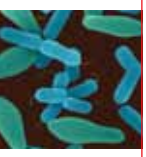

Fung Double Tube Method

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Four-hour rapid evaluation of fecal contamination of recreational water in seaside beaches by the Fung Double Tube Method.

Recreational waters such as those found at seaside beaches, rivers, lakes and ponds, are key areas for recreational use such as swimming, bathing, splashing, water games, etc. However, many of these waters may be contaminated with discharge, sometimes with fecal material from towns, cities, and small and large municipalities. These waters may contain fecal bacteria and other microbes which may pose a public health risk for swimmers, bathers and the general public who use these waters. Thus, it is important to monitor these waters regularly for the presence or absence of fecal contamination to protect the safety of the swimming public.

Conventional detection of fecal contamination in water takes one to two days. By the time the results are available, the quality of the water being tested may have changed substantially, making the data of very limited value.

In the past two years (2011–2013), the Water Research Group, a division of the Department of Public Health in Honolulu, Hawaii, under the direction of Dr. Roger Fujioka, has been testing newer and faster methods to ascertain potential fecal contamination of beach water. Using the unique Fung Double Tube Method, which can generate four-hour data from the time the water is applied to the system to the time results are read, allows a positive or negative result for fecal contamination. Currently, this is the fastest known method in the world for detection of fecal bacteria in water.

The heart of the test is as follows. *Clostridium perfringens*, an anaerobic bacterium which produces spores, is ubiquitous in fecal material (animal and human). Thus, the presence of *C. perfringens* in seawater would indicate a high probability of fecal contamination. The conventional method to detect the presence of *C. perfringens* in water takes 24 to 48 hours, making the results of very little value to warn the public of the potential danger of the water. Daniel Y. C. Fung, Ph.D., Professor of Food Science at Kansas State University, developed a Double Tube Method which can detect and enumerate *C. perfringens* in water in only four hours, making this the fastest test for fecal indicator organisms in the world. The reason for this success is that *C. perfringens* has the remarkably fast generation time of only 7.1 minutes at 41 °C, making it the fastest growing bacterium known to microbiologists at this time.

The group in Honolulu has been testing the Fung Double Tube System for about two years, and has found the method to be clean and very easy-to-use. It can indeed obtain four-hour results so that decisions can be made in four hours to open or close the beach for recreational use.

Did you know…

The production of *Clostridium perfringens* toxins starts under certain conditions?

Only one serotype out of five produces toxins, and the toxin production doesn't start until a concentration of 10^8 vegetative germs per gram of food or beverage is reached.

Figure 1: Keeping a pot simmering between 5–65 °C can provide good conditions for the growth of *C. perfringens*.

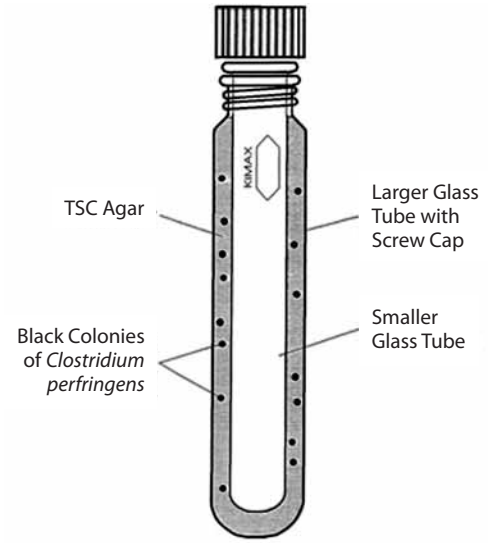

Figure 2: The Fung Double Tube System (for cultivation of anaerobes aerobically)

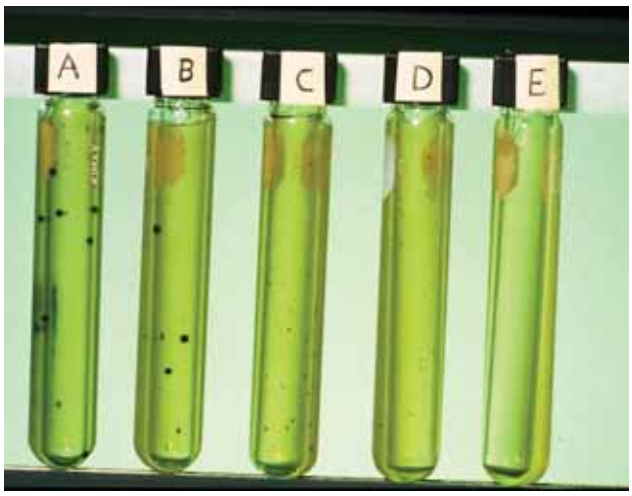

Figure 3: Each large tube contained 10 mL of the water sample selected for experimentation added into 10 mL of double strength (2X) melted sterile SFP agar. After rotating the larger tube with the sample and melted agar for 10 seconds to mix, a sterile inner tube is carefully inserted into the larger tube, thereby squeezing the liquid sample and SFP agar between the space of the two tubes, forming a thin film of sample and agar between the two tubes. The cap being tightly screwed, as well as a very tight, thin agar, makes the system very anaerobic, ideal for growth of *C. perfringens*. After about four hours of incubation at 41 °C, each single cell is visible as a black colony. *C. perfringens* is the fastest growing bacterium known; the generation time, under ideal conditions, is only 7.1 minutes.

The Fung Double Tube System involves two tubes—the smaller inside tube and a larger outer tube. Into the larger tube, 10 mL of liquefied Shahadi Ferguson Perfringens (SFP) agar (specially formulated to detect *C. perfringens*) was first placed and then sterilized. The medium is kept at 42 °C so that it will be in a liquid form. When a water sample is to be tested, 10 mL of the water (seawater, river water, etc.) will be carefully introduced into the large tube with the SFP agar. Then, the final step is to insert a smaller tube into the larger tube which contains both the SFP agar and the water sample. As the smaller tube is inserted into the large tube, the medium and sample will become sandwiched between the two tubes and form a thin film in the cavity. The material will be pushed up to the tip of the two tubes, then a screw cap will be applied, making this a very anaerobic system. In four hours, if there are *C. perfringens* in the water sample, tiny black colonies will form. The colonies will grow in size as time progresses, but at four hours, a researcher can start to ascertain if the water does or does not have *C. perfringens* and how many per mL of water.

If there are no black colonies found in the water, then it can be determined that the tested water is free of fecal material and safe to use for recreational purposes. If the number of colonies is 1–10, then the contamination level would be small and may be safe for swimming with caution. However, if the number is 10–100 colonies, the water is not safe for swimming, and the beach should be closed.

Rapid Detection of *Clostridium perfringens* by a New Chromogenic Media

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Selective chromogenic media for isolation and enumeration of *Clostridium perfringens* in water samples using membrane filtration.

CP *ChromoSelect* Agar is a selective chromogenic media for isolation and enumeration of *Clostridium perfringens* in water samples using membrane filtration. This new agar is more reliable and easier to handle than m-CP and TSC agars. The color does not diffuse in the agar and confirmation is not required since the green coloration is specific for *C. perfringens*. *C. perfringens* is an anaerobic, Gram-positive, spore-forming rod-shaped bacterium. It is widespread in the environment and is also found in the digestive systems of humans and domestic and feral animals. Perfringens poisoning, usually the result of ingesting under-cooked food, especially meat, is one of the most commonly reported foodborne illnesses. Early detection of *Clostridium* in food and water is important to control outbreaks. *C. perfringens* produces an extensive range of invasins and exotoxins. The enterotoxins cause the undesirable, mostly meat-associated food poisoning, and wound and surgical infections that lead to gas gangrene. *C. perfringens* plays a subsidiary role in water examination.[2] Clostridia are spore builders and are resistant to heating, chlorination and other stress factors. In contrast to vegetative cells like coliforms

Did you know…

***Clostridium perfringens* is a special anaerobe?**

It is a strictly anaerobic bacterium but is able to survive when exposed to oxygen for short periods of time. A complex adaptive response to reactive oxygen species was observed but not completely understood.

Figure 1: A photomicrography of a Gram-stained culture specimen from a patient with gas gangrene, showing numerous *Clostridium perfringens*. (Source: CDC 1979).