EPA Science Forum 2003
Partnering to Protect Human Health and the Environment

RAPID VIRUS DETECTION IN WATER

Emerging Technologies

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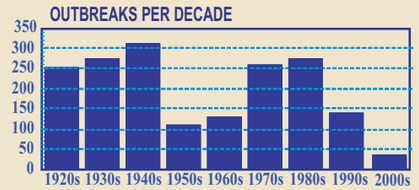
Environmental Issue:

WATERBORNE DISEASE (1920-2000)



- Waterborne disease outbreaks occur every year
  - Annual average is 23 outbreaks with 19,000 cases
  - Over half of the outbreaks are caused by viruses
- The actual disease burden from waterborne viruses is unknown because of a lack of detection methods.

Collaborative Approach:

A partnership of scientists and engineers from ORD, the Office of Ground Water and Drinking Water's Technical Support Center (TSC) and the American Water Works Association Research Foundation (AWWARF) was formed to improve every aspect of standard virus sampling procedures and to develop a new molecular virus detection method. The steps that are needed to detect viruses in water are as follows:

1. Sample Collection:

Virus in water are collected on filters

2. Filter Processing

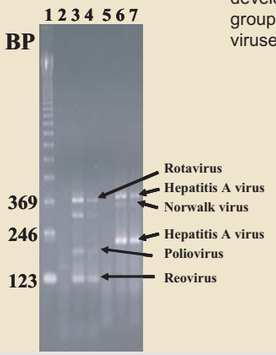
Viruses are removed from filters with over 90% recovery.

3. Virus Purification

Virus are purified and concentrated over 500,000 times with greater than a 70% recovery.

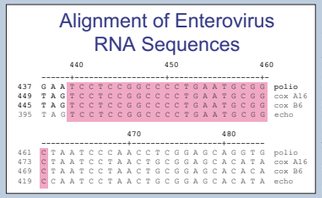
4. Virus Detection

RT - PCR Analysis of Samples



A new method was developed to detect major groups of waterborne viruses.

Alignment of Enterovirus RNA Sequences



The emerging tools of molecular genomics and bioinfomatics was used to develop the new method.

Results

Ground Water:

The virus method developed under the partnership between ORD, TSC and AWWARF was used to examine 29 ground water wells on a monthly basis for 12 months. Results showed that 16% of 321 groundwater samples and 72% of 29 groundwater sites were positive for viruses.

RESULTS

Virus	% Positive Samples	% Positive Sites
Enteroviruses	5	38
Reovirus	10	62
Rotaviruses	0	0
Hepatitis A virus	1	14
Norwalk virus	3	21
Total	16	72

Surface Water:

A second partnership was developed between ORD and the U.S. Geological Survey to test the methods using stream waters from five rural to urban sites across the U.S. The method proved effective for surface water with all sites being positive for viruses as shown below.

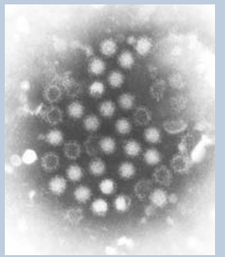
Site	Land Use	Population Density[a]	Percent Virus Enterovirus	Positive Samples Rotavirus	Positive Samples HAV
EIWA	Rural[b]	16	100	0	0
KANA	Rural[c]	88	33	0	0
SANT	Rural[d]	544	100	100	33
LERI	Urban[e]	2849	100	67	67
PUGT	Urban	15540	100	33	0

[a] Population per square kilometer
[b] Agriculture and confined animal facilities near site
[c] Mining areas and septic tanks near site
[d] Agriculture only
[e] Site includes some agricultural contributions

Outbreaks:

A third partnership was developed between ORD, EPA Region VIII, CDC and state Departments of Health to investigate two groundwater-related outbreaks of disease in the state of Wyoming in 2001. The method proved valuable for outbreak investigations in that norovirus strains were detected in the groundwater and shown to be identical to patient strains. The two outbreaks were caused by different norovirus gene groups.

Calicivirus



Viruses in Group:
Norovirus
Genogroup I
Norwalk
Genogroup II
Snow Mountain

Nucleic Acid:
Single-Stranded RNA

Disease:
Gastroenteritis

Impact:

- The virus detection method is available to collect virus occurrence data needed to support the development of regulations to protect the American public.
- The virus detection method is available to support exposure studies designed to determine the disease burden caused by waterborne viruses.
- Data from using the virus detection method will lead to improvement of drinking and recreational water quality.
- The method is adaptable to testing for new viruses in water, such as the recently emerging SARS corona-virus.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Partnering to Protect Human Health and the Environment