THE INTERNATIONAL BOTTLED WATER ASSOCIATION

Guidance Summaries for the U.S. Environmental Protection Agency’s Candidate Contaminate List 4 (CCL4) - Microbiological Contaminants and Other Microbes of Drinking Water Interest*

Introduction

The U.S. Environmental Protection Agency’s Candidate Contaminate List 4 (CCL4) is a list of contaminants that are currently not subject to any proposed national primary drinking water regulations, that are known or anticipated to occur in public water systems, and which may require regulation under the Safe Drinking Water Act (SDWA).

The list includes, pesticides, disinfection byproducts, chemicals used in commerce, waterborne pathogens, pharmaceuticals, and biological toxins. The Agency considered the best available data and information on health effects and occurrence to evaluate thousands of unregulated contaminants. EPA used a multi-step process to select 116 candidates for the CCL 4 list. The final CCL 3 includes 104 chemicals or chemical groups and 12 microbiological contaminants. ¹The draft CCL4 list contains no new microbial contaminants and is currently being revised by the EPA. The finalization of CCL4 will be in the near future. More details on CCL4 can be found here: http://www2.epa.gov/ccl/microbial-contaminants-ccl-4

The International Bottled Water Association’s Virus and Microbial Subcommittee has prepared this summary document on the 12 microbiological contaminants to provide guidance for bottling facilities. In addition, there is an Addendum to this document that holds information on other microbes of interest to the bottled water industry that are not on the CCL4 list currently. This document is meant to be an educational tool and provide guidance. For further information on these organisms and the SDWA, please go to http://www.epa.gov/ogwdw000/ccl/ccl3.html or http://www.epa.gov/fedrgstr/EPA-WATER/2009/October/Day-08/w24287.htm for the federal register notice.

*Note: Bottled water, source water and finished product, should meet the standards of the IBWA Code of Practice. To review the Code of Practice, CLICK HERE.

¹ http://www.epa.gov/ogwdw000/ccl/ccl3.html
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CCL4 Agent Summary: Adenovirus- Bottled water has a negligible risk for Adenovirus contamination. Any threat of contamination can be further reduced with secondary disinfection or filtration. Modified membrane filtration procedures may need to be developed and evaluated to detect viruses in large volume. Ultrafiltration with a nominal pore size of 0.01 mm is capable of achieving 4-log or more virus removal.

1. Microbe/pathogen type (e.g. parasite, bacteria, virus): Virus

2. Why is organism important in the infectious disease world? Adenovirus is highly resistant to UV disinfection and survives in water for long periods of time. There are many different Adenovirus serotypes, each having different symptoms—commonly cause gastroenteritis, respiratory illness, and conjunctivitis.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources?
   a. Outbreaks associated with swimming pools and recreational waters have been reported for Adenoviruses causing conjunctivitis. Adenoviruses have been detected in finished drinking waters (US, Canada, South Africa and South Korea), river and lake waters, and coastal waters. Prevalence in each is not known.
   b. There are no known outbreaks associated with bottled water and there are no published surveys.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? Adenovirus infection leading to gastroenteritis (Adenoviruses 40 and 41) is transmitted by the fecal/oral route. In addition, ingestion of contaminated water or food can lead to infection as well as secondary transmission from human to human. Aerosolized water droplets can act as vectors for other Adenoviruses through inhalation or direct contact with mucosa (eyes).

5. Viability (Stability) in bottled water or BW sources. Adenovirus does not grow outside the body of their host, however it can withstand inactivation under environmental conditions. All adenoviruses are resistant to lipid solvents, sodium deoxycholate, trypsin, 2% phenol and 50% alcohol. They are resistant to exposure at pH 3 to pH 9, but are inactivated by 1:1,000 formalin.

   a. Virus more resistant than most bacteria but fairly susceptible to oxidants and UV. Disinfection likely following $Ct$ recommendations of EPA guidance document ("$Ct^*$- C is the residual disinfectant concentration(mg/L) and t is the
time (in minutes) that water is in contact with the disinfectant. Ct values describe levels of inactivation under various operating conditions)


c. Ultra filtration/ Reverse Osmosis: Virus particle size: 80-90 nm. *Note, microfiltration can be an effective removal technology but likely prohibitively expensive. Viral removal cartridge filters (20 nm) tend to have low flows (1L/10") and very high prices. Microfiltration systems may reduce viral level and these are not prohibitively expensive. Although, some studies do suggest microfiltration is not the recommended method of removal (MicroRisk EU project) many agree that microfiltration, ultrafiltration, and nanofiltration in addition to reverse osmosis is capable of removing these particles.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? See above: question 3.

How easily is the infection spread from one person to another? Transmissibility of Adenovirus is considered to be as possible as other enteric viruses.

8. Relevance as an indicator—microbe may be considered as model indicators if they represent the worst case.

   a. Adenoviruses may be a good indicator for UV process controls as they are highly tolerant but not for oxidation process controls. However, many serotypes are difficult to culture, especially Adenoviruses responsible of gastroenteritis.

   b. Adenoviruses are considered as indicators of viral contamination in surface waters (rivers, lakes, etc).

   c. HACCP approach to consider? Water filtration and/or disinfection by oxidation should be considered.

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. Infection in cell culture is cumbersome. DNA sequencing of amplified products is required for confirmation of Reverse Transcriptase (RT)-PCR results.* Note, whether detection is by PCR or included viability confirmation, the presence of nucleic acid alone does not confirm viable infectious organisms.
**CCL4 Agent Summary: Caliciviruses** - Bottled water has a negligible risk for *Calicivirus* contamination when source waters are potable. Any threat of contamination can be further reduced with secondary disinfection or filtration, however there is debate on filtration techniques that are efficient in complete removal of Caliciviruses. Membrane bioreactors (MBRs) which combine biological processes with membrane filtration for water treatment have been shown to be effective.

1. Microbe/pathogen type (*e.g.* parasite, bacteria, virus): **Virus**

2. Why is organism important in the infectious disease world?

   **Two genera in the Family Caliciviridae affect humans:**
   a. *Norovirus* - the leading cause of acute gastroenteritis in the developed world
   b. *Sapovirus* - is much less common cause of acute gastroenteritis, typically causes milder illness than *Norovirus*

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources?
   a. **Norovirus:**
      i. Previous outbreaks associated with municipal drinking water, well water, recreational water, and ice. Food borne outbreaks due to use of contaminated water has occurred (*e.g.* shell fish, produce). *Noroviruses* have been detected in surface waters, sea water and shallow wells. Prevalence in each is not known.
      ii. No known outbreaks associated with bottled water. There has been one survey of prevalence, but detection of virus was not confirmed (Lamothe et al, 2003. AEM 69(11);6541-9.)
   b. **Sapovirus:**
      i. No known outbreaks due to water source but contaminated outbreaks due to shell fish have been reported. Has been detected in wastewater and river waters. Prevalence is not known.
      ii. No known outbreaks associated with bottled water and no published surveys.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? *Water borne and food outbreaks have are typically associated within in dense populations such as in camps, schools, and on cruise ships. Person to person contact is also a method of transmission. Both viruses are also transmitted by the fecal/oral route and through aerosolized vomit. Reducing food-borne transmission can be achieved by improving production and food handler hygiene, although there have been no reported cases of its presence in bottled water.***

5. Viability (Stability) in bottled water or BW sources.
a. *Norovirus* can survive for weeks to greater than 1 month in water at refrigerated and ambient temperatures. *Noroviruses* are resistant to acid and have been shown to still be infective when exposed to a pH of 2.7 for 3 hours at ambient temperature. The virus can survive in shellfish for extended periods (possibly months). It is resistant to desiccation, and is reported to persist on dry surfaces for up to 12 days.

b. *Sapovirus* survival is not well characterized.

   a. Oxidants: more resistant than most bacteria but fairly susceptible. Disinfection likely following Ct recommendations of EPA guidance document
   b. UV: more resistant than bacteria but fairly susceptible.
      i. 20-41 mj/cm² for 4 log reduction in *Norovirus* surrogate organisms.
      ii. Unknown for *Sapovirus*.
   c. Ultrafiltration/ Reverse Osmosis: Virus particle size: 27 nm for *Norovirus*, 41-46 nm for *Sapovirus*. Viral removal cartridge filters (20 nm) tend to have low flows (1L/10") and very high prices. Within validation packages there will be a listing for MS-2 phage removal as a representative model for viruses.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? See above: question 3.
   How easily is the infection spread from one person to another? *Norovirus* is extremely transmissible. As few as 1-10 viral particles are enough to cause infection.
   Transmissibility of Sapovirus is not clear.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. Neither virus is ideal indicators for process controls. There is no culture method for human *Norovirus*. Less is known about *Sapovirus* inactivation characteristics.
   b. HACCP approach to consider? Water filtration and/or disinfection should be considered

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. * Note, whether detection is by PCR or included viability confirmation, the presence of nucleic acid alone does not confirm viable infectious organisms.
a. *Norovirus*: There is no infectivity model for human strains (there is no method to detect infectious Noroviruses). Surrogates are required for inactivation studies. Reverse Transcriptase (RT)-PCR is required for detection. DNA sequencing of amplified products is required for confirmation of RT-PCR results.

b. *Sapovirus*: can be cultured in mammalian cell culture. Reverse Transcriptase (RT)-PCR is required for detection. DNA sequencing of amplified products is required for confirmation of RT-PCR results.
CCL3 Agent Summary: *Campylobacter* - Bottled water has a negligible risk for *Campylobacter jejuni*. Contamination would likely originate post-process. There is no known documented cause of human illness associated with bottled water. Routine product monitoring for this organism is not necessary.

1. Microbe/pathogen type (*e.g.* parasite, bacteria, virus): **Bacteria.** There are 18 species and of these species, several are considered pathogenic to humans, causing enteric and extra-intestinal illnesses. *Campylobacter* spp. are Gram-negative, non-spore forming; curved or small spiral-shaped cells that have characteristic rapid, darting reciprocating motility. *C. jejuni* and *C. coli* are the most common species.

2. Why is organism important in the infectious disease world? **There is a significant number of human food borne illness cases reported annually.** The organism species (depending on year) is among the leading causes of bacterial human food borne illness in the U.S (second to the cause of foodborne illnesses after Salmonella). There are an estimated 1.3 million cases of illness caused by *Campylobacter* per year in the U.S.

Most infections occur by the fecal-oral route. *C. jejuni* is the etiological agent in approximately 95% of outbreaks with *Campylobacter coli* accounting for approximately 4% of outbreaks and other species accounting for the other 1% of outbreaks annually. Studies have shown that as few as 500 cells can cause illness. Recent foodborne outbreaks have shown that responsible *Campylobacter* strains harbor more and more antibiotic resistance.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources? **Water is a common vehicle in the reported Campylobacter outbreaks.** *Campylobacter* are frequently isolated from aquatic environments, especially in surface waters. Most infections occur by the fecal-oral route. *Campylobacter* spp. can be classified as zoonosis, because animals (birds) are the main reservoir of these organisms.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? **Water consumption is the secondary mode of entry.** Entry is usually associated with consumption of undercooked or raw meats or cross contamination of cutting surfaces.

5. Viability (Stability) in bottled water or BW sources. **Campylobacter** are sensitive to ozone treatments, however they do possess regulatory systems used to aid in oxidative and aerobic stress responses for survival. *Campylobacter* have been shown to enter into a viable but non-culturable state in water and survive for several months. If post-process contamination did occur the organism may be viable, but most likely in a continuous state of decline due to insufficient nutritional requirements.
6. Sensitivity/Resistance to common BW production processes - likelihood of being in a bottle in an infectious form. In general, the genus is very sensitive to common bottled water production processes such as filtration, UV, and oxidants. Studies have shown that certain strains can exhibit elevated tolerances to oxidants.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? Outbreaks are mainly associated with consumption of undercooked or cross-contamination of raw meat and poultry products. Outbreaks have been associated with water, but no documented case with bottled water or potable water. Therefore, in regards to human illness, consumption of bottled water presents very low to no risk of human illness.
How easily is the infection spread from one person to another? Transmission from person to person rarely occurs.

8. Relevance as an indicator - microbe may be considered as model indicators if they represent the worst case.
   a. Routine product monitoring for this organism is not necessary and provides little to no information in regards to processing efficiency.

   b. HACCP approach to consider? Primary control is the process control

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. Campylobacter spp. is very difficult to culture in the laboratory and requires highly trained personnel for routine recovery from samples. They are microaerophilic and require special incubation environments containing low oxygen. Routine testing usually takes between 2-4 days depending on method. PCR-based methods are now widely used and available but there is no distinction between live and dead cells upon detection.
**CCL3 Agent Summary: Enteroviruses** - Bottled water has a negligible risk for Enteroviruses contamination when source waters are potable. Any threat of contamination can be further reduced with secondary disinfection or filtration.

1. Microbe/pathogen type (e.g. parasite, bacteria, virus): *Virus*

2. Why is organism important in the infectious disease world? There are many different types of Enteroviruses; the most common types are Poliovirus, Coxsakieviruses and Echoviruses. They can differ dramatically in their susceptibilities to disinfectants. Different Enteroviruses have different symptoms; commonly cause poliomyelitis, gastroenteritis, respiratory illnesses, and conjunctivitis.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources?
   a. Outbreaks associated with swimming pools and recreational waters have been reported for Enteroviruses. They have been detected in fresh water and coastal waters, but prevalence in each is not known.
   b. There have been no known outbreaks associated with bottled water or any published surveys.
   c. *Poliovirus* infection from drinking water is of little risk in areas of eradication.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? *Enteroviruses* are mainly transmitted by the fecal/oral route and also by aerosolized water droplets.

5. Viability (Stability) in bottled water or BW sources. *Enterovirus* can survive in water and on surfaces for long periods of time. Enteroviruses grow at a wide pH range (3-10). There is a considerable amount of variability based on conditions of the environment. Stability of the virus in external environmental conditions depends on the temperature, humidity and UV radiation.

   a. Oxidants: more resistant than most bacteria but fairly susceptible. Disinfection likely following Ct recommendations of EPA guidance document. However many Enteroviruses have not been studied and may exhibit greatly different inactivation characteristics.
   c. Ultrafiltration/ Reverse Osmosis: Virus particle size: 27-30 nm. Viral removal cartridge filters (20 nm) tend to have low flows (1L/10") and very high prices.
Within the filtration validation packages there will be a listing for MS-2 phage removal as a representative model for viruses.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source?
   See above: question 3. Transmissibility from person to person has been well documented. *Enteroviruses* can be found in respiratory secretions, such as saliva, sputum or nasal secretions, and in the feces of infected persons. Persons may become infected by direct contact with secretions from an infected person, or by contact with contaminated objects such as drinking and eating utensils.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. *Enteroviruses* are not ideal for process controls as their inactivation characteristics are mostly unknown. (With the exception of Poliovirus)
   b. HACCP approach to consider? *Water filtration and/or disinfection by oxidation should be considered*. Also, UV provides a good mechanism for removal of most enteroviruses.

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. *Note, whether detection is by PCR or included viability confirmation, the presence of nucleic acid alone does not confirm viable infectious organisms. Infection in cell culture can be achieved for many *Enteroviruses* but confirmation requires highly specialized staff. Reverse Transcriptase (RT)-PCR or a combination of cell culture and RT-PCR promotes more rapid detection. DNA sequencing of amplified products is required for confirmation of RT-PCR results.*
CCL3 Agent Summary: *Escherichia coli (O157- highly pathogenic)* - Bottled water is not likely to be a source due to FDA zero tolerance rule for *Escherichia coli*, this negates risk in source water and finished product. There are multiple barriers and quality testing performed, thus risk is reduced for *E. coli* including those producing the shiga toxin. There have not been any reported cases of exposure from ingestion from bottled water.

1. Microbe/pathogen type (e.g. parasite, bacteria, virus):  
   **Gram-negative enterotoxin secreting bacteria (the toxins themselves do not persist in the environment without living E. Coli present)**. *Escherichia coli* (STEC) that produce the toxins are associated with a plasmid that may be transmitted to other Genus—not all *E. coli* produce this toxin.

2. Why is organism important in the infectious disease world?  
   STEC are enterotoxin-producing bacteria causing gastrointestinal illness and kidney failure. Disease signs begin with bloody diarrhea and if the toxin is present may lead to kidney failure (HUS, hemolytic uremic syndrome). The route of all infections is the fecal/oral route.

3. Relevance to waterborne disease presence in water--which types?  
   Bottled water (BW) sources? Surface water? FDA rule: Source water containing confirmed *E. coli* positive (+/- toxin) may not be utilized as source water. Thus, there is little to no risk of *E. coli* (O157) intoxication or infection in bottled water.

   Municipal water prior to boil water notice may be a source of *E. coli* (+/- toxin). This is often associated with cracks in the distribution system and pressure differentials. Additionally, distribution systems located near dairy farms may be associated with fecal contamination and the presence of microorganisms transmitted by the fecal-oral route. Pipe damage from freeze/thaw cycles has a high association with transmission of such organisms to the distributed water system.

   Processed water: specifically water coming from a distributed source has a lower risk than would municipal water prior to a boil water alert due to treatments. However, the FDA zero tolerance rule for *E. coli* (0157) completely negates this risk.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? **The majorities of infections are the result of eating contaminated ground meat (beef), vegetables and fruit that were contaminated with E. coli (O157).** The majority of illnesses associated with infection from water are associated with municipal water.

5. Viability (Stability) in bottled water or BW sources. **In water, E. coli may last 2-3 weeks, possibly longer if water temperature is low. E. coli can also keep their ability to cause infection despite their inability to grow on classical growth media.** As no water
containing *E. coli* may be used for source, any risk would come from active tampering or municipal source and incomplete treatment.

6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. *E. Coli* has a very high susceptibility to oxidants and UV. However, lack of compliance with FDA rule for source water could lead to potential consumer exposure.

In addition, for purified waters from municipal source the following could lead to potential exposure:
- Incomplete testing, ineffective filtration (MF/RO), ineffective UV disinfection, and ineffective ozone disinfection.
- Another risk to consider for any type of water could be incompletely treated rinse water for bottles.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? There have been many outbreaks but none associated with bottled water. There was a high profile outbreak in Walkerton, Ontario associated with water. Also, there are many outbreaks from food sources.

How easily is the infection spread from one person to another? *E. coli* can be spread from person to person by the fecal-oral route

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. As bottled water employs multi-barrier treatments, the relevance of indicators maybe less important when the overall process is considered.
   b. HACCP approach to consider? *E. coli* (not necessarily O157+) is a good indicator of all fecal organisms. Application of HACCP and cGMP are important regardless of final food product.

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. One can culture *E. coli* but depending on the level (CFU/mL) within the source the sample size may be high. Culturing *E. coli* is relatively easy but growth or selective growth in liquid or agar media takes a significant amount of time. There are rapid screening methods based on PCR, when coupled with viability confirmation, to provide an indication of the presence of viable cells. The shiga toxin may be identified by PCR and antigenic tests.
**CCL3 Agent Summary: Helicobacter pylori** - *Helicobacter pylori* contaminating bottled water is of no risk. Surface waters are suspected to be a reservoir but transmission routes still to be validated. There is no known documented case of human illness associated with bottled water. Routine product monitoring for this organism is not necessary and provides no benefit as an indicator.

1. Microbe/pathogen type (e.g. parasite, bacteria, virus): **Bacteria**: *Helicobacter pylori* is a Gram-negative motile microaerophilic bacterium; either spiral or coccoid-shaped and nonspore-forming bacterium. Environmental coccoid forms of the bacteria are non-cultivable.

2. Why is organism important in the infectious disease world? **H. pylori** is responsible of gastritis and duodenal ulcers and could be linked to gastric cancers (Nobel Prize Marshall & Warren, 2005). Almost 50% of the world’s population seems to be infected by *H. pylori*, although most infections are asymptomatic.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources? *Helicobacter pylori* has low relevance, there are no waterborne outbreaks documented. The health significance of finding *H. pylori* DNA in drinking water is unknown.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process?  
   a. Water is suspected to be one of the potential vectors of *H. pylori* by fecal-oral route.  
   b. Transmission routes remain unclear: fecal-oral, oral-oral (saliva), gastric-oral (childhood) or even through endoscopic examination are all potential routes.

5. Viability (Stability) in bottled water or bottled water sources.  
   a. *Helicobacter pylori* cannot multiply in water but can persist as cultivable for few days in tap water at low temperature (<10°C). Also, *Helicobacter pylori* can survive longer in non-cultivable coccoid form (>1 month).  
   b. There is no data of *Helicobacter pylori* being found in bottled water.

6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. In general, the genus is very sensitive to common BW production processes such as filtration and UV. Some studies have shown that *H. pylori* can survive in a viable but non cultivable form in chlorinated drinking water.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? **There have not been any outbreaks documented.**
How easily is the infection spread from one person to another? **Fecal-oral route is the most probable method of spreading Helicobacter pylori.**

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. Routine product monitoring for this organism is not necessary and provides little to no information in regards to processing efficiency.
   
   b. HACCP approach to consider? Primary control is the process control to consider.

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. **There is no standard culture method for H. pylori in water.** However there have been several molecular methods developed including: PCR for spiral and coccoid forms, fluorescent *in situ* hybridization (FISH), and labeled probes.
CCL4 Agent Summary: **Hepatitis A virus (HAV)** - Bottled water has a negligible risk for *Hepatitis A* contamination. Reducing food-borne transmission of *Hepatitis A* can be achieved by improving production and food handler hygiene, although there have been no reported cases of its presence in bottled water. Typical bottled water treatments will eliminate viable viruses, however hygienic practices are encouraged.

1. **Microbe/pathogen type** (e.g. parasite, bacteria, virus): **Virus**

2. **Why is organism important in the infectious disease world?**
   - *Hepatitis A* viral (HAV) infection can yield a contagious liver disease.
   - *Hepatitis A* is usually spread when a person ingests fecal matter, from contact with objects, food, or drinks contaminated by the feces, or stool of an infected person.

3. **Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources?**
   - *Hepatitis A* can be found in every part of the United States and throughout the world.
   - When any water source is contaminated with feces from infected humans, the water can potentially spread the *hepatitis A* virus. The virus can enter the water through various ways, including sewage overflows, sewage systems that are not working properly, and polluted storm water runoff.

4. **Is water consumption/contact the main or secondary mode of entry in pathogenesis process?**
   - *HAV* is excreted in feces of infected people and can produce clinical disease when susceptible individuals consume contaminated water, beverages or foods (such as fresh produce). Contamination of foods by infected workers in food processing plants and restaurants is also possible.

5. **Viability (Stability) in bottled water or BW sources.** *HAV* can be found in raw or undercooked shellfish from contaminated waters, raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler. It can survive refrigeration and freezing for up to two years and it is resistant to acid (pH 1 for 2 hours at room temperature).

6. **Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form.** *Waterborne outbreaks of Hepatitis A* are unusual in developed countries. Water treatment processes and dilution within municipal water systems are sufficient to inactivate *HAV*.
   - **Oxidants:** *HAV* is more resistant to chlorine under certain conditions than some bacteria. *HAV* is susceptible to ozone inactivation with constant flow (ref: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC203196/?page=1](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC203196/?page=1))
b. UV: UV treatment has been shown to inactivate HAV in wastewater and polluted drinking water

c. Ultra filtration/ Reverse Osmosis: Because of the small size of hepatitis A, using a point-of-use filter will not remove it from water; however, adequate chlorination kills the hepatitis A virus.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? There have been no outbreaks related to bottled water for HAV. The last drinking water outbreak was in the United States in 2009 from a private well water consumption. In 1997, frozen strawberries were determined to be the source of a Hepatitis A outbreak in five states (Hutin, et al., 1999), and in 2003, fresh green onions were identified as the source of a Hepatitis A outbreak traced to consumption of food at a Pennsylvania restaurant (Wheeler, et al., 2005). Other produce, such as blueberries and lettuce, has been associated with Hepatitis A outbreaks in the U.S. as well as other developed countries (Butot et al., 2008; Calder et al., 2003).

How easily is the infection spread from one person to another? Hepatitis A is usually spread when a person ingests fecal matter, from contact with objects, food, or drinks contaminated by the feces, or stool of an infected person.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.

   a. HAV contamination of a food product can occur at any point during processing, distribution, or preparation. Specific methods to detect enteric viruses, such as HAV, are necessary, because water with low coliform counts (commonly used as a measure of fecal contamination) have been shown to contain viable HAV.

   b. HACCP approach to consider? Water filtration and/or disinfection should be considered. Proper hygienic practices by workers should be employed.

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. * Note, whether detection is by PCR or included viability confirmation, the presence of nucleic acid alone does not confirm viable infectious organisms. There is a nucleic acid sequence-based amplification (NASBA) technique for the detection of hepatitis A virus in foods, also ELISA (Enzyme-linked immunosorbent assay) and (RT)-PCR techniques work as well.
CCL4 Agent Summary: *Legionella pneumophila* - Bottled water has a negligible risk for Legionella. Legionella would not be a good indicator for bottlers to test for. *Legionella* species can be found in tiny droplets of water (aerosols) or in droplet nuclei (the particles left after the water has evaporated).

1. Microbe/pathogen type: *Legionella pneumophila* is a gram negative bacterium. It is common in the environment. Legionella has 50 species and 70 serogroups identified.

2. Why is organism important in the infectious disease world? *Legionella pneumophila* is the causative agent for Legionnaire's disease. Outbreaks are associated when contaminated water is sprayed and inhaled (or able to be inhaled). Annually, between 8,000 and 18,000 people are hospitalized with Legionnaires’ disease in the U.S. However, many infections are not diagnosed or reported, so this number may be higher. Legionnaires’ disease can be very serious and can cause death in up to 30% of cases. Most cases can be treated successfully with antibiotics and healthy people usually recover from infection.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources?
   a. *Legionella* bacteria are found naturally in the environment, typically in water. Sources can be any aquatic environment and the growth could occur where within water transmission and plumbing systems it can form in biofilms and multiple among dirt. The bacteria grow best in warm water, like the kind found in hot tubs, fountains, vegetable counters in grocery stores, spas, cooling towers, hot water tanks, large plumbing systems and large air conditioning systems.
   b. *Legionella* can survive for prolonged periods of time in tap water but do not grow or multiply unless supported by other organisms, therefore *Legionella* is typically associated with other forms of contamination. If present, *Legionella* often resides in protozoa, where it can multiply and be protected from normal concentrations of chlorine and/or other disinfects used to treat municipal water. The bacteria multiply between temperatures of 20-45°C. A minimum temperature of 60°C is required to kill *Legionella* in hot water systems.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? Water consumption is not the mode of entry for *Legionella*. *Legionella* cannot be spread from person to person. To be infectious, *Legionella* must enter the body via a respiratory route when contaminated water is sprayed and inhaled. Hence, for infection both amplification of bacterium in storage and “spray” into nose are needed.

5. Viability (Stability) in bottled water or BW sources. *Legionella* lives on its own or in amoebas in warm water, at temperatures of 7-58°C, it multiplies between 20-45°C.
6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. There is little to no risk of Legionella outbreaks from bottled water. There is risk that Legionella could multiply in the plumbing in bottled water facilities if conditions are ideal. Chlorination is not a good method to treat water for Legionella, as the bacteria are protected from chlorination when in an amoeba.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? There are numerous documented outbreaks of Legionella around the world. Most documented cases are in Europe and the US, as these are the regions that have the ability and mechanisms to detect and track outbreaks. Summer 2015, there was an outbreak in Bronx, New York linked to the building water supplies of multiple buildings. The death toll reached seven. All of the victims were older adults with additional underlying medical problems.

How easily is the infection spread from one person to another? Spread of Legionella in this manner is not likely.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. Legionellae can be ingested by trophozoites of certain amoebae such as Acanthamoeba, Hartmanella and Naegleria, which may play a role in their persistence in water environments. However, Legionella does not cause disease if ingested.
   b. System treatment options are increasing the temperature to 60°C; Legionella is highly susceptible to Oxidants and to UV. It is best to manage through risk mitigation of building water systems.

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. Legionella is detected by culture on buffered charcoal yeast extract (BCYE) agar. It requires cysteine to grow and does not grow on common blood agar media. The most common method for detection utilizes a concentration step such as centrifugation and / or filtration, sometimes followed by heat and/or acid treatment to knock down other flora commonly present in these water samples. Culture methods are notoriously inaccurate, due to poor growth of the target organism and overgrowth of non-targets. There are several PCR assays commercially available to test for Legionella; most of these do not differentiate viable from non-viable organism.
CCL4 Agent Summary: Non Tuberculosis *Mycobacterium (NTM)* including *Mycobacterium avium complex (MAC)*- Non Tuberculosis *Mycobacterium (NTM)*, especially *Mycobacterium Avium Complex (MAC)* can be present in water resources and survive long periods of time in water, both have negligible risk for bottled water. No known documented case of human illness associated with bottled water. Routine product monitoring for this organism is not necessary and provides no benefit as an indicator organism.

1. Microbe/pathogen type (*e.g.* parasite, bacteria, virus): *Mycobacterium* are aerobic acid-alcohol resistant bacteria, usually Gram-positive, all harboring a typical thick hydrophobic cell wall rich in mycocolic acids, and is also part of the Actinomycetales. Only some are fast-growing. Non tuberculosis *mycobacteria* are clearly distinguished from the group of highly pathogenic *Mycobacteria*: *M. bovis*, *M. leprae*, *M. tuberculosis*.

2. Why is organism important in the infectious disease world? *NTM* and especially those from the *Mycobacterium Avium Complex* are opportunistic pathogens leading to a wide range of diseases depending on the infected organs: pulmonary disease, Buruli ulcer, gastrointestinal inflammatory diarrhea, septic arthritis mainly in immune-compromised people and/or people with predisposing factors.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources?
   a. Surface and drinking water are considered to be the main routes of transmission for *Mycobacteria*
   b. Inhalation of droplets of contaminated tap waters is a major method of transmission (showers, hospitals, spas).
   c. There is only one paper relating presence of *Mycobacteria* in bottled water in Greece (1997).

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? Transmission of *Mycobacteria* occurs mainly through aerosols and inhalation but ingestion of contaminated water has been involved. There is no evidence for person-to-person transmission of *NTM Mycobacteria*.

5. Viability (Stability) in bottled water or BW sources. There is a lack of data on this because isolation of *NTM* is difficult and time consuming. *NTM* has been known to persist in surface and tap waters, and may multiply intracellularly in amoebae and in
water distribution systems biofilms. There is no data of *Mycobacteria* being found in bottled water in the United States.

   
a. Oxidants: *NTM* has a high resistance to chlorine disinfection (chlorine, chloramine, chlorine dioxide).

b. UV: UV treatments are considered to be efficient against *MAC*.

c. Ultrafiltration/ Reverse Osmosis: Microfiltration could remove these organisms (the same true for all bacteria) the reoccurring issue is membrane rating--0.45, 0.2 or 0.1 micron.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? There are generally reports of sporadic contaminations, but no large waterborne outbreaks have been documented.

How easily is the infection spread from one person to another? There is no evidence for person-to-person transmission.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.

   a. Routine product monitoring for this organism is not necessary and provides little to no information in regards to processing efficiency.

   b. HACCP approach to consider? Primary control is the process control to consider

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. Culture-based methods for isolation of are difficult due to the potential slow growth rate of some *Mycobacteria* (over competed by fast growing organisms). Decontamination steps to reduce more rapidly growing organisms can lower the number of *NTM*.

There are several molecular methods developed to test for *MAC*: quantitative PCR, PCR-RFLP for tap water and bottled water, FISH probes, IMS-PCR to concentrate cells. Also, there are some commercial kits available but many lack specificity.
CCL3 Agent Summary: *Naegleria fowleri*- Bottled water is not likely to be a source of *Naegleria* due to multiple barriers. Normal disinfection procedures would remove the risk of presence. Infection with *N. fowleri* is almost exclusively contracted by exposure of the nasal passages to contaminated water.

1. Microbe/pathogen type (virus, bacteria, etc.)- *Amoeba (Protozoa)*
2. Why is organism important in the infectious disease world? *Naegleria fowleri* is very common in recreational waters and is the causative agent of Primary Amoebic Meningitis (PAM).
3. Relevance to waterborne disease presence in water—*Naegleria fowleri* can generally be found in untreated water, especially stagnant water exposed to elevated temperature. *Naegleria fowleri* is most frequently isolated from thermal effluents; hot springs; waters with naturally or artificially elevated temperatures
4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process?

Primary exposure is through swimming and diving- entry through nasal passage. Warm water and water without disinfection residual are high risk. Tap water exposure to *Naegleria fowleri* from bathing has also occurred.

5. Viability (Stability) - *Naegleria fowleri* can survive for years in natural waters. Forms a cyst, but susceptible to chlorine, ozone, iodine. *Naegleria fowleri* grows best at higher temperatures up to 115°F (46°C) 10. Although the amoeba may not be able to grow well, *Naegleria fowleri* can still survive at higher temperatures for short periods of time. The trophozoites and cysts can survive from minutes to hours at 122-149°F (50-65°C) with the cysts being more resistant at these temperatures.


a. *Naegleria fowleri* is not likely to be found in bottled water. Water not disinfected and exposed to elevated temperature i.e. hot springs; geothermal water would be more at risk

b. Standard water treatment practices are effective in killing the organism. Standard chlorine residuals found in drinking water can inactivate *Naegleria fowleri*. A free chlorine level of 0.5mg/L readily kills *Naegleria* cysts. Ozone is also effective.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? There has been reported domestic water outbreaks in Australia. In the US there have been
33 reported infections during 10 yrs 1998-2007. This has occurred in the Southern States. In 2014 *Naegleria fowleri* was found in the finished drinking water supply of St. John the Baptist Parish and in 2013 in St. Bernard Parish in Louisiana.

How easily is the infection spread from one person to another? **The infection is not spread person-to-person; primary exposure to *Naegleria fowleri* is by water entering the nasal passages and the amoeba travelling to the brain and spinal cord**

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case?
   
   a. **This organism would not be considered of strict fecal origin as it occurs naturally in soil as well as in aquatic habitats, particularly warm waters.**
   
   b. **HACCP approach to consider? Primary control is the process control.**

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. **It takes weeks to grow and identify the amoeba. Current research is focused on the development of real time PCR diagnostic methods (nested PCR methods).**
**CCL4 Agent Summary: Salmonella** - Bottled water has a negligible risk for Salmonella. There is no known documented cause of human illness associated with bottled water in the U.S. Routine product monitoring for this organism is not necessary and Salmonella would not be a good indicator organism.

1. Microbe/pathogen type (e.g. parasite, bacteria, virus): **Bacteria. Salmonella** are facultative anaerobic Gram-negative non-spore forming rods belonging to the family *Enterobacteriaceae*. Pathogenicity varies between strains and approximately 2450 serotypes have been identified. *S. enterica* subspecies are considered significant etiological agents for human food-borne related illnesses.

2. Why is organism important in the infectious disease world? **Salmonella** is a leading cause of bacterial human gastroenteritis in the U.S. Infection usually occurs through the fecal-oral route. The natural habitat of *Salmonella* spp. are the gastrointestinal tract of animals, however they are ubiquitous in the environment.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources? There is a very low relevance for *Salmonella*. Most infections occur by the fecal-oral route.

4. Water consumption/contact the main or secondary mode of entry in pathogenesis process? **Salmonella** bacteria live in the intestines of people, animals and birds. Most people are infected with salmonella by eating foods that have been contaminated by feces. Commonly infected foods include raw meat, poultry and seafood (Seafood may be contaminated if harvested from contaminated water), raw eggs and some fresh produce (particularly imported varieties, may be hydrated in the field or washed during processing with water contaminated with salmonella).

5. Viability (Stability) in bottled water or BW sources. Most *Salmonella* serotypes are motile and have an optimal growth temperature between 35 and 40°C. However, dependent on the *Salmonella* strain and the type of food matrix, the range of growth can be between 2 and 54°C. Furthermore, *Salmonella* strains have an optimum pH for sustained growth at 6.5 to 7.5.

6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. **In general, the genus is very sensitive to common bottled water production processes such as filtration, UVL, and oxidants.** Studies have shown that certain strains can exhibit elevated tolerances to temperature, pH, and oxidants.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? Historically, *Salmonella* have been thought of as primarily a pathogen of poultry, meat, eggs, swine, and other farm animals but non-meat products (i.e. tomatoes, cantaloupe, and raw milk) are associated with the majority of documented outbreaks.
In regards to water, the majority of waterborne outbreaks have been associated with the municipal water system. Also, no apparent outbreaks of Salmonella have been associated with bottled water in the U.S. A documented outbreak of S. Kottbus occurred in Spain from bottled water in 2006. There is record of one outbreak in Canary Island with mineral bottled water contaminated by Salmonella.

How easily is the infection spread from one person to another? There is very low risk of spread from person-to-person.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. In general, Salmonella are present in low numbers within environmental or product samples and require a pre-enrichment step. Therefore, utilizing Salmonella in routine monitoring or as an indicator organism in bottled water would not be beneficial in evaluating processing effectiveness.
   b. HACCP approach to consider? Primary control is process control to consider

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. Convention testing for Salmonella takes 3-5 days with presumptive test results in 2 days by rapid methods. Rapid methods provide presumptive results within 1-2 days.
**CCL4 Agent Summary: Shigella sonnei** - Bottled water has a negligible risk for *Shigella Sonnei* Contamination would most likely have to originate post-process. No known documented case of human illness associated with bottled water. Routine product monitoring for this organism is not necessary and provides no benefit as an indicator organism.

1. Microbe/pathogen type (*e.g.* parasite, bacteria, virus) **Bacteria: Shigella sonnei is a non-motile, nonspore-forming, facultative anaerobic Gram-negative bacterium in the Enterobacteriaceae family.**

2. Why is organism important in the infectious disease world? *S. sonnei* is a well-recognized cause of gastrointestinal illness and the most common cause of bacillary dysentery in the United States. The organism is not indigenous in foods and transmission is mainly due to infected food handlers. Salad and Salad bars have been implicated in numerous outbreaks.

3. Relevance to waterborne disease presence in water—which types? Bottled water (BW) sources? There is a low relevance for *S. sonnei*— waterborne outbreaks are rare. When waterborne outbreaks do occur they are associated with wells that have been fecally contaminated.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? **Food consumption such as in salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, milk and dairy products, and poultry is primary mode of infection. Contamination of these foods is usually through the fecal-oral route. Fecally contaminated water (thus, water is a secondary medium of consumption) and unsanitary handling by food handlers are the most common causes of contamination.**

5. Viability (Stability) in bottled water or BW sources. **In laboratory conditions, the organisms has a growth range between 10°C-48°C with an optimum growth temperature of 37°C. The organism can grow in the presence or absence of oxygen and between pH 5-8. If post-process contamination did occur, the organism may be viable, but most likely in a continuous state of decline due to insufficient nutritional requirements.**

6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. **In general, the genus is very sensitive to common BW production processes such as filtration, UVL, and oxidants. Control measures that can be applied to manage potential risk include protection of raw water supplies from human waste, adequate treatment and protection of water during distribution. Escherichia coli (or, alternatively, thermotolerant coliforms) is a generally reliable index for *Shigella sonnei* in drinking-water supplies.**
7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source?
   Outbreaks of shigellosis have been attributed to person-to-person, water, and food transmission routes. *S. sonnei* has a low infectious dose (as few as 10 cells).
   Contamination is usually due to poor hygiene. Waterborne outbreaks commonly are associated with wells that have been fecally contaminated. Unlike other bacteria in the *Enterobacteriaceae* family, *S. sonnei* has no known nonhuman animal reservoir. As the organisms are not particularly stable in water environments, their presence in drinking-water indicates recent human fecal pollution.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. Routine product monitoring for this organism is not necessary and provides little to no information in regards to processing efficiency.
   b. HACCP approach to consider? Primary control is the process control to consider

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent.
   Laboratory testing for *S. sonnei* takes 2-4 days. Misidentification of isolates can occur due to genetic similarity with *E. coli*—this can affect PCR, ELISA and FISH methods.
ADDENDUM

Potential Microbes of Interest

Summary: *Acanthamoeba*- Bottled water has a negligible risk for *Acanthamoeba* and exposure is particularly serious for immunocompromised individuals. Ingestion is not the primary method of contact.

1. Microbe/pathogen type (e.g. parasite, bacteria, virus) *Amoeba*

2. Why is organism important in the infectious disease world? *Found in the environment* in water and soil. Most people will be exposed to *Acanthamoeba* during their lifetime, but very few will become sick from this exposure. However, the role of the immune system in resistance to infection remains unresolved. *Acanthamoeba* are the causative agents of granulomatous amebic encephalitis (GAE), amebic keratitis (AK), and cutaneous and sinus lesions. AK can occur in both immunocompetent and immunocompromised, but GAE occurs mainly in the immunocompromised.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources? *Acanthamoeba* have been found in soil; fresh, brackish, and sea water; sewage; swimming pools; contact lens equipment; medicinal pools; dental treatment units; dialysis machines; heating, ventilating, and air conditioning systems, drinking water

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? *The amoeba can be spread to the eyes through contact lens use, cuts, or skin wounds or by being inhaled.*

5. Viability (stability) - *Acanthamoeba* has a feeding, replicative trophozoite, which, under unfavorable conditions, such as an anaerobic environment, will develop into a dormant cyst that can withstand extremes of temperature (-20 to 56 °C), disinfection and desiccation. Depending on the species, *Acanthamoeba* can grow over a wide temperature range in water, with the optimum temperature for pathogenic species being 30 °C. Forms a cyst, but susceptible to chlorine, ozone, iodine.

6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. *Acanthamoeba* are not likely to be found in bottled water. Standard water treatment practices are effective in killing the organism. Standard chlorine residuals found in drinking water can inactivate.

*Acanthamoeba* is relatively large and is amenable to removal from raw water by filtration. Reducing the presence of biofilm organisms is likely to reduce food sources
and growth of the organism in distribution systems, but the organism is highly resistant to disinfection. However, as normal uses of drinking-water lack significance as a source of infection, setting a health-based target for Acanthamoeba spp. is not warranted.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? No outbreaks have been reported. However, the amoeba has been found in bottled (one recent case in mineral water in Southern Brazil) and tap water and vegetation, fish, reptiles, mammals and amphibians.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case?

   a. This organism would not be considered of strict fecal origin as it occurs naturally in soil as well as in aquatic habitats.

   b. HACCP approach to consider? Primary control is the process control to consider

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. PCR has been used for the specific detection of Acanthamoeba and is effective with few specimen present (5-10).
Summary: Enterococci - Bottled water has a negligible risk for Enterococci. The intestinal enterococci group can be used as an index of fecal pollution. Most species do not multiply in water environments.

1. Microbe/pathogen type (e.g. parasite, bacteria, virus)- These bacteria Gram-positive are a subgroup of the larger group of organisms defined as Enterococcus faecalis.

2. Why is organism important in the infectious disease world? Enterococci are used as indicators of fecal contamination of recreational waters throughout the world. Enterococci have been used in testing of raw water as an index of fecal pathogens that survive longer than E. coli and in drinking-water to augment testing for E. coli. In addition, they have been used to test water quality after repairs to distribution systems or after new mains have been installed.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources? Enterococci are typically excreted in the feces of humans and other warm blooded animals. Some members of the group have also been detected in soil in the absence of fecal contamination. Intestinal enterococci are present in large numbers in sewage and water environments polluted by sewage or wastes from humans and animals.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? Water consumption can be a mode of infection, if water is contaminated with feces.

5. Viability (stability) in bottled water or BW sources. The Enterococci are found in soil, plants, and water. When they are found in water it is typically because the water had been contaminated with fecal matte

6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. The presence of intestinal enterococci provides evidence of recent fecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? There is an absence of Enterococci in pure waters, virgin soils, and environments having no contact with animal or human life. There have been reported cases of Vancomycin-resistant Enterococci (VRE) outbreaks (often linked to hospitalized patients).

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case. HACCP approach to consider? See #6 above. Primary control is the process control to consider.
9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. *Enterococci are detectable by simple, inexpensive cultural methods that require basic bacteriology laboratory facilities*. Commonly used methods include membrane filtration with incubation of membranes on selective media and counting of colonies after incubation at 35–37 °C for 48 h. Other methods include a most probable number technique using micro-titer plates. Standardized methods have been approved for the detection of enterococci in water, including the United States Environmental Protection Agency (USEPA) Method 1600 (United States Environmental Protection Agency, 2006), the USEPA Method A (United States Environmental Protection Agency, 2010), and the International Organization for Standardization (ISO) methods 7899-2 (International Organization for Standardization, 2000) and ISO 7899-1 (International Organization for Standardization, 1998). IDEXX (Westbrook, ME)
**Summary: Pseudomonas aeruginosa**- Bottled water has a negligible risk for *Pseudomonas aeruginosa*. Although ubiquitous, *Pseudomonas aeruginosa* is not commonly found in drinking water. The risk of infection from ingesting *P. aeruginosa* in drinking water is low.

1. Microbe/pathogen type (*e.g.* parasite, bacteria, virus)- Gram-negative, aerobic motile rod shaped bacteria

2. Why is organism important in the infectious disease world? *Pseudomonas aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Due to infections being linked to compromised tissues and patients that are immunocompromised, many different epidemiological studies track its occurrence as a hospital-acquired pathogen. *P. aeruginosa* is a major pathogen in burn and cystic fibrosis patients and causes a high mortality rate in both populations.

3. Relevance to waterborne disease presence in water--which types? Bottled water (BW) sources? The bacterium is ubiquitous in the environment. *Pseudomonas aeruginosa* is not often found in drinking water. Its occurrence in drinking water is related more to its ability to colonize biofilms in plumbing fixtures (i.e., faucets, showerheads, etc.) than its presence in the distribution system or treated drinking water.

4. Is water consumption/contact the main or secondary mode of entry in pathogenesis process? Water consumption is an unlikely mode of infection. Modes of transmission include direct contact with water and aerosols, aspiration, indirect transfer from moist environmental surfaces, and via healthcare worker hands.

5. Viability (stability) in bottled water or BW sources. *P. aeruginosa* can grow in deionized or distilled water. Also, it can survive in water and on surfaces for long periods of time.

6. Sensitivity/Resistance to common BW production processes- likelihood of being in a bottle in an infectious form. *P. aeruginosa* does not exhibit any marked resistance to the disinfectants used to treat drinking water such as chlorine, chloramines, UV or ozone.

7. Have there been any reported outbreaks (If yes, when and where)? Was water the source? Was bottled water the source? Was another food product the source? *Pseudomonas aeruginosa* is primarily a soil and waterborne organism that is responsible for many hospital acquired infections. Infections are commonly associated with hospital settings and in individuals with damaged tissues and/or are immunocompromised- in these circumstances water and contaminated hospital equipment has been listed as the source. There have been outbreaks associated with hospitals in Great Britain and Europe. According to Eckmanns 2008, Naze 2010 and a review article, Ferranti 2014, bottled water has been indicated to be the source of infection.* Please note, IBWA is
investigating the scientific aptitude of these studies in order to establish credence in the findings linking bottled water to the referenced outbreaks.

Community-acquired infections among normal healthy individuals are primarily limited to infections of eyes, ears, and skin. These latter infections are most often associated with recreational water (hot tubs, pools, water slides). Have there been any reported outbreaks (if yes, when and where)? Answer is yes, there have been many outbreaks mostly in hospitals in Great Britain and Europe.

8. Relevance as an indicator- microbe may be considered as model indicators if they represent the worst case.
   a. Routine product monitoring for this organism is not necessary and provides little to no information in regards to processing efficiency.
   b. HACCP approach to consider? Primary control is the process control to consider

9. Methods for testing for infectious/viable agent and/or methods to test for protein, RNA or DNA of agent. Testing for the presence of *P. aeruginosa* can be done with the detection of acylamidase activity. This can be accomplished by using a synthetic medium containing acetamide as the substrate for enzymatic action and test reagent, such as Nessler's reagent. *Pseudomonas aeruginosa* is detected by HPC, which can be used together with parameters such as disinfectant residuals to indicate conditions that could support growth of these organisms. In addition, there are rapid PCR testing methods for *Pseudomonas*. 