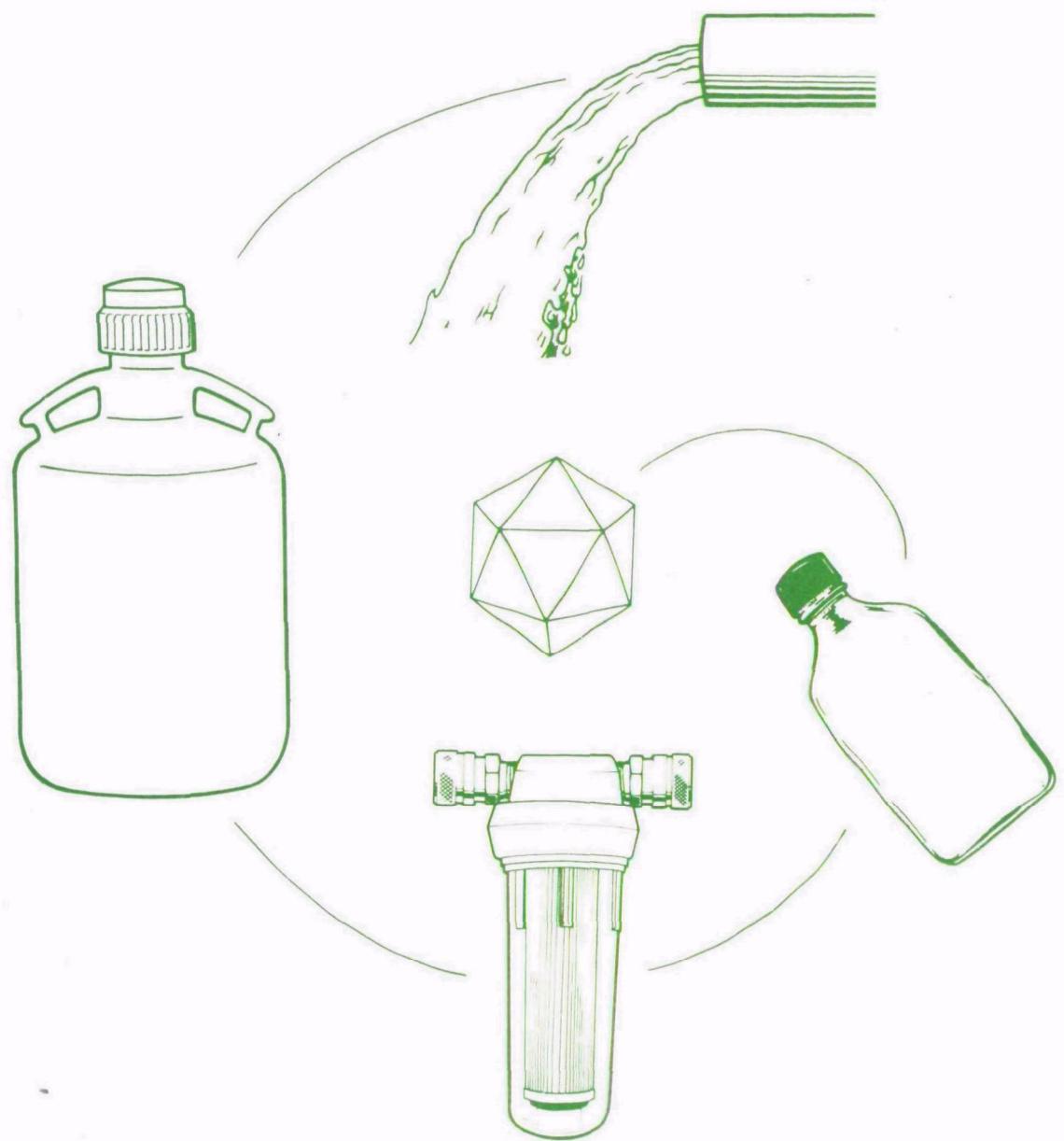




# USEPA Manual of Methods for Virology



## Foreword

Environmental measurements are required to determine the quality of ambient water, the character of effluents, and the effects of pollutants on aquatic life. The Environmental Monitoring Systems Laboratory-Cincinnati conducts research to develop, evaluate, standardize and promulgate methods to:

- Measure the presence and concentration of physical and chemical pollutants in water, wastewater, bottom sediments, and solid waste.
- Concentrate, recover, and identify enteric viruses, bacteria, and other microorganisms in water, waste, soil and air.
- Determine the health and ecological effects of viruses, bacteria and parasites in the environment.
- Measure the effects of pollution on freshwater, estuarine, and marine organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish.
- Automate the measurement of the physical, chemical, and biological quality of water.
- Conduct an Agencywide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

This manual was prepared and updated in order to meet mandates of the Congress of the United States of America as directed in the Water Quality Act of 1987 (PL 100-4), the Safe Drinking Water Act (PL 93-523) as amended by the Safe Drinking Water Act Amendments of 1986 (PL 99-339), the Marine Protection, Research, and Sanctuaries Act (PL 92-532), and the Resource Conservation and Recovery Act (PL 94-580). The manual presents a standardized, step-by-step procedure for recovering viruses from most environmental samples other than air.

Thomas A. Clark, Director  
Environmental Monitoring Systems  
Laboratory—Cincinnati

## Purpose

This manual provides procedures for collecting scientifically valid and legally defensible information on human enteric viruses in water, wastewater and treated effluents and in sludge, sediments and other solids as related to water quality problems, pollution sources and control requirements. It focuses on practical and economical virus monitoring technology and makes it possible for any competent water bacteriology laboratory that can arrange for viral assays (and identifications) to evaluate and quantify enteric viruses in environmental samples.

## Table of Contents

<b>Foreword</b> .....	i
<b>Purpose</b> .....	ii
<b>Figures</b> .....	xi
<b>Tables</b> .....	xiii
<b>Chapter 1 Introduction</b> .....	1-1
1. Perspectives in Environmental Virology .....	1-1
2. The Viruses in Environmental Waters .....	1-2
3. Conclusions and Recommendations of the World Health Organization (WHO) Scientific Group on Human Viruses in Water, Wastewater and Soil .....	1-4
3.1 Conclusions of the Group .....	1-4
3.2 Recommendations of the Group .....	1-6
3.3 Summary .....	1-7
4. History of Methods Selection .....	1-7
4.1 Recommendations of the WHO Working Group and the WHO Scientific Group .....	1-9
4.2 Recommendations in <i>Standard Methods for Detecting Viruses in Various Waters</i> .....	1-10
4.3 Recommendations of the American Society for Testing and Materials (ASTM). ....	1-11
5. The USEPA Manual .....	1-11
6. Bibliography .....	1-13
<b>Chapter 2 Cleansing Laboratory Ware and Equipment</b> .....	2-1
1. Precautions .....	2-2
2. Alternate Procedures .....	2-3
3. Preparation of Cleansing Compounds and Reagents .....	2-4
4. Procedure for Cleansing Laboratory Ware and Equipment .....	2-5
4.1 Cleansing with Detergent .....	2-5
4.1.1 General Laboratory Ware and Washable Equipment .....	2-5
(a) Washing machine procedure .....	2-5
(b) Manual washing procedure .....	2-6
4.1.2 Test Tubes .....	2-7
4.1.3 Pipettes .....	2-8
4.1.4 Automatic Pipettor .....	2-9
4.1.5 Automatic Syringe .....	2-14
4.1.6 Disc Filter Holder .....	2-17
4.1.7 Dispensing Pressure Vessel .....	2-18
4.1.8 Plastic Screw Caps .....	2-19

---

4.2	Cleansing with Acid . . . . .	2-20
4.2.1	General Acid-Resistant Laboratory Ware . . . . .	2-21
(a)	Chromic acid procedure . . . . .	2-21
(b)	Nitric acid procedure . . . . .	2-22
4.2.2	Test Tubes . . . . .	2-23
4.2.3	Pipettes . . . . .	2-25
4.3	Cleansing with Alkalies . . . . .	2-27
5.	Bibliography . . . . .	2-28
<b>Chapter 3 Sterilization and Disinfection . . . . .</b>		<b>3-1</b>
1.	General Procedures . . . . .	3-1
2.	Sterilization Techniques . . . . .	3-1
2.1	Solutions . . . . .	3-1
2.2	Glassware, Autoclavable Plasticware, and Equipment . . . . .	3-1
2.3	Contaminated Materials . . . . .	3-7
3.	Disinfection Techniques . . . . .	3-8
4.	Bibliography . . . . .	3-9
<b>Chapter 4. Quality Assurance . . . . .</b>		<b>4-1</b>
1.	Introduction . . . . .	4-1
1.1	Role in Research . . . . .	4-1
1.2	Scope of Program . . . . .	4-2
2.	Sample Collection . . . . .	4-2
2.1	Water and Sewage Samples . . . . .	4-2
2.2	Chain of Custody . . . . .	4-3
2.3	Sample Handling Procedures . . . . .	4-3
2.4	Transport of Samples . . . . .	4-3
3.	Laboratory Facilities . . . . .	4-4
3.1	Air Handling Systems . . . . .	4-4
3.2	Disinfection of Laboratory . . . . .	4-4
3.3	Space Allocation . . . . .	4-4
3.4	Traffic . . . . .	4-4
3.5	Bench Space Allocation . . . . .	4-5
3.6	Lighting . . . . .	4-5
3.7	Walls and Floors . . . . .	4-5
3.8	Monitoring for Cleanliness in Work Areas . . . . .	4-6
4.	Laboratory Maintenance . . . . .	4-6
4.1	Cleaning . . . . .	4-6
4.2	Storage . . . . .	4-7
5.	Laboratory Personnel . . . . .	4-7
5.1	Professional Level . . . . .	4-7
5.2	Supervisory and Senior Grade Level . . . . .	4-8
5.3	Technical Level . . . . .	4-8
5.4	Supervision of Personnel in Laboratory . . . . .	4-8
6.	Laboratory Equipment and Instruments . . . . .	4-9
6.1	Balances . . . . .	4-9

6.2	pH Meters . . . . .	4-9
6.3	Distilled Water . . . . .	4-9
6.4	Deionized Distilled Water . . . . .	4-10
6.5	Ultraviolet Lights . . . . .	4-10
6.6	Centrifuges . . . . .	4-10
6.7	Downward Flow Laminar Hoods . . . . .	4-11
6.8	Thermometers . . . . .	4-11
6.9	Refrigerators . . . . .	4-11
6.10	Dispensing Apparatus . . . . .	4-11
6.11	Steam Autoclaves . . . . .	4-11
6.12	Gas Sterilizers . . . . .	4-12
6.13	Hot-Air Ovens . . . . .	4-12
6.14	Roller Drum Apparatus . . . . .	4-12
6.15	Freezers . . . . .	4-12
6.16	Incubators . . . . .	4-12
6.17	Security . . . . .	4-13
7.	Laboratory Supplies . . . . .	4-13
7.1	Laboratory Ware . . . . .	4-13
7.2	Media and Chemicals . . . . .	4-13
7.3	Membrane Filters . . . . .	4-14
7.4	Sintered-Glass Filters . . . . .	4-14
8.	Laboratory Procedures . . . . .	4-14
8.1	Cell Cultures . . . . .	4-14
8.1.1	Test for Sterility . . . . .	4-14
8.1.2	Preparation of Cell Lines . . . . .	4-14
8.1.3	Preparation of Cell Cultures . . . . .	4-15
8.1.4	Record Keeping . . . . .	4-15
8.2	Virus Assays . . . . .	4-15
8.2.1	Preparation for Assays . . . . .	4-15
8.2.2	Volume Assayed . . . . .	4-16
8.2.3	Time of Assay . . . . .	4-16
8.2.4	Controls . . . . .	4-16
8.2.5	Counting Plaques . . . . .	4-16
8.2.6	Disposition of Data . . . . .	4-16
9.	Bibliography . . . . .	4-25
Chapter 5.	Virus Adsorption-Elution (Viradel) Disc Filter Procedures for Recovering Viruses from Sewages, Effluents, and Waters . . . . .	5-1
1.	Adsorption—Method One . . . . .	5-1
1.1	Preparation . . . . .	5-1
1.1.1	Apparatus and Materials . . . . .	5-1
1.1.2	Media and Reagents . . . . .	5-3
1.2	Procedure . . . . .	5-3
1.2.1	Assembly of Apparatus . . . . .	5-3
1.2.2	Salt Supplementation . . . . .	5-7
1.2.3	Adjustment of pH . . . . .	5-7
1.2.4	Filtration of Salted, pH-Adjusted Sample . . . . .	5-8
2.	Adsorption—Method Two . . . . .	5-9
2.1	Preparation . . . . .	5-9
2.1.1	Apparatus and Materials . . . . .	5-9
2.1.2	Media and Reagents . . . . .	5-11
2.2	Procedure . . . . .	5-12
2.2.1	Preparation and Implementation . . . . .	5-12
(a)	Assembly of apparatus . . . . .	5-14
(b)	Treatment of prefilters . . . . .	5-17
(c)	Salt supplementation . . . . .	5-23

---

(d) Adjustment of pH . . . . .	5-23
(e) Dechlorination . . . . .	5-24
(f) Fluid proportioner . . . . .	5-24
2.2.2 Filtration of Sample . . . . .	5-27
 3. Elution and Reconcentration . . . . .	5-30
3.1 Procedure for Eluting Viruses from Filter . . . . .	5-30
3.1.1 Apparatus and Materials . . . . .	5-30
3.1.2 Media and Reagents . . . . .	5-30
3.1.3 Procedure . . . . .	5-31
3.2 Procedure for Processing Solids . . . . .	5-32
3.2.1 Apparatus and Materials . . . . .	5-32
3.2.2 Media and Reagents . . . . .	5-33
3.2.3 Procedure . . . . .	5-33
3.3 Organic Flocculation Concentration Procedure of Katzenelson . . . . .	5-36
3.3.1 Apparatus and Materials . . . . .	5-36
3.3.2 Media and Reagents . . . . .	5-36
3.3.3 Procedure . . . . .	5-36
 4. Bibliography . . . . .	5-40
 <b>Chapter 6 Virus Adsorption-Elution (Viradel) Cartridge Filter Procedures for Recovering Viruses from Sewage, Effluents, and Waters . . . . .</b>	<b>6-1</b>
1. Adsorption—Method One . . . . .	6-1
1.1 Preparation . . . . .	6-1
1.1.1 Apparatus and Materials . . . . .	6-2
1.1.2 Media and Reagents . . . . .	6-4
1.2 Procedure . . . . .	6-5
1.2.1 Preparation and Implementation . . . . .	6-5
(a) Assembly of apparatus . . . . .	6-5
(b) Salt supplementation . . . . .	6-9
(c) Adjustment of pH . . . . .	6-10
(d) Dechlorination . . . . .	6-10
(e) Fluid proportioner . . . . .	6-11
1.2.2 Filtration of Sample . . . . .	6-13
2. Adsorption—Method Two . . . . .	6-15
2.1 Preparation . . . . .	6-15
2.1.1 Apparatus and Materials . . . . .	6-15
2.1.2 Media and Reagents . . . . .	6-20
2.2 Procedure . . . . .	6-21
2.2.1 Preparation and Implementation . . . . .	6-23
(a) Assembly of apparatus . . . . .	6-23
(b) Salt supplementation . . . . .	6-24
(c) Adjustment of pH . . . . .	6-25
(d) Dechlorination . . . . .	6-25
(e) Fluid proportioner . . . . .	6-26
2.2.2 Filtration of Sample . . . . .	6-29
3. Elution and Concentration—Method One . . . . .	6-31
3.1 Procedure for Eluting Viruses from Filters . . . . .	6-31
3.1.1 Apparatus and Materials . . . . .	6-31
3.1.2 Media and Reagents . . . . .	6-34
3.1.3 Rearrangement of Apparatus . . . . .	6-34
(a) Rearrangement for Method One . . . . .	6-34
(b) Rearrangement for Method Two . . . . .	6-35

---

3.2	3.1.4 Elution Procedure . . . . .	6-37
	Reconcentration—Method A. Membrane Disc Procedure	6-38
3.2.1	Apparatus and Materials . . . . .	6-38
3.2.2	Media and Reagents . . . . .	6-39
3.2.3	Procedure . . . . .	6-40
	(a) Assembly of apparatus . . . . .	6-40
	(b) Adjustment of pH of eluate . . . . .	6-40
	(c) Filtration of eluate . . . . .	6-43
	(d) Elution of viruses from filter . . . . .	6-43
3.3	Reconcentration—Method B. Aluminum Hydroxide-Hydroextraction Procedure . . . . .	6-45
3.3.1	Apparatus and Materials . . . . .	6-45
3.3.2	Media and Reagents . . . . .	6-46
3.3.3	Procedure . . . . .	6-47
	(a) Preparation of dialysis bag . . . . .	6-47
	(b) Flocculation and hydroextraction . . . . .	6-48
4.	Elution and Concentration—Method Two . . . . .	6-53
4.1	Procedure for Eluting Viruses from Filters . . . . .	6-53
4.1.1	Apparatus and Materials . . . . .	6-53
4.1.2	Media and Reagents . . . . .	6-55
4.1.3	Rearrangement of apparatus . . . . .	6-55
	(a) Rearrangement for Method One . . . . .	6-55
	(b) Rearrangement for Method Two . . . . .	6-56
4.1.4	Elution Procedure . . . . .	6-57
4.2	Organic Flocculation Concentration Procedure of Katzenelson . . . . .	6-58
4.2.1	Apparatus and Materials . . . . .	6-58
4.2.2	Media and Reagents . . . . .	6-59
4.2.3	Procedure . . . . .	6-59
5.	Bibliography . . . . .	6-62
<b>Chapter 7 Method for Recovering Viruses from Sludges (and Other Solids) . . .</b>		<b>7-1</b>
1.	Extraction of Viruses from Sludges . . . . .	7-1
1.1	Preparation . . . . .	7-1
1.1.1	Apparatus and Materials . . . . .	7-1
1.1.2	Media and Reagents . . . . .	7-2
1.2	Procedure . . . . .	7-3
1.2.1	Conditioning of Sludge . . . . .	7-3
1.2.2	Elution of Viruses from Sludge Solids . . . . .	7-6
2.	Concentration of Viruses from Sludge Eluates . . . . .	7-9
2.1	Organic Flocculation Concentration Procedure of Katzenelson . . . . .	7-9
2.1.1	Apparatus and Materials . . . . .	7-9
2.1.2	Media and Reagents . . . . .	7-10
2.1.3	Procedure . . . . .	7-10
3.	Bibliography . . . . .	7-14
<b>Chapter 8 Method for Reduction of Cytotoxicity of Sample Concentrates . . .</b>		<b>8-1</b>
<b>Revised April 1986</b>		
1.	Virus Recovery from Samples . . . . .	8-1
2.	Storage of Sample Concentrates . . . . .	8-1
3.	Predetermine Cytotoxicity of Sample Concentrate . . . . .	8-1

4. Processing of Deeply Colored Sample Concentrates . . . . .	8-1
5. Reduction of Toxicity of Sample Concentrate . . . . .	8-1
5.1 Apparatus and Materials . . . . .	8-1
5.2 Media and Reagents . . . . .	8-1
5.3 Procedure . . . . .	8-2
6. Plaque Procedure for Titrating Viruses . . . . .	8-2
6.1 Preparation . . . . .	8-2
6.1.1 Apparatus and Materials . . . . .	8-2
6.1.2 Media and Reagents . . . . .	8-2
6.2 Procedure . . . . .	8-3
6.3 Counting Viral Plaques . . . . .	8-3
7. Bibliography . . . . .	8-3
<b>Chapter 9 Cell Culture Preparation and Maintenance . . . . .</b>	<b>9-1</b>
<b>Revised</b>	
<b>Jan. 1987</b>	
1. Introduction . . . . .	9-1
2. Medium Preparation . . . . .	9-1
2.1 Apparatus and Materials . . . . .	9-1
2.2 Media and Reagents . . . . .	9-2
3. Preparation of Cell Culture Media . . . . .	9-2
3.1 Technique . . . . .	9-2
3.2 General Procedures . . . . .	9-2
3.3 Medium Preparation . . . . .	9-2
3.3.1 Sources of Cell Culture Media . . . . .	9-2
3.3.2 Procedure for Preparation of EDTA-Trypsin . . . . .	9-2
3.3.3 Procedure for Preparation of Growth Medium . . . . .	9-3
3.3.4 Procedure for Preparation of Trypan Blue Solution . . . . .	9-3
3.3.5 Procedure for Preparation of Stock Antibiotic Solutions . . . . .	9-3
4. Procedure for Verifying Sterility of Liquids . . . . .	9-4
4.1 Procedure for Verifying Sterility of Small Volumes of Liquids . . . . .	9-4
4.2 Procedure for Verifying Sterility of Large Volumes of Liquids . . . . .	9-4
4.3 Visual Evaluation of Media for Microbial Contaminants . . . . .	9-4
5. Procedures for Preparation of Stock BGM Cell Cultures . . . . .	9-4
5.1 General Procedures . . . . .	9-4
6. Procedure for Passage of BGM Cells . . . . .	9-5
6.1 General Procedure . . . . .	9-5
6.2 Procedure for Performing Viable Cell Counts . . . . .	9-5
6.3 Procedure for Changing Medium on Cultured Cells . . . . .	9-6
7. Procedure for Preparation of BGM Cell Cultures for Virus Assay . . . . .	9-6
7.1 Preparation of Cell Culture Bottles or Flasks . . . . .	9-6
7.2 Preparation of Cell Culture Tubes . . . . .	9-6

---

<b>8. Procedure for Preservation of BGM Cell Line . . . . .</b>	<b>9-6</b>
8.1 Preparation of Cells for Storage . . . . .	9-6
8.2 Procedure for Freezing Cells . . . . .	9-7
8.3 Procedure for Thawing Cells . . . . .	9-7
<b>9. Bibliography . . . . .</b>	<b>9-7</b>
 Chapter 10 Cell Culture Procedures for Assaying Plaque-Forming Viruses . . . 10-1	
Revised	
Dec. 1987	
1. Introduction . . . . .	10-1
2. Cell Monolayer Procedure . . . . .	10-1
2.1 Sample Inoculation of Cell Monolayer for Virus Assay . . . . .	10-1
2.1.1 Apparatus and Materials . . . . .	10-1
2.1.2 Media and Reagents . . . . .	10-2
2.1.3 Procedure for Preparation of Stock Antibiotic Solutions . . . . .	10-2
2.1.4 Preparation of Maintenance Media . . . . .	10-2
2.1.5 Procedure for Inoculating Test Sample . . . . .	10-3
2.2 Agar Overlay Procedure for Plaque Assay . . . . .	10-3
2.2.1 Apparatus and Materials . . . . .	10-3
2.2.2 Media and Reagents . . . . .	10-4
2.2.3 Preparation of Medium 199 . . . . .	10-4
2.2.4 Preparation of Pancreatin Solution for Use in Detecting Reovirus . . . . .	10-5
2.2.5 Preparation of Overlay Medium for Plaque Assay . . . . .	10-5
2.2.6 Preparation of Overlay Agar for Plaque Assay . . . . .	10-5
2.2.7 Preparation of Agar Overlay Medium . . . . .	10-5
2.2.8 Addition of Overlay Agar to Cell Culture Test Vessels . . . . .	10-6
2.3 Counting Viral Plaques . . . . .	10-6
2.3.1 Counting Technique . . . . .	10-6
2.3.2 Calculation of Virus Titer . . . . .	10-6
2.4 Procedure for Verifying Sterility of Liquids . . . . .	10-6
2.4.1 Procedure for Verifying Sterility of Small Volumes of Liquids . . . . .	10-6
2.4.2 Procedure for Verifying Sterility of Large Volumes of Liquids . . . . .	10-6
2.4.3 Visual Evaluation of Media for Microbial Contaminants . . . . .	10-6
3. Suspended Cell Procedure . . . . .	10-7
3.1 Sample Inoculation of Suspended Cells for Virus Assay . . . . .	10-7
3.1.1 Apparatus and Materials . . . . .	10-7
3.1.2 Procedure for Inoculating Test Sample . . . . .	10-7
3.2 Agar Overlay Procedure for Plaque Assay . . . . .	10-7
3.2.1 Apparatus and Materials . . . . .	10-7
3.2.2 Media and Reagents . . . . .	10-8
3.2.3 Procedure for Preparation of Stock Antibiotic Solutions . . . . .	10-8
3.2.4 Preparation of Medium 199 . . . . .	10-9
3.2.5 Preparation of Overlay Medium for Plaque Assay . . . . .	10-9
3.2.6 Preparation of Overlay Agar for Plaque Assay . . . . .	10-9
3.2.7 Preparation of Agar Overlay Medium . . . . .	10-9
3.2.8 Procedure . . . . .	10-10
3.3 Counting Viral Plaques . . . . .	10-10

3.3.1 Technique . . . . .	10-10
3.3.2 Virus Enumeration . . . . .	10-10
3.4 Procedure for Verifying Sterility of Liquids . . . . .	10-10
3.4.1 Procedure for Verifying Sterility of Small Volumes of Liquids . . . . .	10-10
3.4.2 Procedure for Verifying Sterility of Large Volumes of Liquids . . . . .	10-10
3.4.3 Visual Evaluation of Media for Microbial Contaminants . . . . .	10-10
4. Bibliography . . . . .	10-11
 <b>Chapter 11 Virus Plaque Confirmation Procedure . . . . .</b>	<b>11-1</b>
<b>Revised</b>	
<b>March 1988</b>	
1. Introduction . . . . .	11-1
2. Recovery of Virus from Plaque . . . . .	11-1
2.1 Apparatus, Materials and Reagents . . . . .	11-1
2.2 Procedure . . . . .	11-1
2.2.1 Procedure for Obtaining Viruses from Plaque . . . . .	11-1
2.2.2 Procedure for Inoculating Viruses Obtained from Plaques onto Cell Cultures . . . . .	11-1
(a) Cell culture processing . . . . .	11-2
(b) Procedure for Samples Tested Immediately . . . . .	11-2
(c) Procedure for Stored Samples . . . . .	11-2
3. Bibliography . . . . .	11-3
 <b>Chapter 12 Identification of Enteroviruses . . . . .</b>	<b>12-1</b>
<b>Revised</b>	
<b>May 1988</b>	
1. Introduction . . . . .	12-1
2. Procedure for Typing Viruses . . . . .	12-1
2.1 Apparatus and Materials . . . . .	12-1
2.2 Media and Reagents . . . . .	12-1
2.3 Procedure . . . . .	12-2
2.3.1 Preparation of Microtiter Plates . . . . .	12-2
2.3.2 Preparation of Virus for Identification . . . . .	12-2
2.3.3 Addition of Antiserum Pools to Microtiter Plate . . . . .	12-2
2.3.4 Addition of Virus to Microtiter Plates . . . . .	12-2
2.3.5 Preparation of Cell Suspension and Completion of Microtiter Test . . . . .	12-3
3. Bibliography . . . . .	12-4
 <b>Appendix</b>	
List of Vendors . . . . .	A-1

## List of Figures

Figure No.		Page
5-1	Flow Diagram of Method for Recovering Viruses from Small Volumes (100 mL to 20 Liters) of Water, Sewage, or Effluent	5-4
5-2	Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Small Volume Filtrations	5-5
5-3	Photographic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Small Volume Filtrations	5-6
5-4	Flow Diagram of Method for Recovering Viruses from Large Volumes (More than 20 Liters) of Water, Sewage, or Effluents	5-13
5-5	Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations	5-15
5-6	Photographic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations	5-16
5-7	Schematic Representation of Apparatus for Treatment of Prefilters with Tween 80 to Prevent Adsorption of Viruses to the Prefilters in the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations	5-18
5-8	Photographic Representation of Apparatus for Treatment of Prefilters with Tween 80 to Prevent Adsorption of Viruses to the Prefilters in the Virus Adsorption-Elution (VIRADEL) Disc Filter Procedure for Large Volume Filtrations	5-19
5-9	Flow Diagram of Reconcentration Procedure (Organic Flocculation Procedure of Katzenelson)	5-35
6-1	Flow Diagram of Method One for Concentrating Viruses from Large Volumes (More than 200 Liters) of Clean Waters	6-6
6-2	Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Clean (Non-Turbid) Waters	6-7
6-3	Photographic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Clean (Non-Turbid) Waters	6-8
6-4	Schematic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Turbid Waters	6-18

6-5	Photographic Representation of Apparatus for Recovering Viruses by the Virus Adsorption-Elution (VIRADEL) Cartridge Filter Procedure for Large Volume Filtrations of Turbid Waters	6-19
6-6	Flow Diagram of Method Two for Concentrating Viruses from Large Volumes (More than 200 Liters) of Turbid Waters	6-22
6-7	Flow Diagram of High pH Procedure (Basic Glycine, pH 10.5) for Eluting Viruses from Cartridge Filters and for Reconcentrating Viruses from Clear Eluates by the Membrane Filter Procedure	6-32
6-8	Flow Diagram of High pH Procedure (Basic Glycine, pH 10.5) for Eluting Viruses from Cartridge Filters and for Reconcentrating Viruses from Turbid Eluates by the Al(OH) <sub>3</sub> -Hydroextraction Procedure	6-33
6-9	Schematic Representation of Apparatus for Reconcentration—Method A, a Membrane Disc Procedure for Reconcentrating Viruses from Glycine Eluates	6-41
6-10	Photographic Representation of Apparatus for Reconcentration—Method A, a Membrane Disc Procedure for Reconcentrating Viruses from Glycine Eluates	6-42
6-11	Flow Diagram of Beef Extract Method for Eluting Viruses from Cartridge Filters with Buffered 3% Beef Extract and for Concentrating Eluted Viruses by the Katzenelson Organic Flocculation Procedure	6-54
7-1	Flow Diagram of Method for Recovering and Concentrating Viruses in Sludges	7-4
12-1	Representation of Microtiter Plate Preparation	12-2
12-2	Photographic Representation of Microtiter Plate Preparation	12-3

## List of Tables

Table No.		Page
3-1	Quantities of Deionized Distilled Water to be Added to Vessels to Facilitate Sterilization During Autoclaving	3-3
3-2	Time-Temperature Couplings for Dry Sterilization	3-4
4-1	Monitoring Laboratory Equipment	4-17
4-2	Standards for Deionized Distilled Water	4-23
4-3	Laboratory Ware Maintenance	4-24
9-1	Guide for Preparation of BGM Stock Cultures	9-5
9-2	Guide for Preparation of Virus Assay Cell Cultures	9-7
10-1	Guide for Virus Inoculation, Suspended Cell Concentration and Overlay Volume of Agar Medium	10-3