United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA-600/9-83-0[illegible] Sept. 1983

EPA

Research Priorities for Monitoring Viruses in the Environment

EPA-600/9-83-010
September 1983

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by

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NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Foreword

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid wastes.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report was prepared at the invitation of the USEPA and is consistent with the USEPA's commitment to provide the scientific public an opportunity to be heard on the major scientific issues that face the Agency and to offer to the Agency appropriate recommendations. To this end, non-EPA leaders in environmental virology focused on research priority needs in monitoring for viruses in the environment; they addressed the direction and rationale of these needs. It is the expectation of the USEPA that the results of their efforts will prove useful to regulatory, regional and research planners.

Robert L. Booth, Acting Director
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Working Group on Research Priorities for Monitoring Viruses in the Environment

September 22, 1982

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**This document does not necessarily reflect the agency views of participant.*

I. Introduction

On May 29 and 30, 1980, the USEPA invited a group of experts to participate in a Conference On Monitoring Viruses In the Environment.[1] Following that conference, EPA convened a smaller working group of seven persons[2] to prepare specific recommendations setting research priorities for monitoring viruses in the environment. The working group met on September 22, 1982. This document is their report recommending research priorities for monitoring viruses in the environment.

This report is organized in two sections:

1. Summary and Recommendations
2. Rationale for the Recommendations[3]

1 The following persons participated in the 1980 Conference: Georges Belfort, Gabriel Bitton, Kerby Fannin, Charles Gerba, John Herrmann, Joseph Karaganis, Edward Larkin, Joseph Melnick, Theodore Metcalf, Bernard Sagik, Pasquale Scarpino, Stephen Schaub, James Smith, Mark Sobsey, Charles Sorber, Robert Sullivan, James Vaughn, Craig Wallis and Flora Mae Wellings.

2 Joseph Karaganis, Edward Larkin, Pasquale Scarpino, Stephen Schaub, Charles Sorber, Robert Sullivan, Flora Mae Wellings and Joseph Melnick, Chairman.

3 In writing the rationale for the research priorities, the working group drew heavily on the 1980 conference. However, the 1982 group has expressly focused on research priorities.

II. Summary and Recommendations

A. Summary

The following facts have been established about human viruses in the aqueous environment.

1. Viruses of public health significance are abundantly present in wastewater, and can be found even after activated sludge treatment and chlorine disinfection.
2. Viruses are concentrated in wastewater sludges which may be ultimately used on land as fertilizers and soil conditioners.
3. Viruses are present in surface waters, especially rivers which many communities use as sources of potable water.
4. In addition to intentional wastewater reclamation, water is being recycled inadvertently as one community pollutes the water source of a second community.
5. Viruses have been detected in the treated water supplies of large and small communities in a number of countries, including the United States.
6. Increasing numbers of communities in different parts of the country are turning to land application of wastewater and sludges. Soils vary greatly in their capacity to adsorb viruses, and thus, to preclude groundwater contamination.
7. Enteric viruses can remain viable for months in water and probably longer when associated with solids found in water.
8. Waterborne dissemination of infectious viruses may occur without being detected.

Research should be conducted in several important areas designed to address the problems described above.

B. Recommendations

1. High Priority Research Needs

a. Monitoring and evaluating the effectiveness of water and wastewater and sludge treatment unit processes for virus removal are essential to developing appropriate virus control strategies. Process evaluations should be accomplished through use of present methods and new methods as they are developed.

b. Current methods often fail to detect viruses of human origin present in water and wastewater. Emphasis should be placed on:

(1) improving the sensitivity of existing methods especially in the presence of particulates;

(2) developing relatively simple methods or adapting existing methods for viruses of particular significance such as hepatitis A virus, rotaviruses and the Norwalk-like gastroenteritis virus group; and

(3) developing a quality assurance program that includes round robin testing for existing and newly proposed methods.

c. Currently available methods for monitoring shellfish for the presence of viruses fail to detect hepatitis, Norwalk, or rotavirus and gastrointestinal viruses. Thus, there is a need to develop more sensitive, quantitative shellfish methodology to detect these and other viruses of importance to human health.

2. Priority Research Needs

a. Develop improved, sensitive, quantitative methods for the detection of viruses in sludges and soils associated with the land application of wastewaters and sludges.

b. Search for more suitable indicators of the presence of viruses in water, wastewater, sludges and soils. Some of this effort can be accommodated through research appropriately designed to accomplish the high priority research recommendation stated in 1.a. above.

III. Rationale

Public health scrutiny of disease causing agents -- be they pathogens or toxic chemicals -- is always beset with unanswered questions. Indeed, that is the *raison d'etre* for pursuing and focusing on public health research.

The agents of concern to this working group are enteric viruses -- particularly those viruses associated with the digestive tract and typically excreted in human wastes. Enteric viruses are responsible for a wide range of serious illnesses ranging from hepatitis (liver disease) to myocarditis (heart disease) to pleurodynia (chest disease) to central nervous system disorders (e.g. polio), to acute gastroenteritis (intestinal cramps, diarrhea), to death.

While much remains unknown about numerous aspects of viruses -- just as much remains unknown about asbestos, vinyl chloride, polychlorinated biphenyls (PCBs) and other toxic chemicals -- there are many known facts which allow us to recommend research priorities at this time. The known facts about viruses in the water environment which form the factual predicate for the working group's research priority recommendations are set forth in the Summary, above.

Based on the eight facts set forth in the Summary, the working group has identified certain health hazards and research priorities. These research priorities and recommendations are an outgrowth of the working group's analysis of research needs in four areas:

1. monitoring for viruses in the environment;
2. the development and identification of indicator organisms or tests to serve as surrogates for direct measurement of viruses;
3. improvement of analytical methods for virus concentration and assay; and
4. improvement and development of quality assurance procedures for viral analysis.

These four areas are closely interrelated and are chosen for analytical convenience. The following discussion under these four areas collectively presents the rationale for the working group's research recommendations listed above.

A. Monitoring

Given the health risk presented by viruses, it is essential to develop more information on the nature and extent of viral contamination in our nation's waters. This information can only be provided through increased monitoring of each major pathway leading to the deposition of viruses into the nation's waters.

In establishing a virus monitoring program, several factors ought to be considered:

Site Selection. The selection of monitoring sites around the country should be made under the combined guidance of EPA and other scientists.

Laboratory Procedures — Round Robin Analysis. A procedure should be developed for gathering, allocating and distributing the samples to various laboratories for round robin testing.

Standard Protocols. In performing viral analysis, all laboratories should follow standard sampling and operational protocols agreed upon by the round robin participants. However, each laboratory should also be free to use and compare other methods of their own choosing.

Parallel Biological and Chemical Analysis. The participating laboratories should also monitor biological indicators of pollution (for example, fecal coliforms, fecal streptococci, mycobacteria), and the chemical and physical constituents of the water being sampled which are significant.

Media to be Sampled. The monitoring effort should encompass sampling at all locations where viruses may be present including sewage, water, shellfish and aerosols. In each medium, the following factors should be considered:

1. Sewage
   a. Untreated raw wastewater (influent). These samples are usually rich in viruses and results of their analyses should serve as a control of the sensitivity of the virus concentration and virus enumeration method. These samples also serve to indicate the virus load requiring treatment.
   b. Effluent. Effluent from the wastewater treatment process before and after dilution in the receiving water (recreational water) should be monitored.
   c. Sludge. Sludge monitoring is essential where sludge may come in contact with humans or their food crops.
2. Water
   a. Untreated source water. This includes river, lake or ground water immediately prior to treatment.
   b. Potable water immediately after treatment.
   c. Potable water at the tap. Selected locations should be sampled to determine if defects exist in the distribution system.
3. Shellfish

   Monitoring shellfish should be the responsibility of the federal and state agencies concerned (EPA, FDA and NOAA, and the state agencies).
4. Aerosols

   The emphasis at this stage of methods development should be on water that forms the aerosol.

B. Indicators

Ideally, isolation of the viruses themselves is the most appropriate means of virus detection. However, at this time, widespread direct testing for virus is hampered by such factors as the long time required to obtain test results, variations in the precision and accuracy (i.e. detectability) of various virus types, the shortage of competent personnel, and the high cost of viral analysis.

Consequently, it is desirable to identify if possible, reliable indicator organisms and analytical methods to serve as surrogates for the presence of viruses. The use of such biological or chemical indicators is often used in public health. For example, public health and environmental health practitioners currently use fecal coliform bacteria as an indicator organism for fecal bacterial pathogens.

No universal indicator presently exists that is suitable for detection of all viruses. Virus occurrence in the environment is sporadic and there are differences between viruses and indicators in survival capabilities, ease of detection responses to environmental stress, and susceptibility to disinfection. In addition, there are differences in the response to environmental stresses between enteric viruses and candidate indicators.

Indicators may be drawn from bacterial, yeast and viral groups. Candidate bacterial indicators may be the coliform group (total and fecal), fecal streptococci, anaerobic spore formers (clostridia species) and non-spore formers (bifidobacteria), acid fast forms, and standard plate counts. Candidate viral indicators may be either bacteriophages or selected enteric viruses.

Conceptually, any of the above may serve as a surrogate indicator for the viruses. Certain candidate indicators may prove more useful than others in a given situation, such as in the examination of sludges, soils, leachates, or water. The choice of the surrogate virus indicator should be determined by the nature of the environmental sample to be tested.

Candidate chemical indicators such as the fecal sterols (coprostanol) have also been suggested as surrogate indicators of fecal pollution.

Historically, coliforms have gained wide acceptance as indicators of fecal pollution. The acceptability of members of the coliform group as indicators of viruses has been based upon the argument that there are many more coliform bacteria present in sewage than viruses. While some general relationship may exist between indicator and virus numbers in grossly polluted waters, discrepancies may occur in high quality waters perhaps due to differences in sample sizes used in tests for viruses and coliform bacteria (e.g., 400 liters vs 50 to 100 ml, respectively for drinking water) or perhaps due to the lack of nutrients to sustain bacterial life as opposed to the inert viruses.

There are other limitations on the use of bacterial indicators. Viruses generally are more resistant to disinfection than coliform bacteria. Moreover, the greater persistence of viruses in receiving water and sediment may lead to a further disproportionate relationship between surviving bacteria and the presence of viruses. In addition, some members of the total coliform group such as *Klebsiella* may manifest regrowth which is impossible for viruses.

Fecal streptococci are more resistant to disinfection than coliform bacteria. Some strains of fecal streptococci persist for days in irrigation waters, sludges and landfill leachates, but fecal streptococci do not multiply in the environment. Some fecal streptococci biotypes are also ubiquitous in aquatic environments. Since fecal streptococci do not multiply in the environment, they would appear in some circumstances *a priori* to be more suitable indicators of enteric viruses than fecal coliforms (e.g. in sludges).

The spore-forming clostridia have the disadvantage of much greater persistence than other bacteria indicators and viruses in the environment. Consequently, at best, certain clostridia may be used as tracers of remote pollution rather than as indicators of viruses.

Although some species of bifidobacteria are specifically associated with human fecal pollution and although they are less likely to regrow in the environment, they may be subject to environmental stresses, especially the presence of oxygen. Bifidobacteria are unsuitable as indicators because they are difficult to detect and because their relationship to enteric viruses is unknown.

Two acid fast bacteria (*Mycobacterium phlei* and *M. fortuitum*) and a yeast (*Candida parapsilosis*) have demonstrated their usefulness as indicators of disinfection effectiveness. Their usefulness is related to their greater resistance to chlorine disinfection than *Escherichia coli, Salmonella typhimurium* and poliovirus type 1. Two disadvantages are associated with the use of these indicators. The minimum incubation time required for growth of mycobacteria is 3 days, and commercially prepared growth media are unavailable.

Standard bacterial plate counts have been suggested as an indicator of the quality of reclaimed water. However, only limited information exists on the suitability of this heterogeneous group of bacteria as an indicator of viruses.

Coliphages such as f2, MS2 and members of the T series may have a restricted, yet useful, indicator function. These phages may serve as laboratory or field models to assess the rate or extent of virus removal in water and wastewater treatment plants, and may possibly be used as tracers. Two areas of investigation remain unanswered. One concerns the ecological relationship that exists between phages and bacterial host cells (i.e., multiplication of phages in the test samples). The second concerns the relationship between the rate of survival of phages and enteric viruses. There have been conflicting results in comparative studies conducted on the survival of phages and enteric viruses, which casts further doubt on the usefulness of phages as indicators of enteric viruses.

A vaccine strain of poliovirus (type 1) has been suggested as a potential indicator for other enteric viruses. This vaccine strain is shed in large numbers by vaccinated individuals, is relativley safe to handle (in seeding studies), and may be more readily detected in environmental samples than wild enteric viruses. One of the most serious objections to the use of a single virus indicator, such as poliovirus type 1, is that neither poliovirus type 1 nor any other enteric virus is always found in fecal samples.

At present, the entire enteric virus group itself is the most meaningful, reliable and effective virus index for environmental monitoring.

In summary, the search for the most suitable surrogate indicator for the presence of enteric viruses should continue. Candidate indicators should be evaluated as to their similarity to viruses on such qualities as regrowth, patterns of survival and disinfectant resistance.

C. Methods Development

There are currently several analytical laboratory methods for the detection and quantification of enteric viruses in environmental samples. There is general agreement that when such methods indicate the presence of virus such an affirmative finding is reliable. However, there is serious concern that existing methods underestimate the quantity of virus or alternatively produce false negative results when viruses are actually present in the sample.

Several factors have been identified which may account for these under-estimation or false-negative problems. These include, but are not limited to, the following:

1. The development of most of the current methods has emphasized the recovery of members of the human enterovirus group (i.e., polioviruses, echoviruses, coxsackieviruses). In addition, most development studies have utilized laboratory strains of the respective virus types. Several recent studies have suggested that such strains may not always represent an appropriate model for naturally occurring viruses.

2. The myriad of sample types and qualities under consideration (e.g., "dirty" water, "clean" water, sludges, soils, marine sediments, etc.) lead to a wide variability in technique efficiencies. This variability is further noted in samples of similar types taken from different locations and from the same location at different times of the year.

3. Current methods may not be appropriate for all of the viruses of public health significance (e.g. at present, assay techniques are not generally available for the recovery and enumeration of Norwalk-like agents, human rotaviruses, most of the Coxsackie A viruses and hepatitis A viruses in environmental samples).

4. Some current methods may not be adequate for the detection of solids-associated or solids-occluded viruses.

5. Methods for enumeration of viruses in samples may exhibit variability among different testing laboratory groups, which may result from the use of different host-cell systems and differing methods of enumeration.

6. Methods have not yet been standardized, quality control is limited, personnel involved in methods application are often not well-trained. No single method is sufficiently meritorious to be put forth as a fully adequate method at this time.

Specific areas for the application of research in improved virus recovery techniques are:

1. Water

   a. The type and quality of water (i.e., marine, fresh, wastewater) sampled has a profound effect on the efficiency of a particular recovery technique. In spite of this, several methods have been developed and successfully used for the recovery of indigenous viruses.

   b. The method most widely used for recovering viruses from aquatic systems is adsorption-elution-reconcentration.

   c. Tangential flow ultrafiltration-reconcentration has also been introduced but has not yet been widely adopted.

   d. The present methods do not detect or quantify all indigenous viruses present in water.

2. Sludge

   a. Most methods for extraction of viruses from sludges involve elution followed by concentration which does not address solids-occluded viruses. A major problem with these and other methods has been the "co-concentration" of a variety of chemical compounds which have proven toxic to the assay tissue cultures and possibly to the viruses under test.

   b. The reduced recoveries noted in sludge-seeding studies correlate with viral toxicity and also with viral complexing to components of the sludge. To date, recovery methods have not addressed the variation posed by the different types of sludge (e.g., aerobically and anaerobically digested, raw, etc.).

3. Aerosols

   a. Application of procedures for the recovery of viruses from wastewater aerosols lags some years behind comparable methods development for water. In addition, the physical problems inherent in the collection of virus particles from large sample volumes of air are a complicating factor.

b. An added sampling problem results from variability in meteorological conditions.

c. The methods used have not been developed for the express purpose of recovering viruses from wastewater aerosols, but rather have been adapted from techniques designed for sampling for microorganisms and other types of pollutants.

d. Present methods involve collection of airborne viruses into a liquid medium which must then undergo some form of reconcentration.

4. Shellfish

a. Quantitative extraction of viruses from shellfish meats has proven difficult.

b. At present, no single method can be used with the assurance of recovering viruses from all shellfish types.

c. Present methods have cytotoxicity problems and sometimes are unable to contain bacterial contamination (which further compromises an already stressed tissue culture assay system).

d. While many methods have been described in the literature, most used currently involve some combination of clarification, extraction and reconcentration steps.

e. In addition to the need for efficient methods, there is a need to define representative sampling procedures.

5. Solids

The full significance of the relationship between viruses and solids (soils and sediments) has only begun to be evaluated. Proposed methods involve elution followed by concentration. Definition of sample collection methods is required.

In light of the foregoing discussion, specific research needs have been identified within the general area of virus methods development. They are:

1. The development of more quantitative methods for assessing the role of viruses in the variety of environmental systems is essential.

2. Increased emphasis must be placed on methods applicable to the recovery of a wider spectrum of indigenous viruses of public health significance.

3. Recognizing the limitations of present methods, an emphasis should be placed on further development of current methods and the development of new practical methods for all environmental systems.

4. Methods to be used for practical monitoring must be rapid, sensitive, reproducible, simple and economical. They must be capable of processing sample volumes large enough to be significant with regard to detection of expected indigenous viral concentrations.

5. A mechanism should be developed, preferably under EPA sponsorship, for the comparative assessment by a variety of laboratories of the recovery capabilities of different virus monitoring methods.

D. Quality Assurance

Consistent with the need for improved viral detection methods is the need for standardization of quality assurance procedures in viral sample collection and laboratory analysis.

Quality assurance should apply to the collection of all environmental virus data.

With respect to virus monitoring data, the following statement is endorsed by the working group:

"These data must be scientifically valid, legally defensible, representative, comparable, complete, and of known precision and accuracy."

(From: The Quality Assurance Program, An Overview, USEPA, Office of Research and Development, Washington, D.C. 20460, March 1980.)

The objectives and activities of a quality assurance program for monitoring human enteric viruses in environmental samples should include:

1. the provision of methods and materials;
2. the evaluation of monitoring performance; and
3. the enhancement of monitoring performance capabilities.

There are a number of considerations which should be given to a quality assurance program associated with the monitoring of viruses in the environment. Specific points for consideration follow:

1. General
   a. General quality assurance criteria, including statistical considerations, should be developed for the operation of an environmental virology laboratory.
   b. Quality assurance should apply to both reference and candidate methods and procedures.
   c. Quality assurance for enteric virus monitoring should consider:
      (i) the variability in types, amounts and state of viruses present in field samples;
      (ii) the characteristics, quality and size of the sample; and
      (iii) each step of all stages of virus recovery including sample collection, processing, assay procedures and data analysis.
   d. Laboratory procedures should be developed with respect to facilities, operators, and sample handling in order to assure the validity of results by prevention of extraneous virus contamination.
2. Laboratory Evaluation of Virus Monitoring Methods
   a. There should be two types of test samples for intra- and inter-laboratory methods comparison, one containing laboratory-grown viruses and, when feasible, another containing indigenous viruses.
   b. The types, quantities and states (e.g., free, solids-associated) of viruses used in test samples should reflect those that would be expected in field samples.
   c. Size and quality of test samples should be consistent with the size and quality of field samples.
   d. The number and frequency of quality assurance samples (including positive and negative test samples) should be governed by statistical considerations. An important reason for including negative controls is to assure the integrity of the test system with respect to extraneous and/or cross-contamination.

3. Field Monitoring

The following should be compatible with the field monitoring objectives:

a. the location, placement and number of monitoring stations;

b. environmental conditions, sample size and frequency of sample collection;

c. quality assurance procedures for collecting, processing, handling, transporting, preserving and storing field samples, and all aspects of virus procedures; and

d. consideration should be given to the development and use of internal controls (markers, tracers) in field samples for quality assurance of field monitoring.

4. Coordination of Effort

There should be coordination of virus monitoring quality assurance activities among environmental laboratories and with other organizations involved in similar efforts.

☆U. S. GOVERNMENT PRINTING OFFICE: 1983/659-095/07