Laboratory and Research Methods in Biology and Medicine, Volume 5

NEW DEVELOPMENTS IN PRACTICAL VIROLOGY

Editor
Colin R. Howard

Alan R. Liss, Inc., New York

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Alan R. Liss, Inc., 150 Fifth Avenue, New York, NY 10011

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Library of Congress Cataloging in Publication Data
Main entry under title:
New developments in practical virology.
(Laboratory and research methods in biology and medicine; v. 5)
Includes bibliographies and index.
Contents: Advanced methods in electron microscopy of viruses/M.V. Nermut--ELISA techniques in virology/A. Voller, D.E. Bidwell, and A. Bartlett--Radioimmunoassay in diagnostic virology/Pekka Halonen and Olli Neurman--[etc.]
1. Virology--Technique. I. Howard, Colin R. II. Series. [DNLM: 1. Virology--Trends. W1 LA205T v.5]
QR385.N48 616'.0194'028 81-20904
ISBN 0-8451-1654-1 AACR2

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Preface

The past decade has witnessed the exponential development of virology as a modern science. Both virologists and microbiologists interested in the diagnosis of infections, virus structure, or the products of virus replication have been quick to assimilate techniques newly developed in the allied fields of immunology, genetics, and molecular biology. The field has moved so rapidly, however, that descriptions of new methodology invariably are found only by reference to original articles and publications, where authors describe their own preferred protocols with respect to particular research or diagnostic problems without necessarily indicating the rationale behind the chosen experimental approach. Thus students new to the subject often have particular difficulty in either understanding the basic concepts behind the application of new techniques or their selection from many variations in order to obtain the approach best suited to their needs. This volume is intended therefore to bridge this gap and is aimed mainly at newcomers to the field, although it is anticipated that established virologists also would benefit from timely reviews of selected techniques.

Authors were invited to review those methods most often requested by students attending the virology component of the M.Sc. course in Medical Microbiology at the London School of Hygiene and Tropical Medicine, where the idea for this volume originated. However, no attempt has been made to cover the techniques of molecular cloning of virus genes as much of the methodology in this area is adequately described elsewhere in the scientific literature.

I would like to take this opportunity to thank the authors, who willingly agreed to undertake the onerous task of reviewing the various techniques covered herein, Professors A. J. Zuckerman and D. I. H. Simpson for continuing encouragement and advice, and Alan R. Liss and his publishing staff for their assistance in the production of this volume.

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Advanced Methods in Electron Microscopy of Viruses

M.V. Nermut

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I. INTRODUCTION

The contribution of electron microscopy to our knowledge of viruses has been enormous, but it is also true to say that the function and role of ultrastructural studies in modern virology is sometimes underestimated, or at least is not fully appreciated. There are two main reasons for this attitude. First, the great advances in molecular biology and biochemistry of viruses have widened the gap between ultrastructural and chemical virology, and thus it is often difficult to relate the two types of information. This is due in part to the present preparation techniques for electron microscopy, which do not allow any better resolution than 15 to 20 Å. The second major criticism of electron microscopy is the lack of understanding of preparation artefacts. Our incapability or unwillingness to eliminate, or at least to reduce, these artefacts has been responsible for an atmosphere of distrust and underestimation of electron microscopy in high-resolution structural research. The aim of this chapter is to show how electron microscopy can be used in such a way as to avoid these pitfalls and criticisms. Thus it should be possible to eliminate some of the misunderstanding of the technique and possible misinterpretation of the results it produces. Moreover, the techniques of "non-destructive" electron microscopy, image analysis with 3D-reconstruction, and the artefact-poor cryotechniques ensure that ultrastructural studies remain an equal partner to biochemistry and other disciplines in virus research.

There has been a noticeable trend in this field to move from "descriptive morphology" to "structural analysis" of viruses. We cannot remain satisfied with "pure morphology"; instead we must combine the morphological data with our knowledge of chemical composition and physical chemistry of viruses and their components and so provide a complete structural analysis. If we define viruses as "organized associations of macromolecules," then the importance of physical and chemical characteristics is clear, and the possibilities for characterization by electron microscopy become evident. "Structural analysis" means to determine the shape and dimensions of the virion, its surface structure, and internal organization. Morphologically speaking, viruses are built from "basic structural elements" (eg, capsomers, spikes, etc), which are themselves organized into structural complexes (eg, nucleocapsid, envelope, etc). The aim of structural analysis is to define and describe the structural elements and complexes using the most suitable methods, and the following pages will deal with the methodological and tactical aspects of this task. Although some routine procedures will inevitably be mentioned, the

main attention will be paid to some aspects of negative staining and, in particular, to the cryotechniques.

II. METHODS OF STRUCTURAL ANALYSIS OF VIRUSES

A. Negative Staining

One of the most useful methods for the visualization of virus particles is undoubtedly the method of negative staining. Since its first application in virology [Brenner and Horne, 1959], it has become a technique of choice for ultrastructural studies of purified virus suspensions, as well as for the rapid identification of virus particles in culture fluids or clinical materials. Its main advantages are simplicity, rapidity, and high resolution. The technical aspects of negative staining have been described in several reviews during the last decade [Horne, 1965; Haschemeyer and Mayers, 1972; Oliver, 1973], and there is no need to describe its fundamental features or procedures, since they are now routinely used in most electron microscope laboratories. Therefore, in this part of the chapter, routine techniques will be briefly described, and more attention will be paid to newer developments in the field.

1. Negative stains and their properties. A dozen staining solutions have been described and used by research workers (Table I), but only a few of them are routinely used. This is certainly not ideal, and it is strongly recommended that at least two types of staining solutions should be used in a research laboratory: salts of tungstic acid—eg, phosphotungstic (PTA) or silicotungstic acid (STA)—and uranium salts such as uranyl acetate (UA) and for high-resolution work uranyl formate [Brack, 1973]. Ideally the following solutions should be at hand at all times: 2% PTA pH 5, 6.8, 7.5, and 8.0, 4% STA pH 6.8, 3% ammonium molybdate pH 6.5, and 1% UA pH 4.5. Other solutions such as 1% uranyl formate are prepared fresh before use. Routinely, virus preparations are stained with STA pH 6.5 and UA [see also Ackermann et al, 1974]; ammonium molybdate is often used for enveloped viruses or cell membranes. Different pHs of the solutions are necessary for visualization of either surface structures (eg, glycoprotein knobs, spikes, etc, acid pH) or internal components (nucleocapsids; alkaline pH).

In a brief assessment of the commonly used stains, it should be mentioned, that the salts of uranium give high contrast and penetrate better into the structure but cause a grainy background (crystallites) and often stain positively, whereas tungstates and molybdates give lower contrast but are very fine. Ammonium molybdate can easily

TABLE I. Negative Stains Suitable for Use in Virus Research

Name (abbreviated formula)	Usual %	pH range (optimum)	Reference
Phosphotungstate (Na, K) PTA, $H_3PO_4 \cdot 12\ WO_3$	1–2	5.0–8.0 (6.8)	Brenner and Horne [1959]
Silicotungstate STA, $SiO_2 \cdot 12\ WO_3$	3–4	5.0–8.0 (6.8)	Valentine et al [1968]
Methylamine tungstate $CH_3NH_2\ WO_3$ (approx.)	1–2	6.5–8.0	Fabergé and Oliver [1974]
Ammonium molybdate AM, $(NH_4)_6Mo_7O_{24}$	3	5.2–8.0 (6.5)	Nagington et al [1964]
Uranyl acetate UA, $UO_2(CH_3 \cdot COO)_2$	0.5–2.0	4.2–4.5	Valentine and Horne [1962]
Uranyl formate $UO_2(H \cdot COO)$	1.0–2.0	4.4	Leberman [1965] Brack [1973]
Uranyl nitrate $UO_2(NO_3)_2$	0.5–1.0	4.4	Valentine and Horne [1962]
Uranyl oxalate $UO_2(COO)_2$	0.5–1.0	5.0–7.0	Mellema et al [1967]

Only abbreviations for routine stains are mentioned as they are used throughout this chapter.

overstain and form large crystals or sublimation artefacts under the electron beam. However, the outcome of negative staining is a result of the properties of the staining solutions and the structure under study, as well as the supporting films; this is dealt with later.

2. Preparation of virus suspensions for negative staining. Purified or semi-purified virus preparations contain salts (= buffers) or complex culture media that would disturb observation in the electron microscope. It is therefore essential to remove such ingredients by washing with distilled water or volatile buffer (eg, ammonium acetate or carbonate); this can be done either before or, more frequently, after adsorption onto the supporting film. Many viruses survive washing in distilled water without difficulties, but if disintegration is observed, volatile buffers must be used instead of water. Alternatively, brief fixation with aldehydes can stabilize the particles before washing in

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