Recommendation Regarding the Use of Human DNA Levels as a Water Quality Criterion for Recreational Waters

Based on studies of the incidence rates of illnesses following exposure to recreational waters that were impacted by sources of treated wastewater, EPA's "2012 Recreational Water Quality Criteria" EPA-820-F-12-061 recommended either a Geometric Mean concentration of *E. coli* of 126 cfu/100 ml based on an estimated illness rate of 36 illnesses per 1,000 primary contact recreators or 100 cfu/100 ml based on an estimated illness rate of 32 illnesses per 1,000 primary contact recreators. Texas elected to adopt the less restrictive 126 cfu/100 ml criterion. These same recommendations were retained in the 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria EPA 823-F-18-001.

Of the many potential sources of fecal contamination, those originating from humans pose the greatest threat to human health. Accordingly, the utility of using total *E. coli* counts as a water quality measure is highly problematic. Brinkmeyer *et al.* (2015) found that "the numeric importance of EC [*E. coli*] and ENT [*Enterococcus*] in sediments of the Buffalo Bayou and White Oak Bayou watersheds … can account for as much as 90% of the observed daily loads." Other studies have described the presence of significant concentrations of *E. coli* in the excreta of cold-blooded animals that are present in waterways.

Because some genetic strains of *E. coli* are most frequently associated with human hosts, the presence of the genetic "fingerprint" of these strains in environmental water has been used as an indicator of human fecal contamination. Still, this measure is indirect in nature because the association of a particular *E. coli* variant with a particular host species (*e.g.,* human or animal) can vary from waterbody to waterbody, and over time within a single waterbody. More recently, EPA has approved methods based on the identification of DNA sequences of another group of bacterial strains referred to as "*Bacteroides*-like microorganisms" that are commonly associated with humans. [Method 1696 EPA 821-R-19-002 (2019) and Method 1697 EPA 821-R-19-003 (2019)]. Still, these methods are indirect because they assume stability of the established associations between bacterial strains and host species across space and time.

As is pointed out in the 2017 Five-Year Review of the 2012 Recreational Water Quality Criteria EPA 823-F-18-001, "Risk assessments, epidemiological studies, and outbreak data indicate that viruses [my emphasis added] cause most illnesses associated with recreational waters impacted by human sources." The concept of viral etiology is consistent with the results of a large study of more than 20,000 visitors to four Great Lakes beaches that are impacted by effluent from wastewater treatment facilities. TJ Wade et al. (2008) found that the incidence of gastrointestinal illnesses among these persons did not correlate as strongly with the presence of culturable *Enterococcus* bacteria as it did with the concentration of *Enterococcus* DNA, thus suggesting that viable bacteria were not the cause of the illnesses. This makes sense when it is taken into account that some viruses are much more resistant to inactivation or removal by standard wastewater treatment than are viable bacteria such as *E. coli* or *Enterococcus*. Some disease-causing viruses such as Norovirus are well adapted to humans, are shed in large numbers by infected persons, and can remain infectious for long periods of time in environmental waters.

What is urgently needed is a recreational water quality criterion that does not depend upon established statistical associations of particular bacterial strains with human hosts, and which more closely matches the profile of viral contamination that can arise from treated wastewater. With this in mind, the strongest candidate is the presence and concentration of human DNA. Epithelial cells are constantly being shed from the lining of the human intestine, and their DNA is an inevitable component of human feces. This human DNA can be sensitively and specifically detected and quantified using TaqMan quantitative Polymerase Chain Reaction methodology. Tanvir Pasha et al. (2020) have applied this methodology to water samples from the Upper Cibolo Creek watershed in Kendall County, Texas, and have run these analyses side-by-side with markers for both human-associated *Bacteroidales* DNA and *E. coli* DNA.

For human DNA levels in recreational waters to be applicable as a water quality criterion, population-based studies that can relate them to disease risk are required. I recommend that the Bacteria Implementation Group go on record as being in support of these studies.

by Tom Douglas
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REFERENCES

2012 Recreational Water Quality Criteria EPA-820-F-12-061
https://www.epa.gov/wqc/recreational-water-quality-criteria-and-methods

2017 Five-Year Review of the 2012 Recreational Water Quality Criteria EPA 823-F-18-001
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Brinkmeyer et al. (2015)
I could not locate this paper online, but a copy of it is attached.

Human-associated *E. coli* variants
https://twri.tamu.edu/media/5467/tr-530.pdf

Method 1696 for *Bacteroides*-like microorganisms EPA 821-R-19-002 (2019)
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Pathogenic Waterborne Viruses, including Norovirus
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TJ Wade et al. (2008)
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