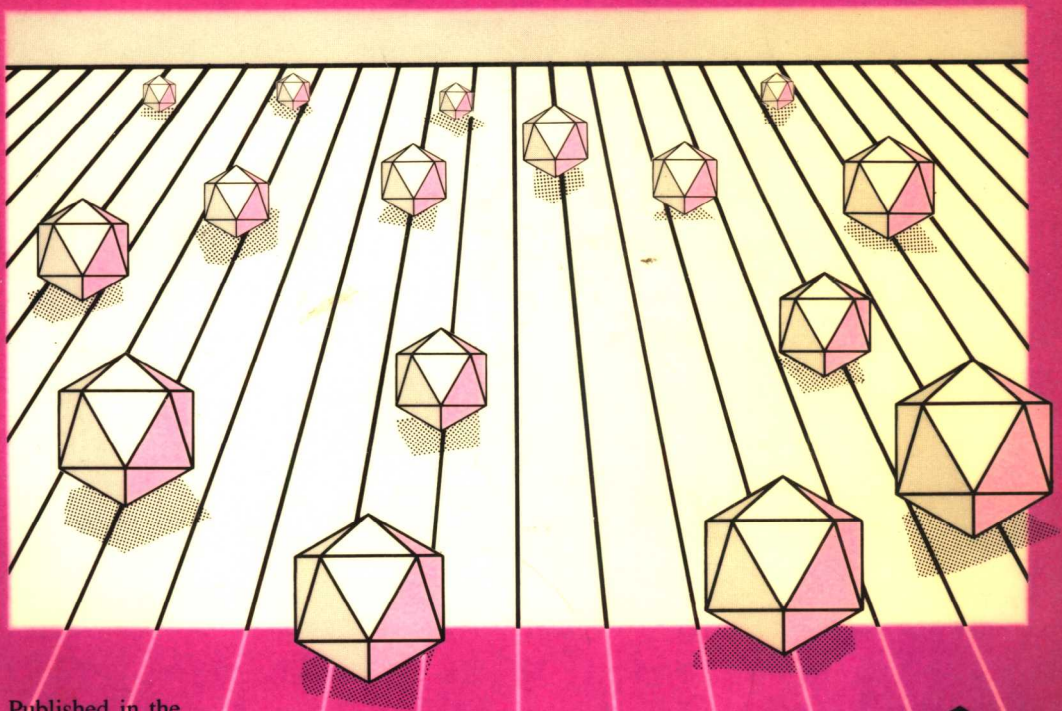
Virology

a practical approach

Edited by
B W J Mahy



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Preface

Virology has long been an esoteric branch of microbiology, fascinating to those pursuing an understanding of the nature of viruses, but a closed book to most biologists. The study of viruses began because they are agents of the most important diseases of plants and animals, as well as man. Despite the elimination of smallpox, many virus diseases such as influenza, foot-and-mouth disease, herpes simplex or rabies are only poorly controlled, and new virus diseases such as Lassa fever, African swine fever or acquired immune deficiency syndrome (AIDS) pose problems which require urgent research effort by virologists. At the same time virology has entered a new phase of growth as a result of the contributions it has made, and is continuing to make, to molecular and cellular biology. Virologists have always exploited technical advances in other branches of science, especially those developed for the study of biological macromolecules, but only recently have molecular and cellular biologists begun to use viruses as tools to probe complex questions concerning cellular structure and function.

It could be argued that the greatest contribution of virology to science and mankind has been the provision of reverse transcriptase, which forms the cornerstone of modern genetic engineering. But many of the current concepts in molecular and cellular biology (such as introns, splicing or oncogenes) arose directly from studies of virus structure and function. It is certain that the study of viruses will continue to expand and the techniques peculiar to virology will need to be used by an ever greater number of scientists.

This book has been compiled with both aspects of virology in mind. It provides practical recipes and protocols for handling most of the animal viruses of current interest either as disease agents or as probes of cell function. Within the space available, it is not possible to give complete coverage of all the virus groups, and I take responsibility for the selection of topics. However, the retroviruses are an important omission: they were deliberately not included since they have recently been covered extensively by two excellent volumes from the Cold Spring Harbor Laboratory.

Instead of devoting each chapter to a different technique, such as assay, radiolabelling, or purification, the chapters have, for the most part, been arranged in virus families. This is because the methods used are frequently peculiar to a particular group of viruses, and whilst some general principles recur, the reader bent on working with a particular virus (e.g., polio) should find all the standard techniques set out in a single chapter. A Dictionary of Virology by K.E.K.Rowson, T.A.L.Rees and B.W.J.Mahy is a useful volume to guide the non-virologist through the nomenclature and jargon of the trade. Recourse may also need to be made to a volume on animal cell and tissue culture methods which is available in the Practical Approach series.

It is a pleasure to thank all the authors for their diligence and co-operation, as well as the series editors and staff of IRL Press for their speedy response to requests, which made the editorial work most enjoyable. I am also deeply grateful to Chris Smale for artistic advice, and to my secretaries, Mary Wright in Cambridge and Hazel West in Pirbright for their forebearance.

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Abbreviations

BBS	borate-buffered saline
BMV	bovine mammilitis virus
BSA	bovine serum albumin
CCV	channel catfish virus
CEF	chick embryo fibroblasts
CFT	complement fixation test
CMC	carboxymethylcellulose
CMV	cytomegalovirus
cpe	cytopathic effect
CS	calf serum
CVS	challenge virus standard
DAPI	4′,6-diamidino-2-phenylindole
DEAE	diethylaminoethyl
DEP	diethyl pyrocarbonate
DGV	dextrose-gelatin-veronal
DI	defective-interfering
DMSO	dimethylsulphoxide
EDTA	ethylenediamine tetraacetic acid
EHV-1	equine herpes virus type 1
EID_{50}	50% egg infectious dose
ELISA	enzyme-linked immunosorbent assay
FA	fluorescent antibody
FCS	foetal calf serum
Fetr	feline rhinotracheitis virus
FITC	fluorescein isothiocyanate
HA	haemagglutination
HAI	haemagglutination-inhibition
HAU	haemagglutinating units
HEL cells	human embryonic lung cells
Hepes	N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid
HFF	human foreskin fibroblasts
HL	haemolysin
HLI	haemolysin-inhibition
HSV	herpes simplex virus
HVS	herpesvirus saimiri
LD_{50}	lethal dose at the 50% end point
MEM	minimal essential medium
m.o.i.	multiplicity of infection
NCS	newborn calf serum
NIEP	non-infectious enveloped particles
NP-40	Nonidet P-40
OMK cells	Owl Monkey kidney cells
OSC	optimum sensitising concentration
PBS	phosphate-buffered saline

PD_{50}	50% protective dose
PEG	polyethylene glycol
PMSF	phenylmethylsulphonyl fluoride
PRV	pseudorabies virus
PTA	phosphotungstic acid
PVM	murine pneumonia virus
rbc	red blood cells
RIA	radioimmunoassay
RIP	radioimmunoprecipitation
RS virus	respiratory syncytial virus
RSB	reticulocyte standard buffer
RuBPC	ribulose bisphosphate carboxylase
SDS	sodium dodecyl sulphate
SNI	serum neutralisation index
SV40	simian virus 40
TCA	trichloroacetic acid
$TCID_{50}$	dilution of virus required to infect 50% of cultures
TD	Tris-Dulbecco
TMV	tobacco mosaic virus
TPB	tryptose phosphate broth
UA	uranyl acetate
VBS	veronal-buffered saline
VSV	vesicular stomatitis virus

Contents

CHAPTER 1

Purification, Biophysical and Biochemical Characterisation of Viruses with Especial Reference to Plant Viruses

ROGER HULL

1. INTRODUCTION

Among the various features used in a descriptive characterisation of a virus are the biophysical and biochemical properties of the particles. Before these properties of a virus can be determined the particles have to be purified. The optimum purification procedure differs from virus to virus and it would not be realistic to list all the various procedures in this chapter. The reader is referred to other chapters in this book for methods of purifying viruses of animals. In this chapter I will discuss the principles behind designing purification procedures for plant viruses and give some examples. Once the particles are purified, the methodology for determining the biophysical and biochemical properties is the same for most, if not all, viruses be they from animals, plants or bacteria. This chapter will be confined to the methods for obtaining these measurements from the simpler viruses comprising a protein coat surrounding the nucleic acid genome.

2. PURIFICATION OF PLANT VIRUSES

Purification procedures for many viruses are given in the CMI/AAB Descriptions of Plant Viruses (1) and in (2). However, although there is no universal technique, there are various basic features and useful facts which can be used in designing methods for virus purification.

2.1 **Host Plants**

Ideally one should have a systemic host in which to grow the virus and a local lesion host for assaying various stages of purification. Choice of a systemic host should take account of the amount of virus produced, the ease of extraction of the virus (e.g., is the plant horticulturally soft? does the plant contain unhelpful substances such as large amounts of polyphenol oxidase?), the ease of growing the plant and the possibility of contamination with other viruses. The local lesion host should be used to ascertain the time of maximum virus content in the propagation host and also to check various stages in virus purification.

In most cases, however, it may not be possible to satisfy many of the above criteria. Often one cannot find a suitable local lesion host. The technique of dot blotting (see Section 3.8) can be used to replace local lesion assays.

2.2 Extracting the Virus from the Plant

To extract virus particles from the plant, the cell walls have to be broken and the cell contents released. This is usually done in the presence of a buffer to control the pH and of additives to prevent the released enzyme activities from damaging the virus particles. The action of enzymes is also slowed by extracting in the cold. Unless otherwise stated all purification steps should be at 0–5°C.

The most frequently used type of instrument for breaking plant cells is the blender, either top- or bottom-driven. There are many types on the market and choice should be governed by the efficiency of breaking up the plant material. This can be affected by the speed at which the blades rotate, the size and angle of the blades and the shape of the vessel. Breakage of plant cells can be increased by prior freezing of the tissue, though in some cases this will reduce virus yield. There may be problems with blenders if the propagation host is very fibrous (a feature to be taken into account in host selection) or if the virus particles are long and flexuous; in the latter case particles of, say, closteroviruses are broken by blending (3). These problems can be overcome by using a sap-press or a pestle and mortar. For viruses which are limited to vascular tissue, for example, phloem-limited luteoviruses, the leaf tissue should be initially disrupted with cellulases and pectinases (4).

The choice of extraction buffer can greatly affect the outcome of purification attempts. Viruses with elongated particles (e.g., potyviruses) which tend to aggregate or to be absorbed onto cellular debris are best extracted in alkaline buffers (pH 8–9) of moderate ionic strength (e.g., 0.1 M); however some rod-shaped viruses [e.g. tobacco mosaic virus (TMV)] can be damaged by alkaline buffers. Acidic buffers of about pH 5 (e.g., 0.1 M sodium acetate adjusted to pH 4.8 with acetic acid) are useful in the extraction of many viruses with isometric particles; there is the added advantage of many of the host proteins being precipitated at this pH. Some viruses with isometric particles, for example, cucumber mosaic and tobacco ringspot viruses, may be precipitated at around pH 5 and so pHs closer to neutrality have to be used for them. On the other hand, particles of other viruses, for example, bromoviruses, swell at around pH 7 making the viral RNA susceptible to nucleases and so, in their case, the use of lower pHs has an additional advantage.

The most common additives are reducing agents to prevent the action of polyphenol oxidases 'tanning' the viral coat protein. Commonly used reducing agents are 10 mM ascorbic acid (the pH of the buffer may need to be re-adjusted), 20 mM sodium ascorbate, 0.5% 2-mercaptoethanol, 20–40 mM sodium sulphite, 10 mM sodium thioglycollate or 10–20 mM sodium diethyldithiocarbamate; for hosts with a high 'tanning' level, hide powder can be used (5). Chelating compounds are also used to reduce enzymic activity and to dissociate ribosomes; 5–50 mM sodium ethylene diamine tetraacetic acid (EDTA) is the most commonly used. However, consideration must be taken of the possible involvement of divalent cations in virus particle stabilisation, e.g., sobemoviruses. The particles of some viruses, for example, caulimoviruses, are contained within proteinaceous inclusion bodies from which they have to be released. With