Using qPCR to Detect Sewage Contamination

Industrial Hygienists and environmental health professionals are frequently asked to determine if fecal contamination exists after sewage overflows, and after flooding from storm events. Many have used the traditional fecal indicator bacteria tests (FIBs) that culture total coliforms, fecal coliforms, *E. coli*, or *Enterococci* for these applications. Even though it is widely recognized that these tests are neither specific nor sensitive enough to be considered good indicators of fecal contamination, they were the only tests available until recently. With the commercialization of Polymerase Chain Reaction (specifically quantitative Polymerase Chain Reaction or qPCR), genomic testing and additional research on using other fecal indicator organisms, we now have more sensitive and specific tests for determining the presence of fecal or sewage contamination.

Feces contain an extraordinary amount of microorganisms: bacteria, viruses, protozoans, and helminthes—some of which are pathogenic. However, it is not technically possible to culture every type of pathogenic organism that exists in feces and it would be prohibitively expense to do so. Because the traditional culture dependent tests for FIBs are easy and cheap, they have been used more than 60 years as indicators of the possible presence of pathogens in feces. Nonetheless, the scientific community has recognized for quite some time that serious limitations exist when these tests are used as strict indicators of fecal contamination in water samples. These limitations are compounded when culture FIBs are used for surface or bulk sample analysis in indoor environments.

The Environmental Protection Agency (EPA), US Geological Survey (USGS), and numerous researchers worldwide are conducting research on new indicator methods using rapid methods rather than culture tests. The most promising to date has been the use of qPCR technology to test for total *Bacteroides*, human *Bacteroides*, and human Polyomaviruses (HPyVs), which have proven to be highly specific and sensitive for this application. According to studies, no association, or at most a weak one, exists between the presence or absence of these new indicators and the counts of culture FIB tests. In other words, culture FIB tests have a high percentage of false positive and false negative results, while *Bacteroides* species are highly specific for fecal contamination, and HPyVs are highly specific for human fecal contamination.

Bacteroides are anaerobic bacteria that predominate in mammalian and some avian feces. Estimates indicate that *Bacteroides* are present in huge concentrations in feces (100,000,000,000 cells per gram of dry feces!)- far outnumbering coliform, *E.coli*, or *Enterococcus* concentrations. Since *Bacteroides* cannot survive in the presence of oxygen, they do not persist or multiply outside of the gut like FIBs. *Bacteroides* are only present in fecal contamination - unlike some coliforms which are soil borne bacteria and *E.coli* and some *Enterococcus* species which may not be specific for fecal contamination (i.e., *E.coli* may be found in clean water and sediments in tropical latitudes, and some *Enterococci* are soil borne). *Bacteroides*' anaerobic nature is a boon when testing for sewage or fecal contamination. Because PCR detects the genetic material (in this case DNA) and does not rely on growing live organisms, these tests do not need special shipping nor do they have particularly short hold times. Moreover, the test is very sensitive compared to FIBS. Therefore, the anaerobic nature of *Bacteroides*, their enormous concentrations in feces, and the sensitivity and specificity of qPCR make testing for these bacteria easier and more effective than using FIB culture tests. (There are qPCR tests for E. coli and Enterococci but due to the relatively low concentrations of these bacteria in feces, they may not be detected if the fecal material is diluted.)

Different types of the *Bacteroides* bacteria exist, allowing analysts to test for specific kinds of contamination. One type, called total *Bacteroides*, encompasses mammalian, some avian, and human sources. Due to the sensitivity of the total *Bacteroides* qPCR test, it is crucial to include background samples from areas that are not contaminated or impacted by sewage or feces. (This test can even detect fecal cross contamination from dirty shoes and mop heads.)

Another type of qPCR test, Human *Bacteroides*, is largely specific for human sources since the test targets human genetic markers. In practical terms, this means that results can be interpreted with a high degree of confidence since this test is specific for human fecal material.

Evidence suggests that HPyVs are even more specific than Human Bacteroides. *Bacteroides* and HPyVs differ in that the former is found in feces, while HPyVs are only found in human urine. Since urine and feces are excreted together, this virus is found in high concentrations in wastewater and urban sewage. In addition, HPyVs are good indicators of human fecal contamination and useful in microbial source tracking since they are not contained in runoff from agribusiness or agricultural feedlot operations. (Note: HPyVs will be present in the runoff from agricultural operations in third world countries where human excrement or "black water" is used as fertilizer or where sanitation practices are lax.)

HPyVs are not found in surface water where there is no known human impact, nor are they found in dairy, poultry or pig waste samples (all of which contain *Bacteroides*). According to studies, these viruses are unique to humans and ubiquitous in humans worldwide. A healthy human can excrete one million of these viral particles in one milliliter of urine, making it easier for detection. All of this portends the possibility of an exquisitely specific and sensitive test for human fecal contamination—even more than human *Bacteroides* analysis.

Despite the difference in sources, *Bacteroides* and HPyVs share one important similarity: testing laboratories can detect them very rapidly. Because qPCR is used to test for both indicators, analysts can examine the samples and provide the client with results within 24 hours. This quick turn-around-time is impossible with the culture-based tests of FIBs because the bacteria must be grown before the analysis even takes place.

Since HPyVs are viruses and not bacteria, they may also prove to be excellent indicators for the presence of enteroviruses (viruses found in feces). If this is the case, it would be a tremendous development in assessing the transmission and prevalence of enteroviruses. The current American Public Health Association Standard Methods procedures for enteroviruses are very labor intensive and require the collection of very large sample sizes (100-1500 liters). This fact complicates the testing process, because current U.S. Department of Transportation (DOT) regulations prohibit the interstate shipment of biohazardous material larger than 4 L. In response to these regulations, samples are often filtered in the field and then the filters are shipped to the lab. These steps create considerable opportunities to contaminate the samples, which could be avoided if HPyVs were used as indicators.

Since a variety of tests are now available for fecal contamination, the question becomes, "How do you choose between the tests?"

If your concern is contamination from surface water, river water, or storm water after a flood, total *Bacteroides* is the best choice since runoff from streets (dog manure), manure contaminated runoff from agricultural operations, and combined (both sanitary and storm water) treatment plant overflows are a concern. In this situation, you have the potential for human disease caused from both animal and human sources of contamination. Total *Bacteroides* will indicate the presence of all of these potential contaminants.

The human *Bacteroides* test is the best choice if your concern is a backed-up toilet or a back-up/overflow from a sanitary sewer (which handles human discharges only). Using human Bacteroides virtually eliminates any background contamination from pets.

Bacteroides may be used for indoor environmental surface or bulk sampling, non- permitted, investigational, or research-driven water, wastewater and soil samples, since there are no stipulated permits or statutory requirements.

However, if you are sampling drinking water, recreational water, or wastewater effluent as part of a compliance evaluation for a regulated National Prevention and Discharge Elimination System (NPDES) permit condition, you are still required to use culture dependent FIB methods for coliforms, *E. coli*, or *Enterococci*.

To date, research has been done using HPyVs PCR tests to determine human fecal contamination in water and wastewater only. Since their use has not been validated for surface, bulk, soil or sediment samples, sampling for these should be restricted to surface and wastewater samples. The benefit of using HpyVs in this instance is the complete elimination of background contamination by animal fecal material.

To summarize, the advantages of using PCR tests in place of culture FIBs are:

1. Current culture FIB tests have a high percentage of false positives and false negative results while total *Bacteroides* are specific for fecal contamination.

2. Unlike the qualitative enrichment tests for detecting FIBS at low concentrations, these PCR tests are quantitative even at low levels of fecal contamination.

3. Human *Bacteroides* and HpyVs are specific for human fecal contamination only. Current culture FIBs cannot distinguish between human or animal sources of fecal contamination.

4. Results can be obtained hours after the samples are received allowing for building closure and remediation efforts to start without a significant delay.

5. Results can be interpreted with a high degree of confidence since PCR tests, when done correctly, eliminate the false positive and negative results that are common with culture FIB tests.