SOURCE MOLECULAR CORPORATION

4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-268-8363, Fax: (1) 786-513-2733, Email: info@sourcemolecular.com

Cow Fecal Virus ID™

Detection of Cow Fecal Viruses by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) DNA Analytical Technology

Submitter: TMDL Watershed AC
Submitter #'s: 31A, 32B, 33C, 34D
Source Molecular #'s: SM 0789, SM 0790, SM 0791, SM 0792
Samples Received: January 3rd, 2011
Date Reported: January 10th, 2011

SM #	Client #	DNA Analytical Results
SM 0789	31A	Cow Fecal Viruses Detected
SM 0790	32B	Negative
SM 0791	33C	Negative
SM 0792	34D	Cow Fecal Viruses Detected

Laboratory Comments

Four 1-liter water samples were filtered and analyzed for the presence of bovine enteroviruses (i.e. cow fecal viruses). These viruses infect the gastrointestinal tract of cows and are excreted in feces in detectable amounts. Furthermore, bovine enteroviruses are found worldwide and they are highly stable in water.

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 poss ible extraneous contaminates, including PCR inhibitors.

Samples 32B (Our Ref: SM 0790) and 33C (Our Ref: SM 0791) tested negative for the presence of bovine enteroviruses. It is important to note that a negative result does not mean that the sample does not definitively have cow fecal contamination. Negative samples should be analyzed further with other microbial source tracking methods such as the Cow E. coli ID™ and Cow Bacteroidetes ID™ services.

Samples 31A (Our Ref: SM 0789) and 34D (Our Ref: SM 07 92) tested positive for bovine enteroviruses suggesting cow fecal contamination is present in these water samples. The analysis targeted and detected virus RNA, strongly indicating the presence of intact, encapsulated viruses since free RNA is quickly degraded in the environment. Additional samples should be analyzed using the two services mentioned above to strengthen the validity of the results. In particular, bovine enteroviruses have been shown to be present occasionally in other ruminants such as white-tailed deer; therefore a positive result should be corroborated with other analyses that target cattle fecal contamination.

DNA Analytical Method Explanation

One-liter water samples were filtered through a negatively charged hydrophobic filter membrane (Millipore Inc.). Filters were collected and a total viral RNA extraction was performed directly from the filter using a commercially available viral RNA extraction kit according to manufacturer's instructions (Qiagen, Inc.).

Each filter concentrate sample was purified and concentrated to 60.0 µl by using spin-column chromatography (RNEasy Mini Kit; Qiagen, Santa Clarita, Calif.). Afterwards, 10 µl of the sample was used for reverse transcriptase PCR (RT-PCR) for the assayed viruses. RT-PCR profiles and master mixes were used according to previously published literature.[1,2,3,4,5] Positive and negative controls were used in each reaction where applicable.

DNA Analytical Theory Explanation

Cattle waste contamination is considered a major environmental concern. Improper or poor cattle waste disposal can lead to excessive nutrients, organics, metals and antimicrobial residuals in water and soils. Furthermore, since cows are known to harbor human pathogens such as *Cryptosporidium parvum* and pathogenic *E. coli,* proper monitoring and remediation of this form of fecal contamination is essential for maintaining viable water systems.

Detection of cattle viruses in water samples can serve as an indicator of cattle contamination.[1,2,3,4,5] Of particular concern are bovine enteroviruses. These viruses infect the gastrointestinal tract of cows, and are excreted in feces in large numbers. Infections in cattle are typically asymptomatic or mild, but they have also been associated with diarrhea and abortions. As such, they are endemic to cattle. Additionally, bovine enteroviruses are found worldwide and they are highly stable in water.

One of the advantages of the Cow Fecal VirusID™ service is that the entire water is sampled and filtered for bovine enteroviruses. 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.

Bovine enteroviruses are RNA viruses; therefore, a PCR (polymerase chain reaction) method called reverse transcriptase PCR (RT-PCR) must be used to transcribe the detected RNA back into DNA. 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 viruses 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 virus or 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 confirm and distinguish viral genomes.

Viruses cannot replicate themselves. They need a host organism to transcribe and replicate their genetic code. Viruses come in two genetic forms, either RNA or DNA based. Their genetic material is protected with a protein coat. Detection of virus RNA or DNA strongly indicates the presence of intact, encapsulated viruses, as free RNA or DNA quickly degrades in the environment.

To strengthen the validity of the results, the Cow Fecal Virus ID ™ service should be combined with the Cow E. coli ID™ or the Cow Bacteroidetes ID™ services. In particular, bovine enteroviruses have been shown to be present occasionally in other ruminants such as white-tailed deer; therefore a positive result should be corroborated with other analyses that target cattle fecal contamination. The Cow E. coli ID™ service is designed around the principle that certain strains of *E. coli* are pathogenic in cattle. As such, this service targets the heat labile toxin IIa (LTIIa) gene from enterotoxigenic *E. coli* as an indicator of cattle fecal contamination. The Cow Bacteroidetes ID™ service analyzes for fecal *Bacteroidetes* that are found in cattle.

[1] Ley, Victoria, Higgins, James, Fayer, Ronald **Bovine Enteroviruses as Indicators of Fecal Contamination** Appl. Environ. Microbiol. 2002 68: 3455-3461.

[2] McCarthy, F. M., G. A. Smith, and J. S. Mattick. 1999. **Molecular characteristion of Australian bovine enteroviruses.** Vet. Microbiol. 68:71–81.

[3] Zell, R., K. Sidigi, A. Henke, J. Schmidt-Brauns, E. Hoey, S. Martin, and A. Stelzner. 1999. **Functional features of the bovine enterovirus 5'-non-translated region.** J. Gen. Virol. 80:2299–2309.

[4] Maluquer de Motes, Carlos, Clemente-Casares, Pilar, Hundesa, Ayalkibet, Martin, Margarita, Girones, Rosina **Detection of Bovine and Porcine Adenoviruses for Tracing the Source of Fecal Contamination** Appl. Environ. Microbiol. 2004 70: 1448-1454.

[5] Fong, Theng-Theng, Griffin, Dale W., Lipp, Erin K. **Molecular Assays for Targeting Human and Bovine Enteric Viruses in Coastal Waters and Their Application for Library-Independent Source Tracking** Appl. Environ. Microbiol. 2005 71 2070-2078.

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