Microbial Activity in aquatic environment

Coliforms and other Indicator organisms

Coliform is a term used to denote a group of gram-negative bacteria that can ferment lactose with a production of gas within 48 hours at either 35ºC or 44/44.5ºC. These characteristics allow for easy isolation, detection, and enumeration in the lab and are the gold standard for microbial water testing. They are always present when enteric pathogens or viruses are detected in water testing. However, a high 'total coliform' count doesn't necessarily mean faecal contamination and requires a second step to identify the faecal coliforms from coliforms found in the environment. *Escherichia, Enterobacter, Klebsiella* are the faecal coliforms, and *Citrobacter* and *Serratia* are found in plants and soil.

The faecal coliforms, also known as thermotolerant coliforms, can survive at temperatures of 44ºC and 44.5ºC, which allows simple differentiation between the two types. The microbiologist will be looking for counts of faecal coliforms such as *E. coli,* whose only habitat is the intestine, and whose life outside it is short-lived; is seen as the ideal indicator organism. Its presence in a sample of drinking water means that the water is unsafe for human consumption.

The presence of faecal streptococci/*Enterococci* is evidence of faecal contamination. Faecal streptococci tend to persist longer in the environment than thermotolerant or total coliforms and are highly resistant to drying. It is, therefore, possible to isolate faecal streptococci from water that contains few or no thermotolerant coliforms as, for example, when the source of contamination is distant in either time or space from the sampling point. Faecal streptococci grow in or on a medium containing sodium azide, at a temperature of 37-44 °C. They are usually detected by the reduction of a dye (generally a tetrazolium-containing compound) or the hydrolysis of aesculin. Conventional methods may give “false positives,” and additional confirmatory tests may be required.

Rapid Methods: Defined substrate technology (DST) developed by IDEXX can produce results in 24 hours. The IDEXX Colilert uses a colourimetric ONPG (o-nitrophenyl-b-D-galactopyranoside) assay to **detect coliforms** and a fluorescence MUG (4-Methylumbelliferyl- ß-D-glucuronide hydrate) assay for *E. coli.* Colilert can simultaneously detect these bacteria within 18-24 hours, depending on the product. It can also suppress 2

million heterotrophic bacteria per 100 mL present. It has been U.S E.P.A approved and is included in the U.S Standard Methods for Examination of Water and Wastewater. As of 2014, this technology from IDEXX has been published as a European Standard Method, and many countries now use this technology as their gold standard for water testing e.g. Finland and Ireland.

The Enterolert Test from IDEXX uses a proprietary Defined Substrate Technology (DST) nutrient indicator to **detect enterococci**. This nutrient indicator fluoresces when metabolized by enterococci. DST improves accuracy and avoids the need for hazardous sodium azide suppressants used in traditional media.

Membrane filtration: A typical MF method for water analysis is performed by passing a known volume of water through a sterile membrane filter with a pore size small enough to retain bacterial cells (typically 0.45µm). The filter is then transferred aseptically to the surface of an agar plate, or an absorbent pad saturated with a suitable selective medium and incubated. Colonies are allowed to develop on the surface of the filter and can be counted and examined directly. MF methods are quick and easy to perform, require little incubator space, and can handle large volumes of water if needed. Over the last 30 years, they have become the preferred methods for the microbiological examination of water for indicator organisms.

Culture media: Much selective media have been developed for the detection of indicator organisms in water by MF methods. Recommended media for coliforms and *E. coli* include membrane lauryl sulphate broth or agar, MI agar and broth, and membrane lactose glucuronide agar. Membrane enterococcus agar (mEA) and membrane-Enterococcus Indoxyl-ß-D-Glucoside Agar (mEI) can be used for detection and enumeration of enterococci, while Tryptose sulphite cycloserine agar without egg yolk can be used to culture *Clostridium perfringens* on membrane filters. *Pseudomonas aeruginosa* can also be detected by an MF method using Pseudomonas agar.

Further culturing, or biochemical testing can then be used to confirm the identity of suspect colonies growing on filters placed on selective media.

Traditional culture: Techniques using pour and spread plate count methods are not sufficiently sensitive for the detection of indicator organisms and pathogens in water, although they are still used routinely for enumerating heterotrophic bacteria. Methods capable of testing a larger volume of water (typically 100 ml) are needed. For many years the method of choice was the multiple tube 'most probable number' (MPN) technique, in which measured volumes of the water sample are added to a series of tubes containing differential media and incubated.

Growth is indicated by a colour change in the medium, and the result is calculated from the distribution of positive tubes. Although the method is simple and inexpensive in terms of equipment and materials, it is labour intensive and requires large amounts of incubator space. It is also an indirect method and does not allow further examination of individual colonies. MPN tests for routine water microbiology have now been largely replaced by membrane filtration (MF) methods, although they may still be useful for occasional tests conducted in small laboratories or the field, and commercial test kits based on MPN methods are available for coliforms and enterococci.

***Legionella* in cooling towers**

Testing for *Legionella* sp. in cooling towers should be carried out regularly and must be part of a plant's programme of testing. *Legionella pneumophila,* for instance, is a heat-loving organism that has a higher tolerance for chlorine than most bacteria and can live within the cells of parasites, protecting itself from these methods of cleaning. Cooling towers provide suitable conditions for *L. pnuemophila* and unfortunately provide a perfect transport for its dissemination via droplets into the atmosphere whereupon it is potentially inhaled by people in the vicinity and cause pneumoniae.

Water quality parameter

Nitrogen

Nitrogen is vital for growth of plants in an aquatic ecosystem and it occurs naturally in both fresh and saltwater. When nitrogen exceeds and comes into the stream ecosystem. It leads to excessive algal growth which ultimately depletes the available oxygen. This becomes deplorable for aquatic life.

Total Coliform

Total coliform bacteria, faecal coliform bacteria, and E. coli are all indicators of water contaminated with faecal matter. Contaminated water may contain other pathogens which are more difficult to test for. Therefore, these indicator bacteria are useful in giving us a measure of contamination levels.

E-Coli

The faecal matter of humans, animals, birds and mammals carry a bacterial species known as E. coli. Total coliform bacteria are an entire group of bacteria species including E. coli species.

There are certain forms of coliform bacteria that do not live-in faecal matter but instead live-in soils. Notably, most of the faecal coliform cells found in faecal matter are E. coli. This is why, all E. coli belong to the faecal coliform group, and all faecal coliform belong to the total coliform group.

Waterborne Pathogens

There are a number of organisms that can be a waterborne pathogen in drinking water. This can include a small sub-group of *E. coli*. and other bacteria, viruses, and protozoans. We are using the term waterborne pathogens which are organisms that can cause disease by way of a drinking water route.

How Do Waterborne Pathogens Become a problem?

Waterborne pathogens become a problem because someone or a pet becomes sick or a person becomes an asymptomatic carrier for the disease that then impacts others. The first line of defence is to "Get Your Drinking Water Tested and to Know Your H20".

The most common waterborne diseases in the United States (2017 Data) were:

Commonly recognized waterborne infections are:

1. Cryptosporidiosis (*Cryptosporidium*)- Cryptosporidiosis (often called "Crypto") is a diarrheal disease caused by the protozoan parasite *Cryptosporidium* spp. *Cryptosporidium* is passed in the stool of an infected person or animal.

- Cyclosporiasis (*Cyclospora* spp.)- Cyclosporiasis is a diarrheal illness caused by the protozoan parasite, *Cyclospora cayetanensis*. People become infected with *Cyclospora* by consuming food or water that has been contaminated with feces that contain the parasite.

- *Escherichia* coli O157:H7 Infection (*E. coli* O157)- *E. coli* O157:H7 is one of hundreds of strains of the bacterium *Escherichia coli*. Although most strains are harmless and live in the intestines of healthy humans and animals, this strain produces a powerful toxin and can cause severe illness.
- Giardiasis *(Giardia)*- Giardiasis is a diarrheal illness caused by *Giardia intestinalis* (also known as *Giardia lamblia* or *Giardia duodenalis*), a one-celled, microscopic protozoan parasite. The parasite is passed in the stool of an infected person or animal.

- Harmful Algal Blooms (HABs)- Harmful algal blooms (HABs) are blue-green algal blooms that contain toxins that can cause illness in humans and animals.
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- Hot Tub Rash (*Pseudomonas* Dermatitis/Folliculitis)- If contaminated water comes in contact with a person's skin for a long period of time, it can cause a rash called hot tub rash, or dermatitis. Hot tub rash is often caused by infection with the germ *Pseudomonas aeruginosa*.
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- L**e**gionellosis (*Legionella*)- Legionellosis includes two diseases, Legionnaires' disease and Pontiac fever, caused by exposure to the *Legionella* bacteria, *Legionella pneumophila*. Legionnaires' disease causes pneumonia and was named after a deadly outbreak of pneumonia in 1976.
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- Norovirus Infection- Noroviruses are members of a group of viruses called caliciviruses, known previously as "Norwalk-like viruses." Norovirus is also sometimes called viral gastroenteritis, food poisoning, and calicivirus. Norovirus infection causes

gastroenteritis, which is an inflammation of the stomach and the small and large intestines.

- Shigellosis (*Shigella*)- Shigellosis is an infectious disease caused by a group of bacteria called *Shigella*. The *Shigella* bacteria pass from one infected person to the next.
- Swimmer's Ear (Otitis Externa)- Ear infections can be caused by leaving contaminated water in the ear after swimming. This infection, known as "swimmer's ear" or otitis externa, is not the same as the common childhood middle ear infection. The infection occurs in the outer ear canal and can cause pain and discomfort.

What are the Standards for Waterborne Pathogens?

The EPA does not regulate all specific waterborne pathogens. The EPA drinking water standards attempt to minimize waterborne disease by providing specific standards for some bacteria, viruses, and protozoans and then technical standards or treatment technique standards to address them: (**Source**).

Total Coliform - Absent or < 1 colony per 100 ml - but for regulated community water supplies no more than 5.0% samples total coliform positive in a month and must be negative for *E. coli* and fecal coliform.

Cryptosporidium - treatment standard that must demonstrate 99% reduction.

Giardia - treatment standard that must demonstrate 99.9 % inactivation/killed.

Legionella - No limit; EPA believes that if *Giardia* and viruses are inactivated, *Legionella* will also be controlled.

Viruses - 99.99% killed/inactivated. Waterborne pathogens like *Legionella* and mycobacteria are associated with biofilms.