



Report Supporting Document

DETECTION OF INDICATOR MICROORGANISMS FOR POSSIBLE SEWAGE CONTAMINATION

(Analysis Code: SSC)

INTRODUCTION

Sewage contamination in drinking and recreational waters is of great concern. Because the presence of enteric pathogens threatens public health, it is extremely important to determine the microbiological safety of the human environment, especially where water is concerned. However, analyses for the presence of waterborne pathogens are extremely difficult and complicated because some pathogens cannot be cultured in the laboratory, or may be injured after exposure to stressful environments. As a result, indicator microorganisms are widely used to detect possible contamination.

Among the indicator microorganisms, fecal coliforms or *Escherichia coli* and enterococci are more commonly used. The total coliform group includes aerobic and facultative anaerobic, gram-negative, rod-shaped bacteria that ferment lactose with gas and acid production in 24 to 48 hours at 35°C. Although total coliforms are traditionally used to detect water pollution of fecal source, some bacteria in the total coliform group can originate from nonenteric environments. Fecal coliform group is a subset of coliforms and is a more definitive indicator of fecal contamination. Among the fecal coliforms, *E. coli* is an even more specific indicator for the presence of fecal contamination. For this reason, *E. coli* has been incorporated into U.S. drinking water regulations as a specific indicator of sewage contamination. Enterococci such as *Enterococcus faecium* and *E. faecalis* exist more specifically in the intestinal tracts of human than fecal streptococci do.

The US EPA recommends testing for *E. coli* and enterococci indicators in place of total and fecal coliforms because these indicators show a direct correlation with gastrointestinal illness rates.

Aemtek is committed to providing fast, accurate and reliable analytical data based on samples and information submitted. As an environmental testing laboratory, Aemtek's role is to provide analytical data to facilitate microbiological investigations.

Aemtek does not conduct sample collection, site evaluation or consultation. Interpretation of the data presented in this report should best be left to the primary investigator.

MATERIALS AND METHODS

As a part of the investigation project, samples were collected and transported to the Aemtek microbiology laboratory for analysis. Data on sample identification, location, and air volume (for air samples) on this report are from the original **Chain of Custody Form**.

Total coliforms and *E. coli* detection

The Colilert® method was used for simultaneous testing of total coliforms and *E. coli*, based on SM9223 (American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*). Colilert® medium is manufactured by IDEXX using patented Defined Substrate Technology®. When total coliforms metabolize Colilert's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert's nutrient-indicator, MUG, the sample fluoresces. According to IDEXX Product Literature, this method can detect bacteria at the level of 1 organism/100 ml within 24 hours incubated at 35°C ± 0.5°C.

Colilert® is approved by US EPA for drinking water testing and incorporated in the testing methods (FOT101) of Microbiology of Drinking Water by California Department of Health Services.

Enterococci detection

Enterolert™ method was used for the detection of enterococci such as *E. faecium*, *E. faecalis*. The testing product, manufactured by IDEXX, is based on Defined Substrate Technology® and utilizes a nutrient indicator that fluoresces when metabolized by enterococci. This technology improves accuracy and avoids the need for hazardous sodium azide suppressants used in traditional media. According to IDEXX Product Literature, when the reagent is added to the sample and incubated at 41°C ± 0.5°C, bacteria down to 1 organism/100 ml sample can be detected within 24 hours.

Enterolert is approved by American Society for Testing and Materials (ASTM) for detecting enterococci in drinking water, source water, recreational (fresh and marine) water, bottled water, and wastewater. This method is incorporated in the testing methods (FOT126) of Microbiology of Recreational Water by California Department of Health Services and recommended for the analysis of recreational water for compliance with Health and Safety Code §115880 [Assembly Bill 411 (AB 411),

Statutes of 1997, Chapter 765]. Enterolert is proposed for approval by US EPA for detecting biological pollutants in ambient water.

RESULTS

The results of the laboratory analysis are shown on the **Data Sheet**. "Positive" indicates the presence of tested microorganisms based on the method used. "Negative" indicates the absence of tested microorganisms according to the procedure. The presence of *E. coli* and/or enterococci in the sample is an indication of fecal contamination and the possible presence of enteric pathogens.

QUALITY CONTROL

Quality control procedures are conducted on each lot of Colilert and Enterolert. Control agents include *E. coli* (positive control), *Klebsiella pneumoniae* (total coliforms), and *Pseudomonas aeruginosa* (non-coliforms) for Colilert, and *Enterococcus faecium* (positive control), *Serratia marcescens* (gram negative), and *Aerococcus viridans* (gram positive) for Enterolert.

REFERENCES

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Department of Health Service, California. 2000. *Recommended Methods for the Analysis of Recreational Marine Water to Comply with AB411*. DHS website, http://www.dhs.ca.gov/ps/ddwem/beaches/ab411_methods.htm

Colilert® - Product Literature by IDEXX Co.

Enterolert® - Product Literature by IDEXX Co.

Toranzos, G. A., and G. A. McFeters, 1997. Detection of Indicator Microorganisms in Environmental Freshwaters and Drinking Waters, p184-194. *In Hurst et al.* (eds.), *Manual of Environmental Microbiology*. American Society of Microbiology Press, Washington, D.C.

US EPA, 2001. Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Ambient Water; Proposed Rule. **Federal Register** 66 (169): 45811-45829.

ABOUT AEMTEK

Aemtek, Inc. is an environmental microbiology laboratory serving the indoor air quality industry. Its mission is to provide accurate, fast, and reliable expert services for detection, identification, analysis of fungi and bacteria in human environments. Aemtek also provides contracted research and expert testimony services. Aemtek is committed to the highest level of quality, scientific integrity, and customer service.

Aemtek, Inc. implements a series of quality assurance procedures. All analytical protocols are developed based on scientific merits and are validated regularly. Aemtek is accredited by the American Industrial Hygiene Association (AIHA) Environmental Microbiology Laboratory Accreditation Program (Lab No.: 167620)

ABOUT THE ANALYSTS

Florence Q. Wu, PhD

Dr. Florence Wu earned a Ph.D. degree in mycology from the University of Tennessee in 1991, and has vast experience in fungal identification, taxonomy, biodiversity, and biogeography. Dr. Wu was a principal investigator of a National Science Foundation research project on fungal biodiversity. She has published many scientific papers and is an active member of several professional organizations. She specializes in identifying fungi using microscopic examination, culture methods, and DNA techniques.

Steven Y. Huang, PhD

Dr. Steven Huang received a Ph.D. degree in plant pathology focused on forest mycology and microbiology. Before joining Aemtek, Dr. Huang was an associate professor in the Institute of Microbiology, CAS, a visiting scholar in the International Mycological Institute (United Kingdom) and Duke University, and a research associate in the University of Pittsburgh. His expertise includes microbial isolation, recovery, and identification, fungal biodiversity, PCR and DNA sequencing techniques.

Suvarna Gandlur, PhD

Coming from a clinical microbiology background, Dr. Gandlur received her PhD degree in molecular genetics and biochemistry from Georgia State University and postdoctoral training in microbiology and immunology in Stanford University. She has extensive laboratory experience in bacterial identification and molecular biology.

AyeAye Win

Ms. Win received a Bachelor of Science degree in microbiology from the Arizona State University in year 2000, with academic training in advanced bacteriology, medical microbiology, bacterial physiology, etc. She had over three years laboratory experiences in mycology and microbiology, particularly fungal spore trap analysis, bacterial identification, endotoxins, and DNA extraction, before joining Aemtek in February 2005. She is currently a senior microbiology technician at Aemtek and primarily responsible for culture preparation and bacteria testing.

CONTACT INFORMATION

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