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Pig E. coli ID™

Detection of the STII Toxin Gene Biomarker for Swine Fecal Contamination by
Polymerase Chain Reaction (PCR) DNA Analytical Technology

Submitter: TMDL Watershed XY

Submitter #'s: 15A, 16B, 17C, 18D

Source Molecular #'s: SM 0125, SM 0126, SM 0127, SM 0128

Samples Received: April 16, 2003

Date Reported: April 23, 2003

SAMPLE

SM #	Client #	E. coli (CFU/100mL) ⁶	DNA Analytical Results
SM 0125	15A	1,500	Negative
SM 0126	16B	15	Negative
SM 0127	17C	5,500	Swine Biomarker Detected
SM 0128	18D	24	Swine Biomarker Detected

Laboratory Comments

Four water samples were filtered for *E. coli*. The *E. coli* was then cultured, eluted and centrifuged for DNA analysis. DNA was extracted from each sample and analyzed for the STII swine gene biomarker.

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 15A (Our Ref: SM 0125) and 16B (Our Ref: SM 0126) tested negative for the STII swine gene biomarker. It is important to note that a negative result does not mean that the sample does not definitely have swine contamination. In order to strengthen the result, a negative sample should be analyzed further for swine fecal contamination with other DNA analytical tests such as the Pig Bacteroidetes ID™ and Pig Fecal Virus ID™ services.

Samples 17C (Our Ref: SM 0127) and 18D (Our Ref: SM 0128) tested positive for STII swine gene biomarker suggesting that swine fecal contamination is present in these water samples. Although the STII biomarker has on rare occasions been associated with other mammals, it is widely accepted that it is an indicator of swine fecal contamination. The client is nonetheless encouraged to conduct other DNA analytical tests such as the services mentioned above to further confirm the results.

DNA Analytical Method Explanation

Cultivation, extraction, PCR amplification and confirmation of the *E. coli* STII toxin gene was performed similar to the Khatib et al. method.¹ Briefly, 100ml of water was filtered through a 0.45µm nylon membrane. The captured cells were grown on mTEC agar (Difco) and incubated according to Dufour et al.⁶ The cells from appropriate dilutions were harvested and resuspended in 1 X PBS, centrifuged at 12,000 g; the supernatant was discarded, and the pellet resuspended as before in 1 ml of lysis buffer. Afterwards, the DNA was extracted using a Qiagen DNA extraction kit.

PCR amplification of the STII toxin gene was performed in an Eppendorf gradient thermal cycler using a total volume of 50 µl containing 10 µl of the DNA extract. Reagents (Qiagen, Inc.) were used according to manufacturer's instructions and MgCl₂ was 2.0mM. The reaction cycle was an initial denaturation of 1 min at 95°C, followed by 30 cycles at 95°C for 30 s, 61°C for 30 s, and 72°C for 30 s, and a final extension of 6 min at 72°C. Each reaction set contained positive and negative controls.

Aliquots of PCR products (10 µl) were visualized on a 2.5% agarose gel supplemented with 5 µg/ml ethidium bromide. A molecular weight marker ranging from 25-500 bp with markers at 25 bp intervals (Gibco Life Technologies) was used to size the DNA fragments in the gel.

DNA Analytical Theory Explanation

Since swine are known to harbor human pathogens such as *Salmonella spp.* and pathogenic *E. coli*, proper monitoring and remediation of this form of fecal contamination is essential for maintaining viable water systems.

The Pig *E. coli* ID™ service is designed around the principle that certain strains of *E. coli* are specifically pathogenic in swine. These enterotoxigenic *E. coli* (ETEC) can be used as indicators of swine fecal contamination.¹ Enterotoxigenic *E. coli* have toxin genes that render them pathogenic. These ETEC toxin genes can serve as DNA biomarkers.

It has been shown that the heat stable toxin II (STII) gene from enterotoxigenic *E. coli* can serve as a reliable indicator of swine fecal contamination.^{2,5} Although this toxin gene has on rare occasions been associated with other mammals such as cattle or humans, it is on the whole endemic to swine.³ As such, the STII toxin gene is used as an indicator of swine fecal contamination because 1) it is relatively species-specific, 2) its nucleotide sequence is unique to swine, and 3) of its more frequent occurrence in swine than other toxin genes such as StaP (heat stable toxin I associated with pigs), LT (heat labile), and StaH (heat stable toxin associated with humans).⁴

One of the advantages of this method is that the entire population of *E. coli* of the selected portion of the water sample is screened. As such, this method avoids the randomness effect of selecting isolates off a petri dish. It has been shown that if the total *E. coli* count (irrespective of the volume of water) of the sample is equal to or greater than 35, the reliability of the analysis is significantly greater, particularly in regards to negative results.

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 STII toxin gene from enterotoxigenic *E. coli*.

These banding patterns confirm or negate the presence of the STII toxin gene from the *E. coli* of the water sample. As such, the banding patterns provide a reliable indicator of swine fecal contamination. To strengthen the validity of the results, the Pig *E. coli* ID™ service should be combined with other DNA analytical services such as the Pig Bacteroidetes ID™ and Pig Fecal Virus ID™ services.

¹ Khatib L, Tsai YL, Olson BH. 2003. **A biomarker for the identification of swine fecal pollution in water using the STII toxin gene from Enterotoxigenic *E. coli***. Applied Microbiology and Biotechnology.

² Olson BH, Khatib L, McGee C. 2001. **Comparison of DNA Fingerprinting Methods of *E. coli*, Genotyping Male Specific Phage Serotypes and the Use of Toxin Genes as Biomarkers to Differentiate Human and Animal Waste**. American Water Works Association, WQTC Proceedings.

³ Moon HW, Schneider RA, Moseley SL. 1986. **Comparative prevalence of four enterotoxin genes among *Escherichia coli* isolated from swine**. Am J Vet Res 47:210-212.

⁴ Shin SJ, Chang YF, Timour M, Lauderdale TL, Lein DH. 1994. **Hybridization of clinical *Escherichia coli* isolates from calves and piglets in New York State with gene probes for enterotoxins (STaP, STb, LT), Shiga-like toxins (SLT-1, SLT-II) and adhesion factors (K88, K99, F41, 987P)**. Veterinary Microbiology. January 38(3):217-25.

⁵ Chern, Eunice C., Tsai, Yu-Li, Olson, Betty H. **Occurrence of Genes Associated with Enterotoxigenic and Enterohemorrhagic *Escherichia coli* in Agricultural Waste Lagoons** Appl. Environ. Microbiol. 2004 70: 356-362.

⁶ Dufour AP, Cleseri LS, Greenberg AE (1981) **Membrane filter method for enumerating *Escherichia coli***. Appl. Environ. Microbiol 41:1152-1158.

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