Basic Laboratory Procedures In Diagnostic Virology

Mary Christensen M.S., Ph.D.

Research Fellow, Department of Biochemistry
Northwestern University Medical School
Former Chief, Clinical Virology Laboratory
Northwestern University Medical Center
Passavant Hospital, Chicago, Illinois

The two major approaches to the laboratory diagnosis of virus diseases receive detailed treatment in this manual: virus isolation with subsequent identification, and virus diagnosis by serologic means. The step-by-step procedural instructions and the explanatory text will be extremely useful to all laboratory personnel, including microbiologists, medical technologists and laboratory technicians.

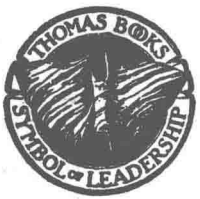
CHARLES C THOMAS • PUBLISHER • SPRINGFIELD • ILLINOIS

Basic Laboratory Procedures In Diagnostic Virology

By

MARY CHRISTENSEN, M.S., Ph.D.

Research Fellow, Department of Biochemistry
Northwestern University Medical School
Former Chief, Clinical Virology Laboratory
Northwestern University Medical Center
Passavant Hospital, Chicago, Illinois



CHARLES C THOMAS · PUBLISHER
Springfield · Illinois · USA

Published and Distributed Throughout the World by
CHARLES C THOMAS • PUBLISHER
Bannerstone House
301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.

ISBN 0-398-03617-9

Library of Congress Catalog Card Number 76-48213

With THOMAS BOOKS careful attention is given to all details of manufacturing and design. It is the Publisher's desire to present books that are satisfactory as to their physical qualities and artistic possibilities and appropriate for their particular use. THOMAS BOOKS will be true to those laws of quality that assure a good name and good will.

Library of Congress Cataloging in Publication Data

Christensen, Mary
Basic laboratory procedures in diagnostic virology.

Bibliography: p.
1. Virus diseases—Diagnosis. 2. Virology—
Laboratory manuals. I. Title. [DNLM: 1. Virology—
Laboratory manuals. 2. Virus diseases—Diagnosis—
Laboratory manuals. QW25 C554b]
QR46.C5 616.01'94'028 76-48213
ISBN 0-398-03617-9

Printed in the United States of America
C-1

PREFACE

THIS MANUAL was introduced at Northwestern University Medical Center as a basic laboratory manual in a new clinical virology program offered to medical technology students. No manual emphasizing the more routine and fundamental diagnostic virology techniques was available for the teaching of this subject to medical technology students or to laboratory technicians; the few diagnostic virology publications available were reference-type publications, for use by a diagnostic virology laboratory supervisor or director.

This manual will also fulfill the need for a basic manual for use in the training of technical personnel in a virus diagnostic laboratory, and as reference material. As technical personnel in a virus laboratory frequently have one of several possible diverse backgrounds academically and in experience, i.e., biology, zoology, medical technology, or general microbiology, one infrequently has the good fortune to obtain an individual with a background in virology at the technical level. It is usually necessary to train such an individual in serology, tissue culture techniques, or basic virologic techniques, or any combination of these three. This manual will help fulfill this need.

Thirdly, many community hospitals are now undertaking the more routine laboratory virus diagnoses in their diagnostic microbiology and serology laboratories, frequently assigning one microbiologist to carry out the procedures. This manual will thus be of aid to these microbiologists who have a solid background in microbiology but are not trained in virology per se. Not only will this manual (1) provide step-by-step methods for the more routinely used procedures, usually to which a community hospital is limited, but will (2) serve as an introduction to diagnostic virology, providing an overall view of this field. Since diagnostic virology is a relatively new and rapidly expanding field, it is anticipated that this manual will serve in the three areas described above.

It must be emphasized that this manual is meant primarily as a guide for the most frequently used routine methods carried out in a virus diagnostic laboratory. It is by no means an attempt to cover every procedure or method, or to take into account every approach used for the determination of the viral etiology of particular disease syndromes by isolation and identification of a viral etiologic agent or by serologic means. For specific methods used in the laboratory diagnosis of particular individual virus infections, one should consult *Diagnostic Procedures for Viral and Rickettsial Infections,* edited by E. H. Lennette and N. J. Schmidt, Fourth Edition, New York, American Public Health Association, 1969.

Although numerous laboratories throughout the country are now routinely engaged in the performance of the rubella hemagglutination-inhibition test, I have purposely omitted this test procedure from this manual. It is an exacting procedure which should be carried out by well-trained, experienced, and knowledgable individuals. It is recommended that laboratories consult the Center for Disease Control Standard Rubella Hemagglutination-Inhibition Test Training Manual for this procedure. In addition, I have purposely described only the use of tissue culture techniques for the isolation and identification of viral etiologic agents of disease since tissue culture is now predominantly used, and techniques utilizing embryonated eggs and living animals are reserved today in special instances only.

I am deeply grateful to Opal E. Hepler, M.D., Ph.D., Professor of Clinical Pathology and Director of the Medical Technology Program at Northwestern University Medical School for the time she spent in critically reviewing the manuscript, and for her many helpful suggestions regarding it. In addition I would like to thank the students who reviewed for clarity those sections containing specific laboratory procedures, and the publishers of this book for their consideration and cooperation.

M.C.

CONTENTS

Basic Laboratory Procedures In Diagnostic Virology

Part I

VIRUS ISOLATION AND IDENTIFICATION FOR THE DIAGNOSIS OF VIRAL INFECTIONS

INTRODUCTION

SINCE THIS MANUAL concerns the laboratory diagnosis of virus disease, it is necessary to first understand certain fundamental concepts regarding viruses. Viruses, as defined:[1-3]

1. contain ribonucleic acid (RNA) *or* deoxyribonucleic acid (DNA), but not both
2. cannot produce high energy bonds
3. reproduce from their nucleic acid
4. are unable to grow and undergo binary fission
5. make use of the ribosomes of host cells

Due to their lack of necessary enzymes and their unique mode of reproduction, viruses multiply intracellularly only. In addition viruses are characterized by their extremely small size, with the diameters of the animal virus particles ranging from 18 nanometers (nm) to 300 nm (1 nm = 1 mμ = one-billionth of a meter). In nature, viruses infect a wide range of host organisms, including vertebrate and invertebrate animals, plants, bacteria, and other microorganisms. This discussion will be limited to animal viruses.

Virus particles consist of a relatively simple structure. The virion, the mature virus particle, is composed of a nucleic acid and protein core, surrounded by a protein coat called the *capsid*. The nucleic acid core and capsid together form the nucleocapsid. The capsid is composed of a large number of protein subunits, termed *capsomeres*. In certain virus groups, the virion consists only of the nucleocapsid. In other groups, a lipoprotein "envelope" surrounds the nucleocapsid, thus forming the complete virion.

Most virus particles are classified as (1) "cubic" (icosahedral) or (2) helical in shape, based on their capsid symmetry. In addition, poxviruses are more complex and are brick-shaped. Rhabdoviruses, which in one form superficially appear to be bullet-shaped, are basically helical viruses. Cubic virus particles have a multi-faceted diamond-shaped capsid surrounding a

nucleic acid core. Certain cubic viruses, such as the herpesviruses, possess an envelope surrounding the nucleocapsid, thus forming the complete virion. Helical virus particles consist of a nucleocapsid wound in a helix or spiral, which is surrounded by a lipoprotein envelope. Lipid components of the envelope are probably responsible for the sensitivity of certain groups of viruses to lipid solvents[11, 12] in particular ether and chloroform.

DNA and RNA viruses may be either (1) cytocidal, that is, infection of host cells results in (a) the release of infectious virus particles and (b) usually the eventual death of the cell; or (2) they may transform host cells, in which case they are referred to as tumor or oncogenic viruses. Some viruses are only cytocidal and some viruses are considered to be only oncogenic, whereas many viruses, e.g. Simian virus-40 and certain adenoviruses may exhibit either cytocidal or oncogenic properties, depending on certain factors, such as type of host cell infected. We will discuss here only cytocidal or productive infections.

The first step in virus reproduction probably involves attachment of the virion to receptor sites on a susceptible cell. This is followed by penetration or engulfment of the virus by the cell. The protein coat is removed either at the cell surface or inside the cell, depending on the virus type. Removal of the capsid initiates the eclipse period, during which time the virus cannot be recovered. After uncoating, the mode of replication of DNA and RNA viruses exhibit basic differences.

After a DNA virus is uncoated,[4] messenger RNA (m-RNA) is transcribed off of certain areas of the viral DNA which code for early proteins. Translation of the m-RNA into early proteins, which are primarily virus coded enzymes (especially enzymes necessary for viral DNA synthesis) follows. After translation of m-RNA into early proteins, replication of viral DNA occurs. Transcription of m-RNA continues from parental and progeny viral DNA. Late in the replicative cycle, late proteins, primarily structural proteins, as well as regulatory proteins, are translated. Probably translation of early m-RNA into early proteins is "turned-off" by late-coded protein(s). During the replication cycle, inhibition of host cellular protein, RNA and DNA synthesis usually occur.[4]

Because most RNA viruses contain single-stranded RNA, the mode of replication varies. The replication of small RNA viruses is exemplified by poliovirus. The parental viral RNA acts as one large m-RNA molecule. This m-RNA is translated into one large protein molecule, which then goes through two series of cleavages. This results in the formation of four structural virion proteins and several nonstructural proteins. Two nonstructural proteins inhibit host cellular RNA and protein synthesis. A third protein(s) is an enzyme(s) which catalyzes the replication of new viral RNA. The single-stranded virion RNA is referred to as a *plus strand.* From the plus RNA strands, complementary *minus RNA strands* can be transcribed. These minus strands then form a template for new progeny plus strands. Thus, plus strands can act as m-RNA and be translated into protein, or can be used to read off new minus RNA strands.

Large RNA viruses, such as the paramyxoviruses, differ somewhat in their replication. Here the viral RNA molecule does not act as m-RNA. Instead, minus strands are formed off of the viral plus strands, and short minus strands act as m-RNA.

Reoviruses are unique in that they are one of the few RNA viruses that contain double-stranded RNA.

During the maturation period after the nucleic acid and capsid (coat) proteins of nonenveloped viruses are manufactured, they are assembled into mature virions. Release occurs by lysis of the infected cell, such as occurs in the release of poliovirus. In the case of enveloped viruses, nucleic acid and protein are assembled into the nucleocapsid, and this is followed by the maturation period during which the nucleocapsid is enveloped by a lipoprotein membrane as it leaves the cell by a process called *budding.* Herpesviruses and influenza viruses become enveloped by a membrane as they leave the nucleus. New virions, released either by cell lysis or by budding, can infect new susceptible cells with which they come in contact.

Certain of the criteria used in classification of viruses are also used for the identification of viruses isolated from clinical specimens. The classification of animal viruses[5, 6, 7] is based on:

1. Nucleic acid type (RNA or DNA)
2. Presence or absence of an envelope
3. Ether (or chloroform) sensitivity

4. Diameter of the virion (size)
5. Capsid symmetry (cubic, helical, or complex)
6. Number of capsomeres
7. Site of capsid assembly and of nucleocapsid envelopment

In addition, the determination of sensitivity to acid can be used in the identification of a newly isolated virus.[8]

Using the above criteria, the classification of the major groups of animal viruses[5-7] is as follows on Table I. In parentheses following each of the major virus groups is the family name according to the Linnaean system. For example, herpesviruses are now classified as the Family Herpetoviridae. The family name is not used exclusively since it is not found in publications prior to 1976.

Listed in the table under the major virus groups are those specific viruses with which a diagnostic laboratory is most likely to be concerned, as well as several other individual viruses of interest. In the list of virus groups, many specific viruses are not listed, e.g. many viruses that affect animals other than man. Also, viruses in the togavirus and bunyamwera groups that were formerly referred to as arboviruses cannot be listed as they number approximately 200.

Acid sensitivity is listed where (1) the acid sensitivities have been determined and (2) where it is applicable for laboratory identification of an isolate or (3) for differentiation, as among the picornaviruses.

So that the reader may better understand and remember the names of viruses listed in Table I, a list of derivations of virus names follows in Table II. Viral nomenclature is based on one of several criteria which include type of infection (e.g. rhinitis), tissue or organ infected (e.g. adenoid tissue), and description of the virion (e.g. bullet-shaped).

The laboratory diagnosis of virus infections is carried out by two main lines of investigation:[9, 10] (1) the attempted isolation and subsequent identification of the causal agent from clinical specimens, and (2) the diagnosis of virus infection by serologic means. Serology literally is "the study of serum" (see Part II).

Depending on the clinical diagnosis of a patient's illness, one of

TABLE I

THE MAJOR GROUPS OF ANIMAL VIRUSES

	Ether Sensitivity	*Acid Sensitivity*
RNA Viruses		
Picornaviruses *(Picornaviridae)*		
Enteroviruses	Resistant	Resistant
Polioviruses (Types 1, 2, 3)		
Coxsackie viruses (Group A, 24 types) (Group B, 6 types)		
ECHO viruses		
Enteroviruses		
Rhinoviruses (50+ types)	Resistant	Sensitive
Coronaviruses *(Coronaviridae)*	Sensitive	
Reoviruses *(Reoviridae)* (Types 1, 2, 3)	Resistant	Resistant
Orthomyxoviruses *(Orthomyxoviridae)*	Sensitive	Sensitive
Influenza viruses (Types A, B, C)		
Paramyxoviruses *(Paramyxoviridae)*	Sensitive	Sensitive
Para-influenza viruses (Types 1, 2, 3, 4)		
Mumps virus		
Measles virus		
Respiratory syncytical virus		
Rhabdoviruses *(Rhabdoviridae)*	Sensitive	
Rabies virus		
Arenaviruses *(Arenaviridae)*	Sensitive	
Lymphocytic choriomeningitis virus		
Togaviruses *(Togaviridae)*	Sensitive	Sensitive
Rubella (German measles) virus		
Arboviruses		
Group A		
Eastern equine encephalitis virus		
Western equine encephalitis virus		
Group B		
St. Louis encephalitis virus		
Yellow fever virus		
Bunyamwera super group *(Bunyaviridae)*	Sensitive	
Leukoviruses or Oncornaviruses *(Retroviridae)*	Sensitive	
RNA tumor viruses		
DNA Viruses		
Papovaviruses *(Papovaviridae)*	Resistant	Resistant
DNA tumor viruses		
Papilloma of humans (warts)		
Polyoma		
Simian vacuolating virus (SV-40)		
Adenoviruses *(Adenoviridae)*	Resistant	Resistant
(31 human types)		
Herpesviruses *(Herpetoviridae)*	Sensitive	Sensitive
Herpes simplex virus		
Herpes B virus (simian)		
Herpes zoster-varicella (chickenpox) virus		
Cytomegalic virus		
EB virus of infectious mononucleosis		
Poxviruses *(Poxviridae)*	Resistant or Sensitive	Sensitive
Variola (smallpox) virus		
Vaccinia virus		
Others		
Hepatitis virus (Types A, B)		

TABLE II

DERIVATION OF VIRAL NOMENCLATURE

Name of Virus	*Derivation*
Picornavirus	Pico—small Rna—RNA-containing
Enterovirus	Reproduces in enteric tract; includes newly isolated enteroviruses which are referred to as enteroviruses, rather than Coxsackie or ECHO viruses
Coxsackie virus	First isolated from a patient in Coxsackie, New York
ECHO	*E*nteric *C*ytopathic *H*uman *O*rphan; "orphan" virus indicates a virus without a parent disease
Rhinovirus	Causal agent of rhinitis, i.e. the common cold
Coronavirus	Corona(tion)—crown; periphery of virus has crown-like appearance; infects upper respiratory tract
Reovirus	*R*espiratiory *E*nteric *O*rphan
Orthomyxovirus	Ortho—proper, normal Myxo—mucin
Paramyxovirus	Para—beyond, apart from; larger virion than orthoviruses
Respiratory syncytial	Causal agent of certain respiratory infections; causes formation of syncytia or giant cells in infected cell cultures
Rhabdovirus	Rhabdo—bullet-shaped or rod-shaped; characteristic shape of virion
Arenavirus	Arena—sandy; refers to characteristic fine granules in the viral core
Lymphocytic choriomeningitis	Infiltration of lymphocytes into the choroid plexi, meninges
Toga	Toga—a covering
Arbovirus	*Ar*thropod-*borne*; an ecologic term
Equine	Equine—refers to horses; virus referred to primarily transmitted to horses
Encephalitis	Inflammation of the brain (encephalon)
Bunyamwera	Area of Africa from which first virus of group originally isolated
Leukovirus	Certain viruses of group are causative agents of leukemias
Oncornavirus	Onco—refers to tumors Rna—RNA-containing
Papovavirus	*Pa*pilloma, *po*lyoma, *va*cuolating viruses
Adenovirus	First isolated from adenoid tissue
Cytomegalic virus	Cyto—cell Megalo—enlarged; giant cells observed in virus-infected cells
EB virus	EB—for Epstein and Barr, investigators originally studying virus
Vaccinia	Vacca—Latin for cow; virus probably dervied from the cowpox virus, first used for vaccination against smallpox
Hepatitis	Inflammation of hepatic tissue (liver)

these two general methods may be preferred over the other. Usually, however, it is advisable to follow both types of investigation, since a diagnosis might be made by one method, whereas the other may not yield results for one or more reasons. As examples, when dealing with enterovirus infections, virus isolation is relatively successful, whereas the large number of enterovirus types would make serologic diagnosis unwieldy for the average virology laboratory, and the necessary diagnostic reagents are commercially unavailable. However, a viral agent will frequently not be recovered from a clinical specimen. There are several possible reasons for this. For example, a specimen may not be collected soon enough after onset of clinical symptoms, so that extremely few virus particles are present in the specimen, or none may be present. In these instances use of serologic methods may be more likely to yield results.[9, 10]

REFERENCES

1. Lwoff, A. and Tournier, P.: The classification of viruses. *Ann Rev Micro, 20:*45-74, 1966.
2. Lwoff, A.: The concept of virus. *J Gen Micro, 17:*239-253, 1957.
3. Lwoff, A.: *Bact Rev, 17:*269-337, 1953.
4. Green, M.: Biosynthetic modification induced by DNA animal viruses. *Ann Rev Micro, 20:*189-222, 1966.
5. Wildy, P.: Classification and nomenclature of viruses. In Melnick, J. L. (Ed.): *Monographs in Virology,* Volume 5, S. Karger, Basel, 1971.
6. Melnick, J. L.: Classification and nomenclature of viruses. *Prog Med Virol, 13:*462-484, 1971.
7. Melnick, J. L.: Classification and nomenclature of viruses, 1972. *Prog Med Virol, 14:*321-332, 1972.
8. Hamparian, V. V., Hilleman, M. R. and Ketler, A.: Contributions to characterization and classification of animal viruses. *Proc Soc Exp Biol Med, 112:*1040-1050, 1963.
9. Horstmann, D. M. and Hsiung, G. D.: Principles of Diagnostic Virology. In Horsfall, F. L., Jr. and Tamm, I. (Eds): *Viral and Rickettsial Infections of Man,* Fourth Edition, Philadelphia, Lippincott, 1965, pp. 405-424.
10. Lennette, E. H. and Schmidt, N. J.: *Diagnostic Procedures for Viral and Rickettsial Diseases.* New York, American Public Health Association, 1969.
11. Andrewes, C. H. and Horstmann, D. H.: The susceptibility of viruses to ethyl ether. *J Gen Micro, 3:*290-297, 1949.
12. Andrewes, C. H.: In *Methods in Virology,* Volume IV, New York, Academic Press, 1968, pp. 593-613.