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Horse Bacteroidetes ID™

Detection of the fecal *Bacteroidetes* Horse Gene Biomarker for Horse Fecal Contamination by Polymerase Chain Reaction (PCR) DNA Analytical Technology

Submitter: XYZ Municipal Beach

Submitter #'s: 275, 276, 277 and 278

Source Molecular #'s: SM 0225, SM 0226, SM 0227 and SM 0228

Samples Received: January 3rd, 2011

Date Reported: January 10th, 2011

SAMPLE

SM #	Client #	DNA Analytical Results
SM 0225	275	Horse Gene Biomarker Detected
SM 0226	276	Negative
SM 0227	277	Horse Gene Biomarker Detected
SM 0228	278	Negative

Laboratory Comments

The submitted water samples were filtered for fecal *Bacteroidetes*. The filters were then eluted and centrifuged for DNA analysis. Fecal *Bacteroidetes* are found in abundant amounts in feces of warm-blooded animals. They are considered a good indicator of recent fecal pollution because they are strict anaerobes (i.e. they do not survive long outside the host organism).

All reagents, chemicals and apparatuses were verified and inspected beforehand to ensure that no false negatives or positives could be generated. In that regard positive and negative controls were run to attest the integrity of the analysis. All inspections and controls tested negative for possible extraneous contaminants, including PCR inhibitors.

Samples 276 (Our Ref: SM 0226) and 278 (Our Ref: SM 0228) tested negative for the fecal *Bacteroidetes* horse gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have horse contamination. In order to strengthen the result, a negative sample should be analyzed further for horse fecal contamination with other DNA analytical tests such as the E. coli ID™ service.

Samples 275 (Our Ref: SM 0225) and 277 (Our Ref: SM 0227) tested positive for the fecal *Bacteroidetes* horse gene biomarker suggesting that horse fecal contamination is present in these water samples. The client is nonetheless encouraged to conduct other DNA analytical tests such as the service mentioned above to further confirm the results.

DNA Analytical Method Explanation

The water samples (100 ml each) were filtered through 0.45 micron membrane filters. The filters were placed in separate 15-ml disposable centrifuge tubes containing 2 ml of lysis buffer. DNA extraction was prepared using a Qiagen DNA extraction kit, as per manufacturer's instructions.

Two micro-liter aliquots of purified DNA extraction were used directly as template for subsequent PCR reactions. Amplification of PCR primers were carried out using HotStarTaq polymerase (Qiagen, Inc.) and master mix, which contained a final concentration of 1.5 mM MgCl₂, 150 mM dNTP, and 0.3 mM of each primer.

An Eppendorf Gradient Thermocycler was used with the following cycling parameters: 25 cycles of 94°C for 30 s, appropriate annealing temperature for 30 s, and 72°C for 1 min followed by a final 6-min extension at 72°C. PCR products were electrophoresed on 2% agarose gels, stained with GelStar nucleic acid stain (BioWhittaker, Inc.) and visualized under UV light.

DNA Analytical Theory Explanation

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.¹ Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Horse Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.^{2,3,4,5,6} Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in horse. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in horse.^{2,3,5,6} As such, these bacterial strains can be used as indicators of horse fecal contamination.

One of the advantages of the Horse Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available for detection by gel electrophoresis.

The gel electrophoresis apparatus uses an electrical field to distinguish different DNA fragments according to their molecular weights. Lighter DNA fragments will move farther along the gel than their heavier counterparts. At the end of the procedure different bands of accumulated DNA fragments will aggregate at different parts of the gel. It is this accumulation of DNA fragments that creates a band on the gel. Researchers use these bands to distinguish certain genomes such as the horse gene biomarker from the *Bacteroides* and *Prevotella* genus.

These banding patterns confirm or negate the presence of the fecal *Bacteroidetes* horse gene biomarker. As such, the banding patterns can be a good indicator of horse fecal contamination. Nonetheless, in order to strengthen the validity of the results, the Horse *Bacteroidetes* ID™ service should be combined with other DNA analytical services such as the E. coli ID™ service.

¹ Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

² Bernhard, A.E., and K.G. Field (2000a). **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Applied and Environmental Microbiology, 66: 1,587-1,594.

³ Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

⁴ Kreader, C.A. (1995). **Design and evaluation of Bacteroides DNA probes for the specific detection of human fecal pollution.** Applied and Environmental Microbiology, 61: 1,171-1,179.

⁵ Fogarty, Lisa R., Voytek, Mary **A Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

⁶ Dick, Linda K., Bernhard, Anne E., Brodeur, Timothy J., Sant Domingo, Jorge W., Simpson, Joyce M., Walters, Sarah P., Field, Katharine G. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification** Appl. Environ. Microbiol. 2005 71: 3184-3191.

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