Essential Clinical Virology

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Preface

This small book adopts a clinical approach to virology by emphasising those aspects which will be encountered by the clinician—the presenting symptoms and signs. In this way, family doctors, housemen, registrars, examination candidates and those with an interest in infection, as well as nurses and other health care personnel, will be able to follow the likely pattern of development of the clinical illness. Each section leads on naturally to discuss the best possible use of the expensive and often time-consuming tests available in a modern diagnostic virus laboratory.

New methods of virus diagnosis are evolving year by year and in some clinical situations our understanding of the role of viruses is still in an evolutionary phase. The text indicates these areas where appropriate but does not deal at length with the complexities of highly specialised test procedures. It does not replace standard textbooks of virology but gives, instead, an integrated approach based on clinical findings.

It is necessary, however, to refer to technical methods for making a virus diagnosis and to avoid repetition these are all described together in a concise manner in Chapter 1, which is illustrated appropriately so that the non-specialist can grasp the outline of the test method.

The range of infectious agents included has been extended to cover *Coxiellas, Chlamydiae* and *Mycoplasma pneumoniae,* because the symptoms of infection caused by these are often indistinguishable from those of virus infections and, also, because the diagnosis is usually handled by virus laboratories.

It must never be forgotten, however, that virus infections frequently become complicated by secondary bacterial or fungal infections and that the 'whole patient' must be considered when evaluating a clinical problem. It must also be remembered that the speed of modern travel and the frequency with which holidaymakers explore different and often unaccustomed parts of the world, make it obvious

that uncommon and occasionally 'exotic' infections may turn up as a result in quite unexpected situations. Where appropriate, the text refers to the possible occurrence of such infections and draws attention to the need for additional evaluation of the sick person's background and recent travel experience.

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Chapter 1

Laboratory Tests

This book is intended to give help in different clinical situations and to suggest ways in which diagnostic problems in virology can be overcome. Inevitably, most diagnostic problems can only be resolved completely by making use of virus diagnostic facilities which are frequently complex, time-consuming and the province of the expert. To allow the clinician to discuss the problem rationally with diagnostic laboratory workers, reference is made in each chapter to possible tests which will facilitate the diagnostic process.

A great deal of repetition in the text will be avoided by outlining here the diagnostic laboratory tests available. Detailed technical methods are not, however, given; these can be found in many specialised textbooks and readers looking for additional details are referred to such texts. The intention here is only to indicate the principles on which the different types of test are based so that the non-virologist can comprehend easily what is involved when a request is made for a particular test. In any case of doubt about the correct approach, it is always a good idea to discuss the problem with your local laboratory director to obtain personal advice and to ascertain the correct, most rapid and cost-effective method with which to establish a diagnosis.

There are 2 main routes which can lead to the laboratory diagnosis of a virus infection: to identify the virus, or to prove infection by identifying a virus-specific antibody. Within these 2 categories there is a plethora of different methods. Many are slow and costly and require a high level of scientific skill to obtain a satisfactory result. *It is most important to remember that the virus laboratory cannot distinguish between good and bad specimens, irrespective of how they have been collected.* The clinician must ensure that appropriate, well-taken, specimens are collected and taken to the laboratory under correct storage conditions if the best possible results from the laboratory are to be obtained.

Traditional approaches to virus diagnosis have always been time-consuming. To grow a virus in living cells, either in an egg, animal or

tissue culture system, takes from 2 days to 4 weeks. After it has grown, the virus must be identified and this can also take days, if not weeks. The alternative method has been to demonstrate rising antibody titres in the patient's blood to a particular virus. By definition, this method implies that antibody production will usually coincide with the patient's recovery. For this reason, the serological answer to the diagnostic problem has often arrived in the hands of the clinical worker after the patient has recovered.

During the last few years numerous attempts have been made to speed up virus diagnosis and immunological methods are now being used, with greater freedom and accuracy, either to directly identify viruses (or viral antigens) in infected secretions or to detect virus-specific antibody at an early stage in the illness. The latter approach has concentrated particularly on the presence of IgM class antibody, which usually appears only briefly during the early stages of virus infections. Prolonged IgM antibody formation is the exception rather than the rule. New and rapid immunological methods of detecting virus-specific antibodies in the IgG class have also been introduced.

Varying amounts of success have been claimed for both of these newer approaches.

Electron microscopy has also contributed significantly to virus diagnosis, by allowing direct viewing of a virus in pathological secretions or in biopsy specimens. By itself, however, electron microscopy does not usually allow one to distinguish, for example, between members of the herpesvirus group, unless immune specificity is added. For most routine applications, where direct viewing of a virus in secretions is undertaken (as opposed to biopsy preparations), the rider must be added that the procedure is very labour-intensive and requires a high degree of sophisticated scientific training.

The possible individual approaches will now be considered in detail.

1.1 Direct microscopy

Light microscopy

Viruses are generally too small to be seen with even the highest magnification of a conventional light microscope. The exception to this rule is the poxvirus group, members of which can be seen in

appropriately stained specimens as fine eosinophilic 'dust-like' material, lying extracellularly in scrapings obtained from the base of lesions, preferably taken from vesicles at an early stage and before pustule formation. However, light microscopy can give help in diagnosis in other directions. Specimens for examination are placed on microscope slides, air-dried, fixed (in methanol, usually) and stained. Several stains will show inclusions within infected cells. These are usually the site of virus replication. Stains which are particularly recommended are Giemsa, Macchiavello and Castaneda. Giemsa is the most readily adaptable of these and can be used for different types of specimens to show the following.

Vesicle fluid or vesicle base scrapings. These may show multinucleated giant cells which are typically produced by herpesviruses. Nuclear inclusions may be present (Fig. 1.1).

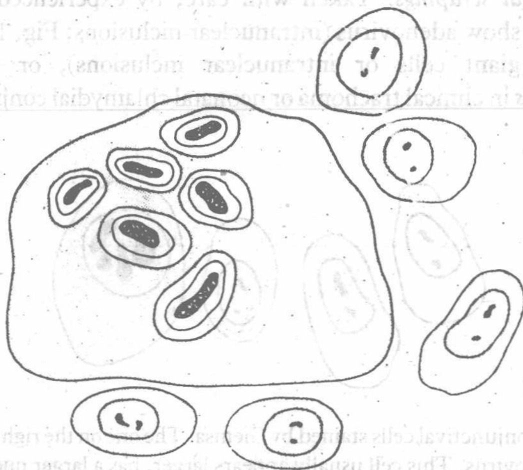

Fig. 1.1. A typical multinucleated giant cell induced by herpes simplex and found in a Giemsa-stained scraping from the base of a herpes simplex vesicle. The individual nuclei within the giant cell show typical inclusions with central condensation of nucleo-protein into a dense band. Normal cells surround the giant one.

Urethral scrapings. These may reveal the presence of *Chlamydia trachomatis* (cytoplasmic basophilic inclusion bodies) in non-specific urethritis (Fig. 1.2).

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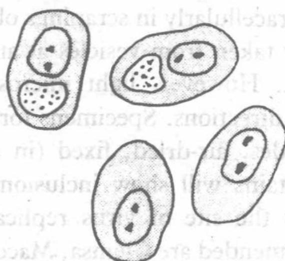

Fig. 1.2. Typical urethral epithelial cells obtained from a case of non-specific urethritis. The top two cells contain typical *Chlamydia trachomatis* inclusions. These are always cytoplasmic, usually relatively transparent and are packed with individual chlamydiae which have multiplied by binary fission.

Conjunctival scrapings. Taken with care, by experienced workers, these may show adenovirus (intranuclear inclusions; Fig. 1.3) herpes simplex (giant cells or intranuclear inclusions), or *Chlamydia trachomatis* in clinical trachoma or neonatal chlamydial conjunctivitis.

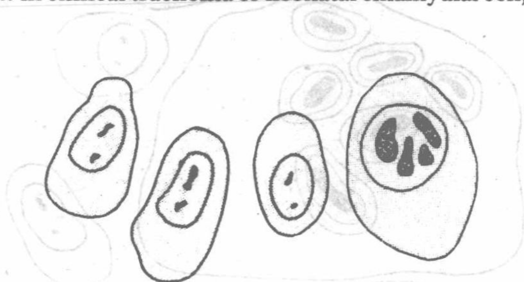

Fig. 1.3. Conjunctival cells stained by Giemsa. The one on the right is infected by an adenovirus. This cell usually appears larger, has a larger nucleus which is literally full of developing virions and, invariably, shows condensation of nuclear chromatin into discrete lumps.

Urinary sediment. Obtained from a normal 'clean' voiding—*not* an early morning specimen (which tends to have crystalline or amorphous phosphate or urate deposits)—this may show cytomegalovirus (intranuclear owl's eye; Fig. 1.4) or human polyomavirus inclusions in cell nuclei. Similar results can be obtained from a sterile bladder-tap in neonates.

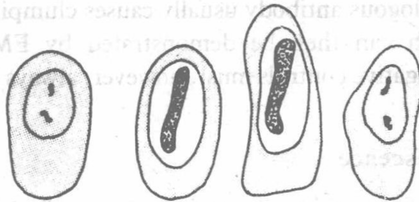

Fig. 1.4. Typical appearance of cytomegalovirus-infected renal epithelial cells, which might be present in Giemsa-stained urine sediment. Nuclear inclusions are present in the two central cells. The nuclear enlargement, its typical 'ground-glass' inclusions and the condensation of nucleo-protein into a dense band which gives the nucleus the typical 'Owl's eye' appearance, are shown.

The presence of typical inclusions in stained specimens may be helpful in an emergency or when virus laboratory facilities are not available. The amount of information which can be obtained is, however, strictly limited, and virological facilities should always be sought to confirm the diagnosis at an early date.

Electron microscopy

The very much higher magnification available by electron microscopy (EM) allows all viruses to be seen; sometimes after much searching. Virus groups can be identified in pathological secretions, biopsies or laboratory tissue culture cells. For example, poxviruses and herpes-viruses can be identified with ease. Appropriate contrast conditions must be obtained by addition of electron-dense material to cause shadowing of the viruses present on the specimen grid of the electron microscope. Both intra- or extracellular viruses may be visualised by EM and this contrasts with observation by light microscopes where intact cells are required for visualisation.

Some indication of the amount of virus present can be obtained with EM but the procedure is very labour-intensive and is not always accurate.

It should also be remembered that with any potentially dangerous virus, the specimen for EM must be inactivated by ultraviolet light prior to examination.

Immunological specificity may be added to EM identification in a

number of situations. Premixing virus in suspension with hyperimmune homologous antibody usually causes clumping of the virion particles which can then be demonstrated by EM. Appropriate positive and negative controls must, however, always be included.

Immunofluorescence

This method of visualising viruses has won much acclaim during the last few years because it is extremely rapid, has the capacity to identify the virus directly in pathological secretions and is highly adaptable to suit different conditions. The main disadvantage of immunofluorescence (FA) is its requirement for intact cells, so that the virus can be visualised at the correct site within the cell.

The technique depends upon the fact that an intracellular virus is antigenic and can react with an appropriate antibody. Cells are either directly obtained from lesions by scraping, or exfoliated cells are used (e.g. respiratory secretions). In all cases, the cells are transferred to a specially cleaned 3 × 1 inch microscope slide. The material to be examined is 'air-dried' and 'fixed' in acetone to fix cellular components to the slide and to render cells permeable to antibodies. The specimen is then overlaid with an appropriate dilution of an antiviral antibody, homologous to the viral antigen which is being sought within the cells and incubated for 20–30 min to allow the antigen and antibody to unite.

Unreacted antibody is removed by gentle and careful washing, first in buffer, then in water and afterwards the slide is dried. A fluorescein-labelled antibody to the species-specific antibody is then applied in appropriate dilution and the incubation and washing processes are repeated. Thereafter, the slide is dried and examined by fluorescence microscopy, either after mounting in buffered glycerol with a coverslip, or unmounted, using immersion oil or water, depending on the microscope objective.

Unmounted preparations can be stored in a dark box at 4°C for several weeks. Mounted preparations have to be examined within 24 h because the fluorescein tends to leach out into the glycerol.

At all stages, appropriate controls must be included. Practical advice about these must be obtained from laboratory workers who are experienced in this type of work.

A fluorescent antibody examination for the detection of viral

antigen in, for example, respiratory secretions, will confirm the presence of virus within 2–3 h of laboratory processing time. Considerable experience is necessary with 'reading' FA slides before firm diagnostic conclusions can be reached but in experienced hands the accuracy of the method is now excellent.

Some examples of possible applications and the viruses which may be found are given below.

Respiratory secretions (including nasal washings; Fig. 1.5): influenza A, B; parainfluenza 1, 2, 3, 4; RSV; adenovirus; measles; mumps.

Fig. 1.5. Immunofluorescence applied directly to cells obtained by suction aspirator from the respiratory tract of a child suffering from bronchiolitis. This peparation has been stained by anti-respiratory syncytial virus antiserum and the bright cytoplasmic fluorescence is typical (×1050).

Conjunctival scrapings: herpes simplex and varicella-zoster; adenovirus; also *Chlamydia trachomatis*.

Vesicular eruptions on skin and mucus membranes. These are not recommended, because non-specific staining may occur between polymorphonuclear leucocyte granules and fluorescent dyes. This can lead to false-positive results and erroneous diagnoses.

Urine or sterile bladder tap: cytomegalovirus (Fig. 1.6); measles; rubella—particularly in congenital infections.

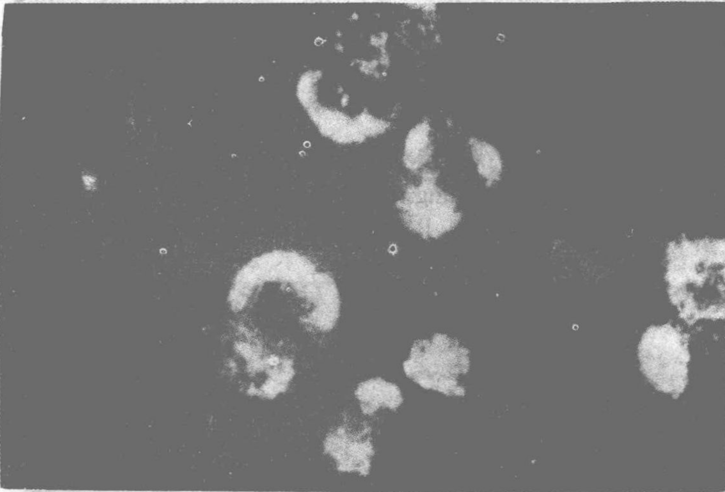

Fig. 1.6. Cytomegalovirus within epithelial cells found in a urine sediment from a marrow transplant recipient who developed a febrile illness 10 days post-transplant. The preparation was stained with anti-cytomegalovirus antiserum and the presence of the virus is easily seen (×1350).

CSF: mumps, measles, enteroviruses.

Amniotic liquor: rubella; cytomegalovirus or herpes simplex.

1.2 Virus isolation procedures—animals

In the early days of diagnositic virology, laboratory animals were used widely as the only available way in which viruses could be propagated. The virus multiplied in tissues of the animal and often produced signs of its presence. Now, better methods are available and laboratory animals are not often used for virus isolation (with the very important exception of arboviruses and haemorrhagic fever viruses).

Probably the most important single animal used for isolation of viruses is the *newborn mouse.* It is susceptible to a number of viruses,

depending upon the route of inoculation, including:

- Coxsackie B viruses
- Coxsackie A viruses (many of which cannot be propagated by any other method)
- herpes simplex virus
- most arboviruses and haemorrhagic fever viruses.

Herpes simplex and the Coxsackie B viruses also grow readily in many different types of tissue culture cell, and animals are not now used.

Coxsackie A viruses (with the main exceptions of A5, A7, A9, A10 and A16) will only propagate in newborn mice. These viruses produce a typical syndrome in the mice of focal muscular degeneration, which leads to a flaccid paralysis. By contrast, Coxsackie B viruses produce a spastic paralysis. The viruses can be recovered in high titre at autopsy from brain, muscle, liver and spleen. Both naked eye and histological patterns are pathognomonic for the Coxsackie virus A group but typing of the isolate depends on performing protection tests using hyperimmune serum in newborn mice. This is costly, very time-consuming and is not a test which is readily available in many centres.

Arboviruses also multiply readily in newborn mice and are usually isolated from pathological material after intracerebral inoculation. The virus rapidly causes a fatal encephalitis in the newborn mice and can be extracted from the brain after removal and emulsification with saline at pH 7.2. *Care is needed because many of these viruses are pathogenic for man.* Identification is usually by a pH-dependent haemagglutination test, using homogenised brain extracted in saline. Many arboviruses can now be propagated in VERO cell tissue culture (see section 1.4).

1.3 Embryonated eggs

The principle which has governed the use of embryonated eggs in diagnostic virology is the ready availability, within the egg, of various types of tissue contained in an aseptic environment. Many of these could be used to grow viruses from diagnostic specimens. The development of tissue culture methods has largely removed the requirement for embryonated eggs but their use is discussed here briefly, within a largely historical perspective. The number of different viruses which could be thus grown was very limited (see below). The use of embryonated eggs is best discussed with reference

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