



# Project Summary

## Improved Methods for Hepatitis A Virus and Rotavirus Concentration and Detection in Recreational, Raw Potable, and Finished Waters

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**A nucleic acid hybridization test for the detection of hepatitis A virus and an immunofluorescence test for the detection of human rotavirus are described. Also specifically detailed are the sampling, concentration and reconcentration steps for processing water, wastewater and associated solids for these viral pollutants. Although the subject matter is delineated in a concise step-by-step form to expedite its usability, the methods described are highly sophisticated technology directed toward the research structured laboratory.**

***This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (See Project Report ordering information at back).***

### Introduction

This research project has resulted in the development of detection methodology for hepatitis A virus and human rotavirus in the water environment. The standardized procedures developed by Baylor College of Medicine are set forth in the report in considerable detail, so that it can be better utilized as a laboratory bench guide. This product is part of the Laboratory's program to upgrade

monitoring methodology for the detection and enumeration of viral pollutants in water, wastewater, and associated solids. The development of these particular procedures is important because human rotavirus and viruses of the hepatitis A type are difficult or impossible to detect by current plaque assay procedures.

Recognizing the occurrence of recreational water-associated type A hepatitis has depended upon development of clinical illness in swimmers or bathers and probably does not reflect the true incidence of this epidemiologic route. In spite of recognition and reporting deficiencies, hepatitis A virus-associated illness is still the most prevalent type of perceived waterborne disease.

Human rotaviruses are excreted in large numbers in the stools of infected individuals. They survive sewage treatment and are released in waste treatment plant effluents. Availability of a monitoring system for detection of rotavirus is important because these fastidious viruses appear to be a major cause of childhood diarrhea.

### Results

Procedures described in this report include sample collection, sample concentration and viral titration for the detection of hepatitis A virus and human rotavirus. They have been found to be well suited to disclose the presence of these virus types in varying qualities of

water. The protocol is applicable in surface and finished waters. Finished water samples may be collected from distribution system outlets (e.g. faucet) or water mains at a municipal water treatment plant. Recreational or raw potable water samples that may be collected include water, solids suspended in water, or the uppermost loose layers of bottom sediments, called fluffy sediments. Water and suspended solids may be collected and processed as a single sample or collected and processed separately. Fluffy sediments are collected and processed separately. The virus adsorption-elution (VIRADEL) method as described in Chapter 6 of the *USEPA Manual of Methods for Virology* (EPA-600/4-84-013, February 1984) was used successfully for the processing of water samples. Water samples processed for suspended solids or fluffy sediments are passed through filters that served as solids-collecting surfaces. Solids-associated viruses were recovered by elution followed by reconcentration of eluates.

Procedures for detecting rotaviruses are dependent on cell culture amplification of one or more viruses in test sample. Assaying for their presence is based on an immunofluorescence test using a monoclonal antibody and fluorescein-isothiocyanate-conjugated antibody staining procedure to visualize virus-infected cells.

The techniques described for detecting hepatitis A virus are based on the use of labeled nucleic acid (gene) probes in hybridization tests to detect virus nucleic acid. Guidelines are provided for preparation of complementary DNA and single-stranded RNA probes used for their detection.

## **Conclusions and Recommendations**

The described methods should enable detection of hepatitis A virus and human rotavirus in polluted surface waters to a degree not currently possible. Where found, solids-associated virus in conjunction with the developed methodology can be exploited for separation and recovery of these viruses.

Improved methods for detection of hepatitis A virus and human rotavirus in water represent tools which can be used to offer better protection against the danger of waterborne virus infections. Environmental assessment of recreational, raw potable and shellfish waters can be extended to include these

viruses. Decisions about the effectiveness of water or wastewater treatment procedures for elimination of hepatitis A virus and human rotavirus can be based on results of tests in which these pathogens rather than simulant viruses were used.

The use of labeled nucleic acid probes for assaying for the type A hepatitis virus places this work at the cutting edge of virus detection technology. It should, therefore, be recognized that any undertaking of this test requires laboratory personnel with a background in molecular biology and a knowledge of molecular cloning theory and practice. Because of the considerable degree of specialization needed, this technology currently is limited to highly specialized research facilities and thus is not within the grasp of today's water monitoring laboratory. Nevertheless, it is an important step forward in virus detection technology and could form the foundation for the production of commercially available, off-the-shelf, gene probe test kits for hepatitis A virus, as well as other enteric viruses.

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The complete report, entitled "Improved Methods for Hepatitis A Virus and Rotavirus Concentration and Detection in Recreational, Raw Potable, and Finished Waters," (Order No. PB 88-158 944/AS; Cost: \$14.95, subject to change) will be available only from:

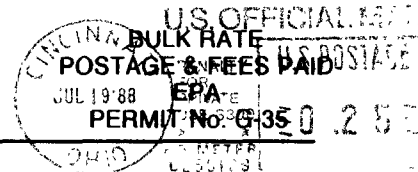
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