University of South Florida Methods for Laboratory Analyses

Fluorometry. From June to August, water samples from fixed sites were tested for the presence of optical brightners, associated with human sewage (Poiger et al. 1998, Boving et al. 2004), using the Aquaflor handheld fluorometer (Turner Designs) by the method described by Hartel (2007). Briefly, optical density of a water sample is taken using a 445nm emission filter, the sample is exposed to UV light (365nm) for 5 minutes, and the optical density was taken again. Samples were considered positive if the optical density of the sample after UV exposure was at least 15% lower than the original optical density.

Enumeration of Indicator Bacteria. Water and sediment samples were processed by membrane filtration (0.45µm pore-size, 47mm diameter) using a vacuum pump to enumerate *Escherichia coli*, fecal coliforms, and enterococci. Prior to filtration, sediment samples were diluted 1:10 in sterile buffered water and sonicated (Anderson et al. 2005) to release bacteria attached to sediment particles. Fecal coliforms were enumerated on mFC agar after 24 h incubation at 44.5°C (American Public Health Association 1995); enterococci were enumerated on mEI agar at 41°C after 24h incubation (United States Environmental Protection Agency 2002a); *E. coli* was enumerated on mTEC media at 35°C for 2h, followed by 22h incubation at 44.5°C (United States Environmental Protection Agency 2002b). Colonies on plates were counted and concentrations were reported as CFU · 100 ml^{-1} or CFU · 100 g^{-1} (wet weight) for water and sediment samples, respectively.

Microbial Source Tracking (MST).

***esp* gene.** The assay for detection of the human-associated *esp* gene of *Ent. faecium* by polymerase chain reaction (PCR) was performed as described previously (Scott et al. 2005). Three hundred ml of water or 25ml of sediment sample (diluted as described above) was filtered through a 0.45µm pore-size membrane and incubated on mEI agar at 41ºC for 48h. Filters were than transferred to 5ml azide dextrose broth (Difco) and incubated at 41ºC with shaking for 3h. A DNA extraction was then performed on 2ml of this culture using the QIAamp DNA Stool Mini Kit according to the manufacturer's instructions (Qiagen). DNA was then used as PCR template using conditions described previously (Scott et al. 2005). The PCR product (680bp) was visualized by agarose gel electrophoresis (1% agarose gel) and captured using the FOTO/Analyst Archiver (FotoDyne). To test for the possibility of matrix inhibition of the PCR reaction, cells of the positive control strain *Ent. faecium* C68 were spiked into environmental water samples and processed in the same way. Sterile buffered water was also filtered and processed as described above as a method blank to ensure that false-positives did not occur as a result of contamination. A negative control for the PCR reaction alone was also included for each sample event.

Human Polyomavirus. Presence of human polyomavirus (HPyV) was detected using a method previously described using 600ml of sample (McQuaig et al. 2006). PCR products (172bp) were visualized by agarose gel electrophoresis (2% agarose gel) and captured as described above. This procedure was repeated four times or until HPyV was detected for each sample to account for possible heterogeneity in the DNA template. Viral particles of the BK strain of the virus were spiked into

environmental waters to test for matrix inhibition. Buffered water was also processed as a method blank, and a PCR negative control was run for each sample event.

Bacteroides. The procedure and PCR conditions used for *Bacteroides* assays was changed during the sampling period. From May through July, PCR was performed on whole cell templates using a modified version of Layton's method (Layton et al. 2006). Briefly, 60ml of sample was pushed through a 0.2μm pore-sized syringe filter and back-flushed into a 2ml microcentrifuge tube with 1ml of AE Buffer (Qiagen). The sample was then centrifuged at 10,000 rpm for 10min, after which 0.9ml of the buffer was removed and the pellet resuspended in the remaining 0.1ml. The resuspension was used as template for PCR reactions using primers described previously for human- (Bernhard & Field 2000), ruminant- (Bernhard & Field 2000), and horse- (Dick et al. 2005) associated *Bacteroides*. In August, 100ml of sample was processed by membrane filtration as described for enumeration of indicator bacteria and DNA was extracted from the filter using a MoBio UltraClean DNA kit following the manufacturer's instructions (MoBio). For the rest of the sampling period, 500ml of sample was processed by membrane filtration and DNA was extracted using a MoBio Power Soil DNA kit following the manufacturer's instructions (MoBio). DNA was used as PCR template beginning in August.

From May through July, PCR products were re-amplified – PCR product from an initial round of PCR was used as template for an identical second round to increase sensitivity of the assay using an initial whole cell template (Roux 1995). From May through August, PCR reactions were prepared using JumpStart Taq Ready Mix (Sigma) and a touchdown PCR program (Don et al. 1991) for all primer sets. The touchdown PCR program was maintained for the human and ruminant assays throughout the study and through the September sampling event for the horse assay. From September through the end of the study, PCR reactions were prepared using GoTaq Green Master Mix (Promega). Also beginning in September through the end of the study, primer concentrations for all *Bacteroides* assays was reduced from 0.5μM to 0.4μM. Beginning in October, the horse assay was conducted using a standard PCR program with an annealing temperature of 55º. Results were visualized by agarose gel electrophoresis (2% agarose) as described above.

Citations

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United States Environmental Protection Agency (2002b) Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC)