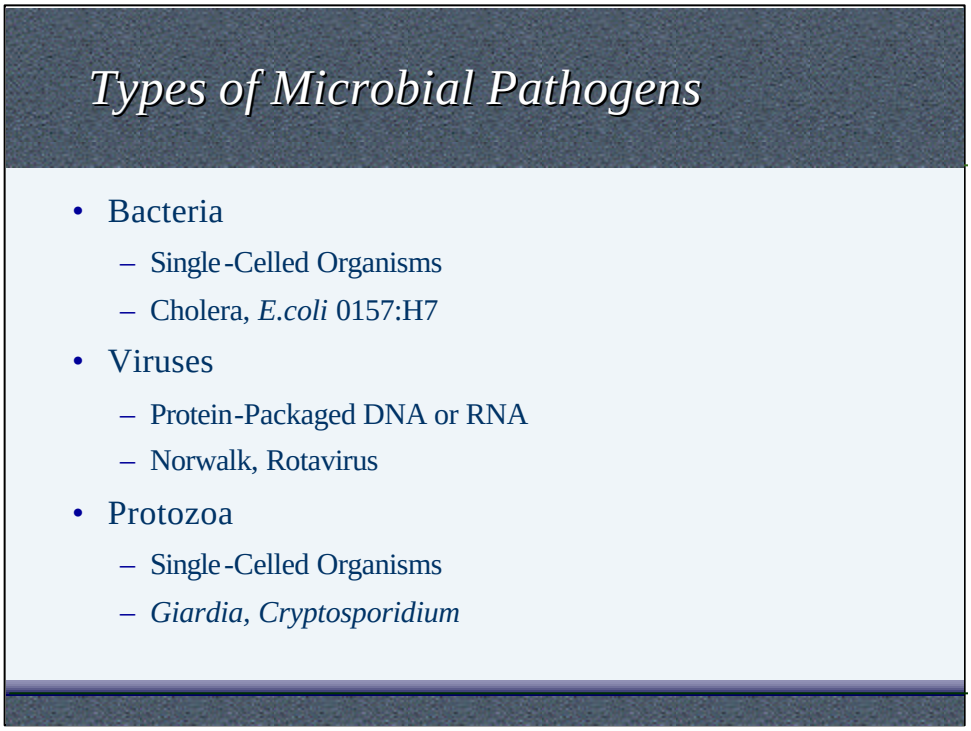
Early reports of health protection measures for microbial organisms included boiling, straining and chlorinating water to avoid exposure to the unknown agents of disease. Improvements in the understanding of the transmission of disease included the classic epidemiological study of John Snow, where in 1854 he revealed a large number of cholera cases centered around the location of the Broad Street well. Rendering the well unusable resulted in a significant reduction in the number of cholera cases by local residents previously reliant on the well.



Diseases caused by microbial pathogens are typically ***acute health effects***. Acute health effects have a relatively rapid onset of disease – usually within a matter of days. Examples of acute health effects include gastroenteritis (gastrointestinal disease) and diarrhea. Intestinal cramping, nausea, vomiting and/or fever are common symptoms.

Acute health effects may also be the result of a single exposure to a disease-causing organism. The amount of exposure required is reliant on the number of organisms contained in the exposure vehicle (such as in a glass of water or in water used for food preparation) as well as the susceptibility of the individual to the disease. Studies have shown it may take as few as 1-10 individual organisms of some microbial pathogens to cause disease.

People who are very young, very old or immune compromised are more susceptible to microbial agents of disease than are most otherwise "healthy" people. They may also be less able to recover from the illness. However, even individuals with no health problems are often victims of microbial waterborne disease outbreaks.



In this presentation, the three most common types of microbial pathogens are addressed. They are bacteria, viruses and protozoa. Other types of organisms (such as multicelled organisms and some algae) are also capable of causing disease, but are not discussed in this overview.

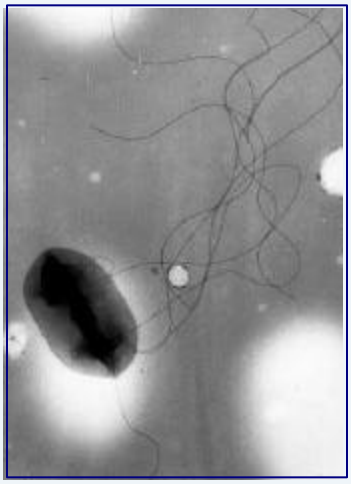
Bacteria are single-celled organisms of around 1 micron (or 1/1,000 millimeter) in length – although this varies greatly. Two common examples are *Vibrio cholera*, the organism responsible for the disease cholera, and *Escherichia coli 0157:H7*, a more recently recognized pathogen which has caused severe illness and death in recent waterborne disease outbreaks in North America. Bacterial disease is often caused by toxins, which are powerful chemical poisons.

Viruses are protein coated DNA or RNA material. They rely on their host-cell to replicate their genetic material and make new viruses. Virus particles are extremely small - typically less than 100 nanometers (or 1/10,000 millimeter) in diameter. Hepatitis and Polio are caused by well-known viruses, and more recently recognized waterborne disease agents include the Norwalk and Rotaviruses. Viruses cause disease by causing their replication in a cell, rupturing that cell to release numerous virus particles and invading other cells. Viruses may also alter the functioning of invaded cells.

Protozoa are single-celled organisms which invade or colonize the intestinal lining of their hosts. They are larger than bacteria, falling in the 3-20 micrometer (3-20/1,000 millimeter) size range. Intestinal illness is experienced as these organisms continue to invade and the hosts' body tries to eliminate them from their system. Protozoa of waterborne disease significance include *Giardia lamblia* and *Cryptosporidium parvum.*

Bacteria

- Detection Requires Growth in Specific Media
- Cell Division Produces "Colonies"or Measurable Enzymatic Reactions
- Colony Characteristics and Unique Metabolic Abilities Identify the Organism
  - Photo: CDC. *E. coli* 0157:H7

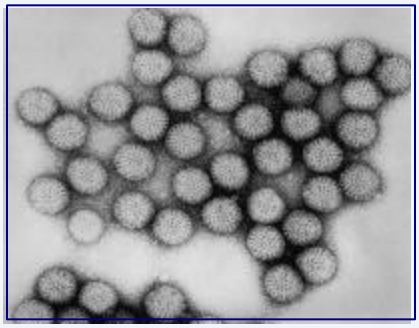


The detection of specific bacteria in a sample of water relies on the multiplication of the bacterial cells under controlled conditions. The controlled conditions may include limited sources of food provided in the growth medium, a specific incubation temperature and the amount of oxygen provided during the incubation period.

Bacterial cells multiply by asexual division – that is, they basically just split in half after internally manufacturing duplicates of each of their internal organelles. One cell becomes two, two become four and four become 8, etc. On a solid growth medium, the bacterial divisions result in a pile of cells, or colony, which is visible to the naked eye or under a microscope. The colony may have a characteristic color or sheen to it which helps identify it as a specific bacterial type. In liquid growth media, the cell culture will appear cloudy and may have a characteristic color resulting from a unique bacterial enzyme reacting with a certain ingredient in the media. Additional steps are usually required to confirm if the growth is of a particular species of bacteria. These steps verify organism-specific metabolic abilities.

Viruses

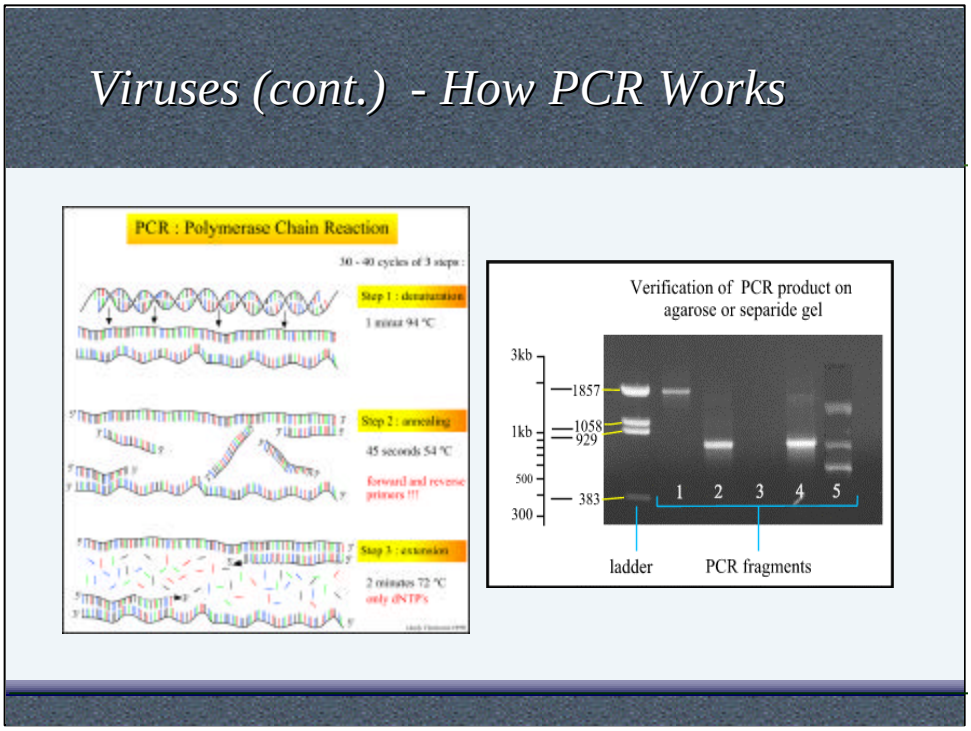
- Detection Requires Cell-Culture Techniques or Polymerase Chain Reaction (PCR).
- PCR Cannot Determine Viability
  - Photo: Rotavirus, ASM Digital Image Collection



The detection of viruses is much more complicated than that for bacterial cells. Because virus particles cannot multiply without a host cell to replicate their genetic material, there are no known growth media available to support their independent division. The small size of the particles further complicates their detection as it takes special slide preparations to see them. The photo provided on this slide is a scanning electron micrograph. This method bounces electrons off of gold-coated virus particles so that an image of the outer surface of the particles can be detected.

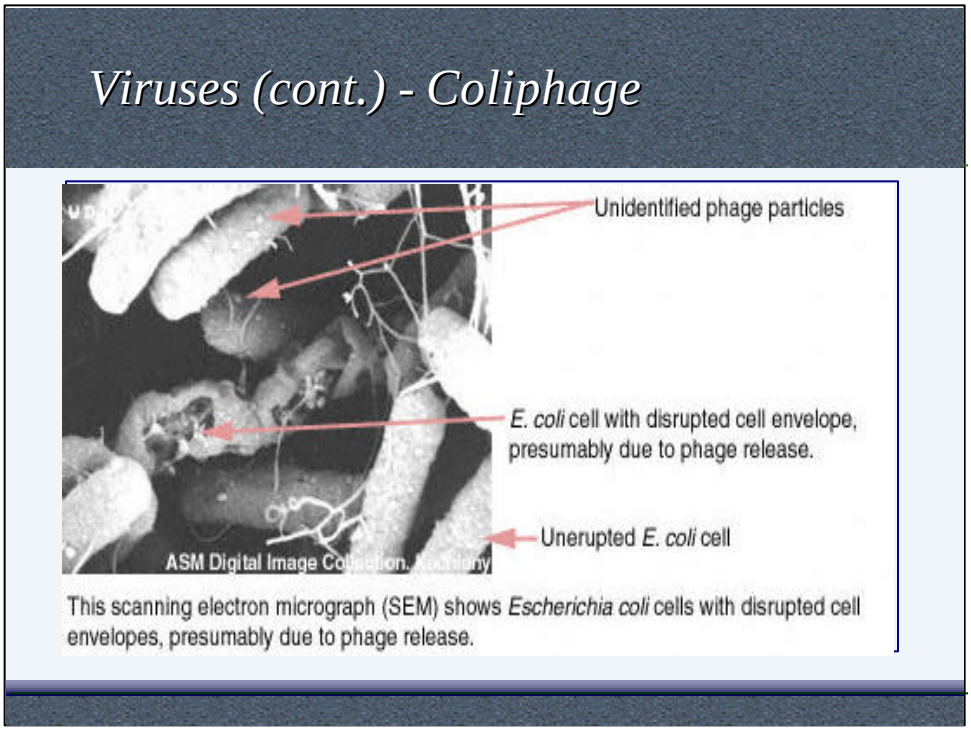
Detection of virus particles in a water sample is typically done through one of two relatively complicated analytical methods - cell culture technique and polymerase chain reaction (PCR).

In cell culture technique, a 'lawn' of host cells is grown on a flat surface. The water sample is applied to the surface of the lawn of cells with the intention that if any viruses are present, which happen to be able to use that particular cell line as a host, they will invade a cell, be replicated, subsequently rupture their host cell, enabling invasion of adjacent cells. Ultimately their detection is reliant on creation of a 'hole' in the lawn of host cells. The cell line used to host the virus particles must be compatible with the virus being sought. Unfortunately, most human viruses do not have known host cell lines identified and available.



Polymerase Chain Reaction (or PCR) is a more recently developed analytical method. In PCR, a machine is used to chemically unwind the virus's genetic material and replicate specific sections of the material millions of times. This replication provides a larger sample of the targeted sections of genetic material, which are then compared to the genetic material from a known virus particle using gel electrophoresis. Gel electrophoresis is used to separate components of the resultant genetic mass by subjecting the mass to a controlled electric field. How far portions of the mass move in the field is determined by the material's molecular weight.

While PCR allows more rapid detection of the presence of genetic material, there is no way to determine the viability of the original virus particles. Care must also be taken to ensure specific viruses are identified by targeting highly-specific sections of their genetic material, otherwise false-positive or false-negative results may be obtained.



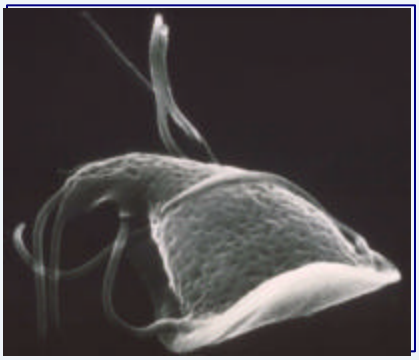
Because of the limitations of the cell culture technique and PCR technology, a method to detect the viruses of bacterial cells (not human hosts) has gained popularity in some arenas.

Coliphage is the name of a type of virus which attacks *E. coli*, a bacteria. Detecting coliphage is believed by some to be a fair indicator of the potential for a virus which is specific to humans to also be present.

As shown in this slide, cells of *E. coli* are ruptured by the release of coliphage. Because *E. coli* is relatively easy to grow under laboratory conditions, a cell culture technique using *E. coli* as the cell line is used to detect the presence of coliphage in water. Since *E. coli* is a typical bacterium found in the intestines of humans and in fecal material or sewage, finding a virus that attacks the bacterium may serve as a surrogate for the presence of viruses that infect humans.

Protozoa

- Detection Requires Identification of Individual Organisms, Usually by Immunofluorescent Antibody (IFA) Techniques.
- IFA Cannot Determine Viability.
  - Photo: *Giardia* Trophozoite, S. Erlandsen

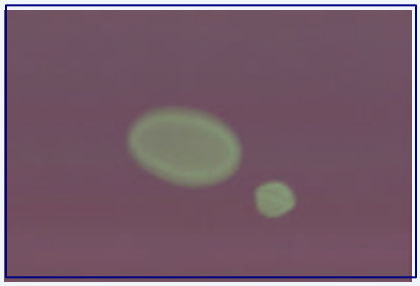


Detection methods for protozoa currently require the identification of individual organisms in a water sample. The protozoa of concern in drinking water cannot currently be cultured in a laboratory media. PCR techniques are under development and have shown some promise for potential future use.

While this slide shows the trophozoite form of *Giardia,* the lifecycle stage which is active and multiplying in the intestines of the host animal, detection in water samples targets the dormant cyst-stage.

Protozoa (cont.) - IFA Techniques

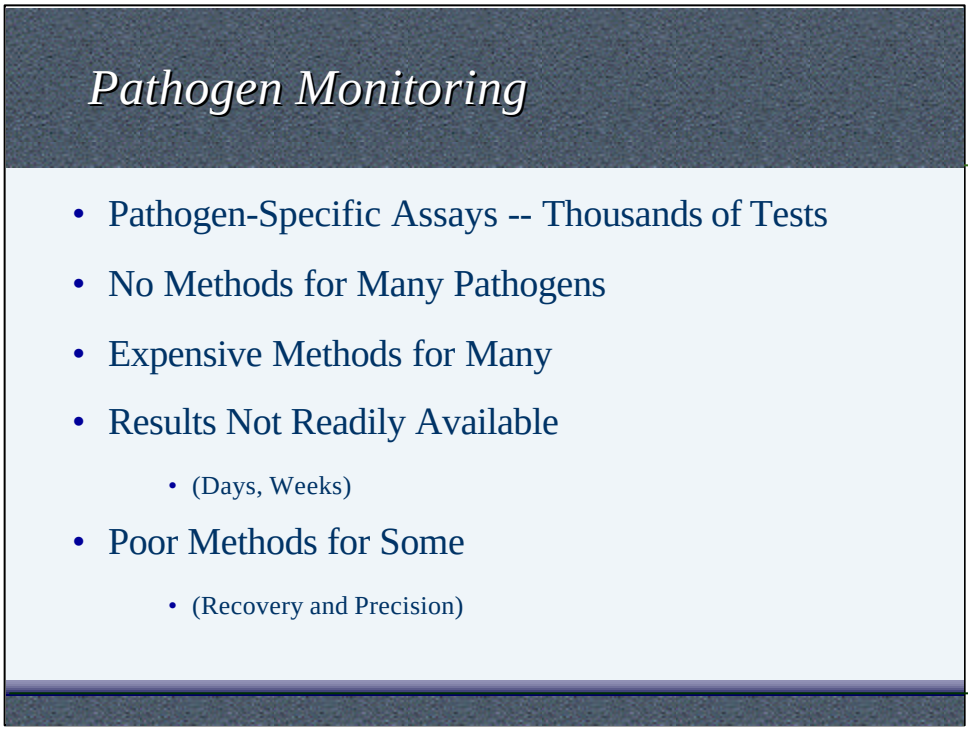
- **Concentrate Particles From Water**
- **Separate Target From Other Particles (Immunomagnetic Beads)**
- **Identify Organism By Epifluorescence; Confirm Internal Structures (DIC)**
  - Photo: Fluorescing *Giardia* and *Crypto.*, CH Diagnostics



Water is analyzed for the presence of protozoans by passing it through a filter which traps the organisms (and a lot of other debris), washing the organisms and other debris from the filter, separating the organism from the contaminating material (currently based on an immunomagnetic method) and looking for the organisms using a microscope. Immunofluorescent antibody techniques are used to help identify specific types of organisms.

This slide shows an immunofluorescing *Giardia* cyst and *Cryptosporidium* oocyst. The *Giardia* cyst is the larger organism, at about 10-12 micrometers in diameter. *Cryptosporidium* oocysts are 4-6 micrometers in diameter. The green color is caused by a fluorescent dye having been attached to a specific immunoglobulin, which in turn is attached to the cyst or oocyst. When analyzing a water sample the microscopist first scans for these green shapes of the appropriate size, then confirms the organism by viewing internal organelles.

Although recent improvements have been implemented in the method, it has a relatively low precision and accuracy, and a labor-intensive sample preparation requirement.



In summary – whether a microbial pathogen is a bacteria, virus or protozoan, methods to recover them from water are varied. If specific pathogens were to be identified in a water sample, literally hundreds of organism-specific methods would have to be applied to each sample.

No methods are available for many pathogens, particularly viruses for which no known cell culture line exists and for which PCR has not been developed.

Analytical costs vary for these assays, from about $25.00 for a bacterial test to over $1,000 for cell culture techniques for virus samples. Protozoan analyses typically cost about $300-400.

The fastest turn-around time for sample analysis-to-result is 24 hours for one type of bacteria test, excluding sample transit times. Due to the complicating factors of cell culture maintenance and sample incubation time requirements, virus assays may take several weeks for results to be available. Results of protozoan monitoring as well as specific bacterial tests often take a week or more from sample collection to organism confirmation.

Lastly, because the test methods for some organisms are limited in precision and accuracy, sample results which are negative for the target organisms may not reflect the absence of that organism from water. This also limits the validity of comparing the concentration of an organism found at one site on one day to other data since the analytical method itself is of limited accuracy.

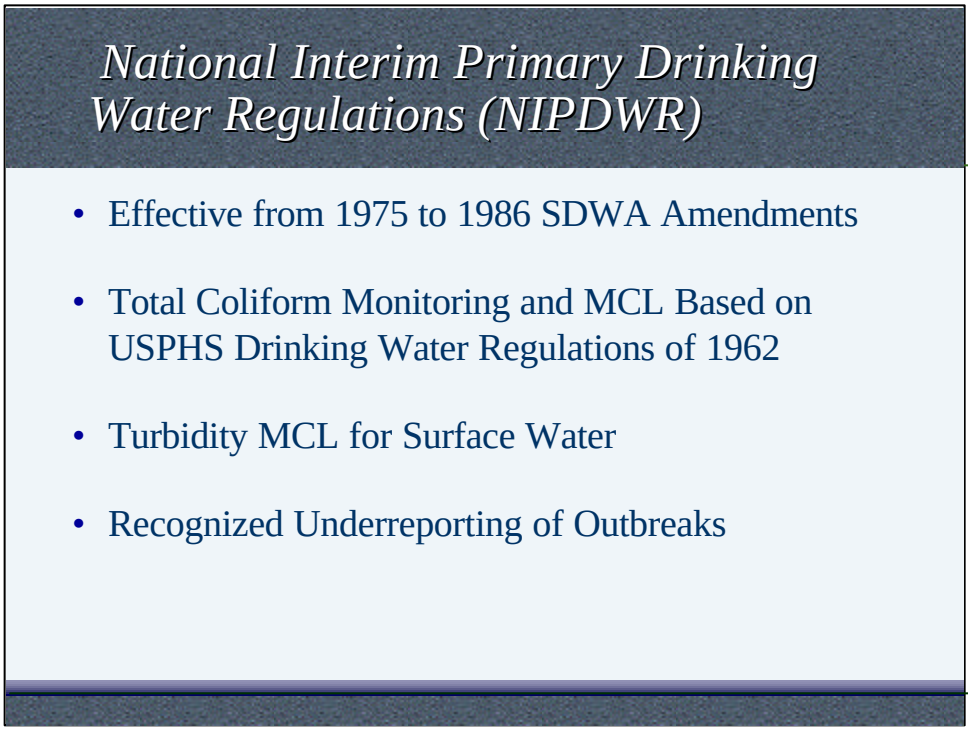
Solution: Indicator Organisms and Treatment Techniques

- Indicator Organisms:
  - The Presence of an Indicator Organism Indicates a Pathogenic Organism May Also Be Present
- Treatment Techniques:
  - Protects the Public Health by Providing Treatment to Control Pathogenic Organisms, Even If They Are Not Detectable

One solution to the problems inherent to analyzing water for individual organisms is to use an indicator organism as a surrogate for a group of pathogens, or a treatment technique method of public health protection.

The presence of an indicator organism is used to indicate a disease-causing organism may also be present. It does not guarantee the presence of the pathogen, but suggests the mechanisms and pathways are in place which could enable contamination by a pathogen.

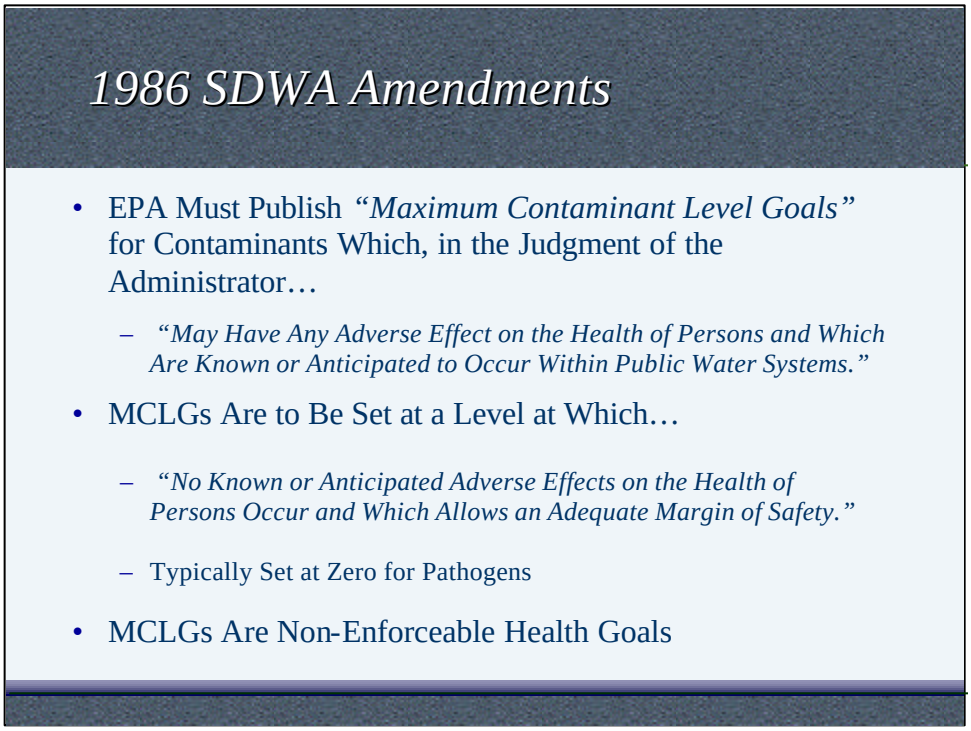
Treatment techniques are regulatory requirements applied to a water source when there are no economical or feasible methods available to monitor for a pathogen or an adequate surrogate for that pathogen. This is the case for *Giardia* and *Cryptosporidium*. There are no indicator organisms known which would adequately represent their presence in water or their response to water treatment methods. Filtration with disinfection is therefore specified as a treatment technique, and watershed control programs with disinfection are specified for high-quality and controllable waters.



The National Interim Primary Drinking Water Regulations were implemented in response to the original Safe Drinking Water Act of 1974. These regulations were in effect from 1975 until the implementation of the 1986 SDWA amendments occurred.

These interim requirements were largely based on the Public Health Service Drinking Water Regulations of 1962 – which regulated interstate carriers of water.

The regulations were relatively limited in scope, addressing total coliforms, turbidity, and a limited number of organic and inorganic chemical contaminants. By 1986, there was a recognized underreporting of waterborne disease outbreaks as well as the occurrence of outbreaks in water systems meeting the requirements of the SDWA.

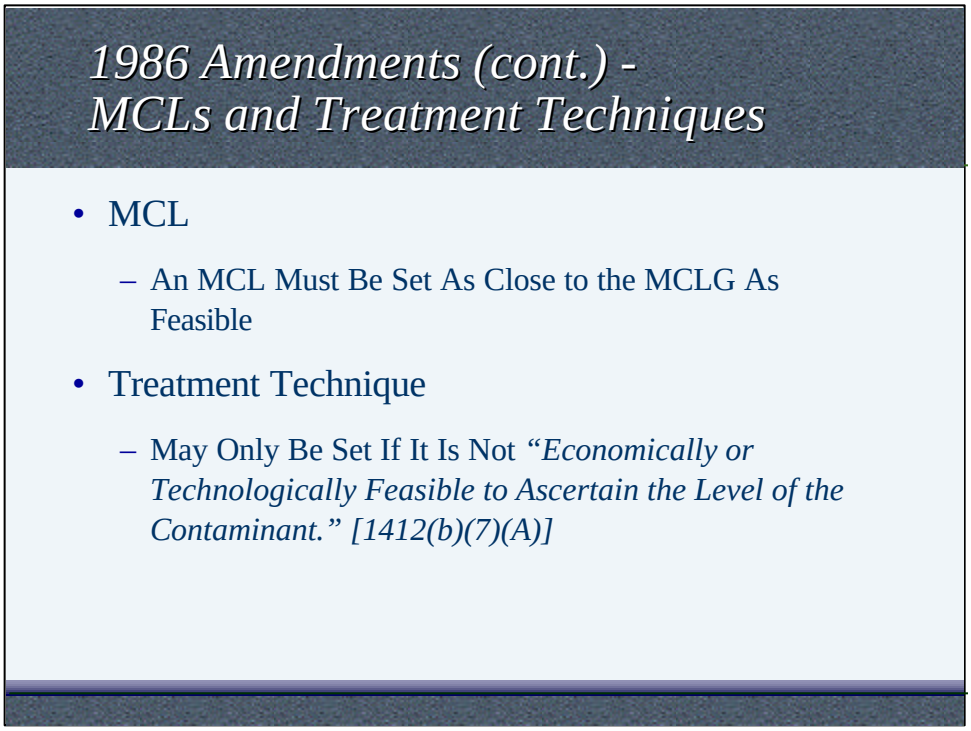


In 1986 Congress passed amendments to the SDWA which significantly tightened drinking water requirements.

Maximum Contaminant Level Goals were established for all regulated contaminants. This is the level at which no known adverse health effects would be expected to occur, allowing for an adequate margin of safety.

For pathogens, for which some are known to cause disease with ingestion of only one organism, the MCGL is typically set at zero – meaning water with one or more of the regulated organisms present would exceed the MCLG.

While MCLGs are non-enforceable health goals, they are used as the target for enforceable Maximum Contaminant Levels (MCLs).



The regulated limit of a contaminant in drinking water is the Maximum Contaminant Level, or MCL. This level is set as close to the MCLG as feasible, taking cost into consideration.

Treatment Techniques were implemented where it is not economically or technologically feasible to determine the level of the contaminant in water. In the SDWA Amendments of 1986, this was applied to systems subject to *Giardia* or virus contamination. Cryptosporidium was not included in the 1986 Amendments as there was insufficient information about the organism and effective treatment technologies to impose a regulatory requirement at that time.

1986 SDWA Amendments (cont.)

- EPA Must Promulgate NPDWRs for 83 Contaminants
  - *"A Group of Related Bacteria Known As Total Coliforms Is One of the 83 Contaminants Which EPA Must Regulate."*
  - Total Coliforms Include Fecal Coliforms and *E. coli*
- Public Notification Requirements

The 1986 Amendments required EPA to promulgate National Primary Drinking Water Regulations for 83 contaminants. Total coliforms, including fecal coliforms and *E. coli*, were specified among those contaminants.

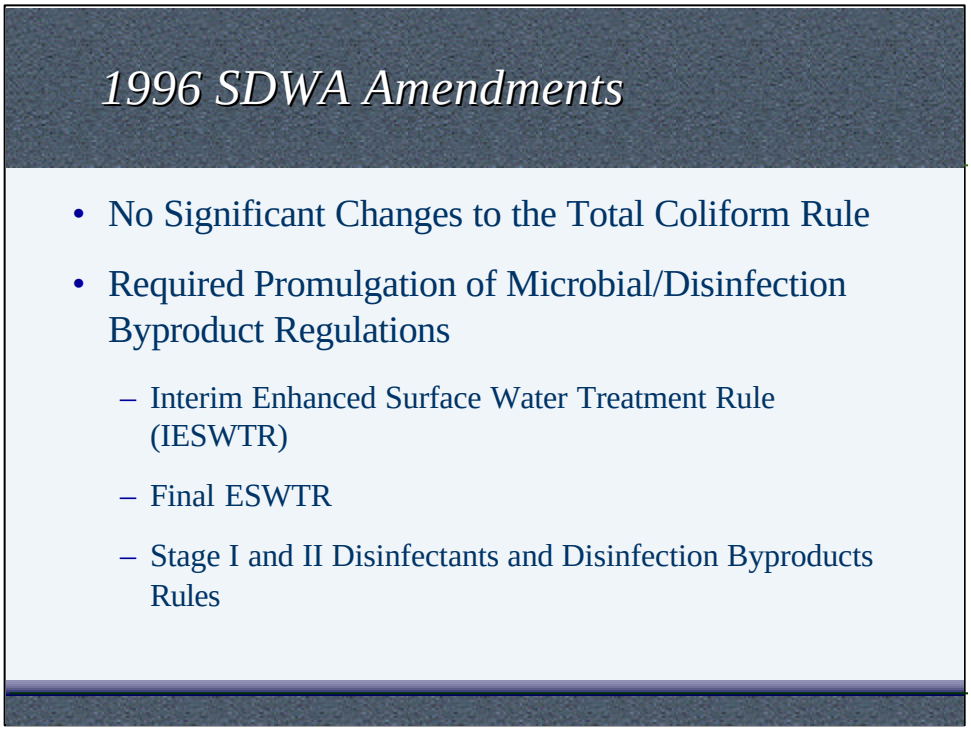
Public notification requirements were also established as part of the public right-to-know provisions. Public notice was required for the following violations:

•Failure to Comply With Monitoring

•Failure to Comply With MCL, Treatment Technique or Testing Procedure

•Existence of a Variance or Exemption

•Failure to Comply With the Schedule Prescribed for a Variance or Exemption



The 1996 amendments added regulation of *Cryptosporidium* to the list of regulated contaminants and required promulgation of regulations governing disinfection byproducts. These changes were to be implemented through:

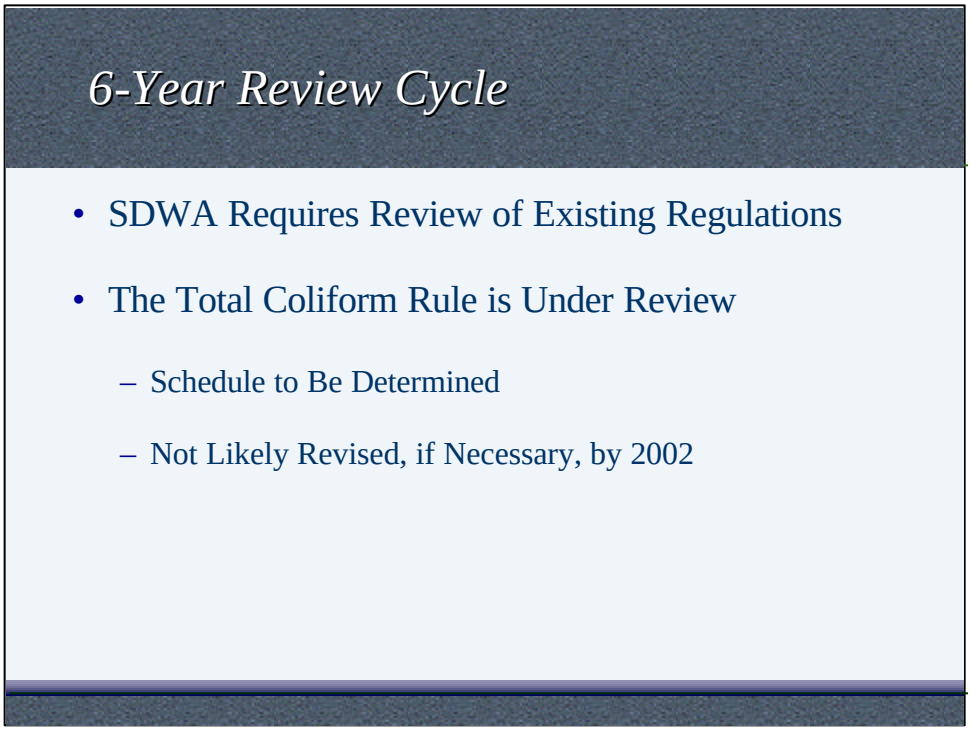
•The IESWTR (affecting systems serving 10,000 or more people and using surface water or ground water under the direct influence of surface water);

•The Final IESWTR (now the LT1 and LT2 ESWTR); and

•Two phases of rules addressing disinfection byproducts.

Disinfection byproducts had become more important due to increasing knowledge of their health effects. This was combined with the application of water treatment practices of relatively high doses of disinfectant held in water for significant periods of time for inactivation of disinfectant-resistant organisms.

This cluster of regulations governing both microbial control and disinfection byproducts was devised to balance the health risks of disinfection byproducts with the health benefits of using disinfectants to protect the public health from microbial pathogens.



As required by the SDWA, each regulation is to be reviewed every 6-years after promulgation. As of 2001, a review of the Total Coliform Rule was underway. As of June, 2001 the schedule for the completion of the review and any potential proposed revisions had not been determined.