Detection of antigens and IgM antibodies for rapid diagnosis of viral infections: a WHO Memorandum*

This Memorandum describes recent progress in the development of simplified and rapid laboratory technology and reviews currently available methods for the direct detection of viral antigens in clinical material and/or quantification of specific IgM antibody in acute serum specimens. Particular emphasis is given to such infections as viral hepatitis, rotavirus gastroenteritis, viral infections of the skin, genital, and respiratory tracts, and rabies. In addition, the standardization and quality control of reagents are discussed and recommendations are made.

The World Health Organization recently devoted attention to the development of methods for rapid laboratory viral diagnosis suitable for use in the laboratories of WHO Member States, especially those in developing countries. Two previous meetings on this topic (1, 2) held during 1976 and 1977 resulted in recommendations for the perfection and testing of various techniques, including immunofluorescence (IF) and immunoperoxidase (IP) methods and the enzyme-linked immunosorbent assay (ELISA) for the detection of viral antigens in clinical material and viral antibodies in human serum. Training courses and collaborative studies have been organized. In the meantime, two groups interested in furthering advancement in laboratory viral diagnosis have been formed—the European Group for Rapid Laboratory Viral Diagnosis (EGLVLD) and the Pan-American Group for Rapid Viral Diagnosis (PAGRVD). These two groups have met and are collaborating with WHO in seeking solutions to common problems.

This Memorandum reviews the latest information on techniques for the rapid detection of viral antigens and antibodies, especially IgM, in clinical material, and recommendations are made concerning standardization and quality control of reagents, collaborative studies, and teaching programmes.

VIRAL DISEASES

Viral hepatitis

Rapid diagnosis of hepatitis A virus (HAV) infection is based on detection of the virus in faeces or on determination of specific IgM for HAV in a single serum specimen obtained in the acute phase of illness. Since most of the HAV in faeces is excreted in advance of the onset of illness and only relatively few virus particles are usually present at onset of jaundice, screening of faeces for HAV at the acute phase of the illness is of limited diagnostic value. Immune electron microscopy (IEM), radioimmunoassay (RIA), and enzyme immunoassay (EIA) procedures are highly sensitive techniques for the detection of HAV in faeces but all have their drawbacks. IEM is a cumbersome method and is not universally adaptable for screening purposes. RIA and EIA procedures are suitable for stool screening if the reagents are carefully chosen to ensure maximum specificity, particularly in situations such as epidemics where persons shedding virus in faeces can be identified prior to onset of illness and appropriately isolated.

The method of choice for the rapid clinical diagnosis of hepatitis A is the detection of specific anti-HAV IgM in serum taken at the acute phase of the illness (3,4). Both EIA and RIA can be modified to detect anti-HAV IgM and for RIA the modified technique can be carried out with commercially available equipment.

Sensitive and specific EIA and RIA techniques have been established, and are, or soon will be, commercially available, for each of the markers of

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hepatitis B virus (HBV) infection, such as HBsAg, anti-HBs, HBeAg, anti-HBe, HBeAg, and anti-HBc. These tests have been supplemented by a latex agglutination test for HBsAg and a passive haemagglutination test (PHA) for HBeAg and anti-HBe, which are of reasonable sensitivity. Determination of HBsAg in serum is the cornerstone of both the clinical diagnosis of HBV infection and the screening of donor blood. The clinical significance of HBeAg, anti-HBe, HBeAg, and anti-HBc as serological markers for infectivity needs to be further elucidated before their routine use can be justified. Further studies of specific anti-HBc IgM are of particular importance in this context. After further clinical evaluation, HBeAg and anti-HBe tests might be valuable for estimating the likely severity and course of chronic hepatitis and as indicators of the risk of horizontal and vertical transmission of hepatitis B.

Diagnosis of non-A/non-B hepatitis can be made at present only by the exclusion of hepatitis A, hepatitis B, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) infections through application of the relevant diagnostic procedures.

Rotavirus gastroenteritis

While electron microscopy is the basic method by which rotaviruses have been detected in faecal samples, its use is limited because of the small number of samples that can be handled and the sophisticated equipment required. The human rotavirus has an incomplete cycle in cell culture and thus virus isolation techniques are not suitable; nevertheless, growth in culture produces an antigen that can be detected by IF following centrifugation or trypsinization (5). Stool from infected patients often contains considerable amounts of free viral antigen as well as large numbers of virus particles. Because of this, serological techniques can be employed for the detection of rotaviruses; in particular the ELISA technique has now been employed in several studies (6). Solid-phase radioimmunoassay (SPRIA) is somewhat similar to the ELISA technique, with the enzyme label being replaced by a radioactive tracer. Both of these techniques are very sensitive and suitable for the screening of large numbers of specimens. SPRIA has the drawbacks that sophisticated equipment is required and that radioactive material gives rise to safety considerations.

Recently a test entitled solid-phase aggregation of coated erythrocytes (SPACE) has been described which includes some of the features of both ELISA and SPRIA but which uses an antibody-coated red cell to detect the presence of virus (7). The method involves coating the U-shaped cells of microtitration plates with specific antiviral antibody; after the plates have been washed, simple 10% faecal suspensions are added and allowed to adsorb. The adsorbed viral antigens are then detected by the addition of erythrocytes coated, by the chromium chloride method, with specific antiviral IgG. The results are read in the same manner as with a conventional haemagglutination test, positives showing a carpet of cells and negatives a button. In a series of faecal samples examined under code, the SPACE test gave very good agreement with both IF and IEM. Since SPACE is a solid-phase test, it has the advantage, in common with ELISA and SPRIA, that the background of extraneous faecal material is washed off after virus adsorption has taken place. This is in contrast to reversed passive haemagglutination (RPHA) which is unacceptable for faecal specimens.

All the tests described here have a place in surveillance for rotavirus infection. Those with the greatest sensitivity recognize antigen in addition to virus and are the ELISA, SPRIA, and SPACE tests. Until tissue culture techniques are discovered for the propagation of rotaviruses of all species, viable virus can be detected only by the immunofluorescence method.

Viral infections of the skin and genital tract

Herpes simplex virus (HSV) grows rapidly in cell cultures; in many cases characteristic cytopathic effects appear following overnight incubation in monkey or human cells. When a more rapid diagnosis is needed, electron microscopy (EM) using negative staining of vesicular or pustular material has proved to be very dependable for identification to group. However, similar results can be obtained from patients with chickenpox or shingles. Concentration procedures are usually not needed. Immunofluorescence techniques have proved suitable for vesicular material or punch biopsies as early as the maculopapular stage of the exanthem but variable for pustular and later stages. IF has also been used for the identification of herpes viruses from other sites (brain, throat, cervix, etc.) A rapid diagnosis may be achieved in some cases of herpes encephalitis by measuring the level of antibodies in cerebrospinal fluid compared with that in serum, but controls must be included. The immunofluorescence technique should not be used to examine cells in cerebrospinal fluid for antigen. An ELISA test has been attempted.
for HSV-1 but not followed by further reports. An ELISA test for varicella-zoster virus has been compared with counter-immunoelectrophoresis (CIE) and immune adherence haemagglutination (IAHA): although antigen was easily detected in cell culture fluid, specific activity could not be evaluated in vesicular fluid owing to the cross-reaction of conjugate with IgG. EM, cytopathology, and IF techniques that detect immediate-early antigens and early antigens in infected cell cultures have proved to be rapid procedures for detecting cytomegalovirus in urine and throat washings collected from congenitally infected infants (8). An ELISA test for Epstein-Barr virus has been attempted but with little success.

The techniques described above for the detection of viruses in lesions (EM and IF) have both been suitable for the rapid diagnosis of vaccinia and molluscum contagiosum viruses; the variola virus, EM has been suitable but IF has been unreliable. There have not been any reports of ELISA being used for the detection of virus in clinical specimens collected from patients infected with these viruses.

In rubella, because the levels of antibodies rise so quickly after the onset of rash and the virus is difficult to detect in specimens, most investigations have examined methods of antibody detection (rising titre or presence of virus-specific IgM). Many techniques for the separation and measurement of immunoglobulins are now available. Inoculation into appropriate cell cultures and haemadsorption combined with serotyping is used for measles detection but IF may be used on secretions for a more rapid diagnosis.

Chlamydia may often be detected in smears from infected eyes by histopathological staining of the smears, but this organism is not readily detected in genital or throat specimens. Cell culture techniques provide the methods of choice for a laboratory diagnosis from clinical specimens. The most rapid method reported so far involves the centrifugation of the specimens onto McCoy cells, treatment with cycloheximide and IF staining 21 hours later (9).

Respiratory virus diseases

Although IF has been used successfully for many years for the detection of respiratory viruses, a fluorescence microscope is needed and the technique has limited value for groups of viruses that have many antigenic types (rhinoviruses, coxsackieviruses, echoviruses). IF has been used effectively for the diagnosis of respiratory viruses difficult to cultivate (coronaviruses, parainfluenza viruses types 4a and 4b, and measles virus when this presents with an atypical clinical picture). Influenza A and adeno-virus have been diagnosed successfully for many years by IF but this test has always been type-specific. Recent research (10) has suggested that, with purified viral antigen as inoculum, a serum can be made that is subtype-specific if treated by affinity chromatography. The technique needs to be evaluated on clinical specimens.

A recent WHO collaborative study (11), which showed limitations of the immunoperoxidase method owing to endogenous peroxidase present in clinical material, has led to investigation of the use of ELISA in the detection of respiratory syncytial virus antigen. A capture-antibody technique was used and 23 out of 29 (80%) culture-positive cells were confirmed directly on secretions. A comprehensive comparison of IF and ELISA for the detection of RS virus is now in progress. The advantage of IF over ELISA at present is that the diagnosis is usually achieved sooner. IF, ELISA, and SPACE should be compared for respiratory infections in a double-blind trial. RIA has so far not played a significant part in the laboratory diagnosis of respiratory viral antigens.

Rabies

A microenzyme immunoassay (EIA) using polystyrene wells coated with either complete virus or viral glycoprotein detects antibodies to rabies virus. The indicator system employs either anti-human IgG (sheep) or staphylococcal protein A; both are conjugated with peroxidase. The protein A conjugate has proved to be a universal detector for a wide range of species. The assay correlated well with other conventional tests (12).

IMMUNOLOGICAL METHODS

Detection and measurement of IgM specific antibody

Usually the first detectable antibody is in the IgM class and this is less persistent than that in the IgG fraction of the serum. At present the main methodological approaches, used together or separately, are: (a) separation of the IgG from the IgM; (b) the use of the IgM-specific labelled antiglobulins; (c) the use of a solid phase coated with anti-IgM.

IgG may be separated from IgM by fractionation
of the serum by column chromatography, by density gradient centrifugation (for example, on sucrose), by the use of staphylococcal protein A which removes IgG, or by destruction of the IgM with 2-mercaptoethanol. Following any of these procedures, the test for antibodies to the virus is carried out in the usual way by means of traditional tests.

Antisera specific to IgM (for example, anti-human IgM) may be labelled with fluorescein, peroxidase, or $^{125}\text{I}$. In all these techniques, the antigen can be immobilized on a solid phase. The test serum (or fraction of it) is incubated with the solid phase and finally the anti-IgM conjugate is added. The techniques are relatively easy to carry out but with unfractionated sera there are pitfalls:

1. IgG-specific antibody and rheumatoid factor (RF) of the IgM class in the sample will both lead to false positive results (i.e., the sample will appear to have IgM antibody). The use of F (ab)$^2$ for preparing the conjugate will largely eliminate the problems of RF. The serum can then be adsorbed with heat-aggregated human immunoglobulin which will remove the RF activity.

2. False negative results can be obtained as a result of high levels of IgG antibody being present together with IgM antibody in the test serum.

Another approach to both of these problems involves a solid phase coated with anti-IgM. The test serum can then be added and any IgM will become fixed to the sensitized solid phase. Labelled antigen, or unlabelled antigen followed by labelled specific antibody, can be used as indicator. This technique has been applied successfully for the direct detection of IgM in hepatitis A and rubella patients, and for the detection of anti-HBc.

**Thin-layer immunoassay**

Thin-layer immunoassay (TIA) is a new technique for assaying antigen–antibody reactions (13). The basis for the technique is that certain macromolecules may be adsorbed as a thin layer to a polystyrene surface. Such an antigen-coated surface has the characteristics of an immunosorbent and is able to bind antibodies. Visualization of antigen–antibody interaction on the surface is accomplished by condensation of water vapour. A surface on which an antigen–antibody interaction has taken place is relatively hydrophilic in comparison with the background area, which is more hydrophobic. The difference in the condensation patterns is easily recognizable with the naked eye and does not require any sophisticated laboratory equipment. The technique has so far been used mostly for the demonstration of antibodies but preliminary attempts have been made to demonstrate HBsAg in serum. The sensitivity of TIA for the demonstration of HBsAg, when an overall IgG fraction is used for coating, is about the same as that of counter-immunoelectrophoresis. The use of specific IgG may improve sensitivity.

**Quality control and standardization of reagents**

Factors limiting progress with many of the rapid techniques discussed are related to the need for high quality, standardized, commercially produced reagents. The EGRLVD, PAGRVD, and other groups are attempting to draw up standards for several of the techniques. Most progress has been made in the field of IF for antisera and conjugates and the following rules have been proposed: (a) Infected and control cells should be tested to determine the optimum dilution for detecting antigen. This dilution must be at least four times greater than that revealing any nonspecific fluorescence with uninfected cells. The control cell cultures should include four different types of cell. (b) A limited titration for final evaluation should be made on positive clinical material appropriate for the virus under test. (c) Antisera should be tested at their optimum dilution on negative pharyngeal secretions and where possible on other negative human clinical specimens. (d) Antisera should be tested on cell cultures infected with a representative collection of viruses, e.g., respiratory syncytial virus, parainfluenza viruses 1, 2, 3, 4a, and 4b, mumps virus, influenza viruses A and B, measles virus, herpes virus, cytomegalovirus, and at least two enteroviruses. There should not be any nonspecific reaction with these antisera at their optimum dilution. Similar criteria are used to establish the efficacy of antispecies conjugates.

These criteria will apply only under the conditions of the test system and it is therefore of great advantage to users of the reagents to use the staining technique including counterstain as suggested in the *Manual on rapid laboratory viral diagnosis*. Similar criteria will be necessary for the assessment of all reagents for rapid diagnostic techniques. When assessments of reagents and methods are made it is important that tests be carried out by individuals having a high degree of expertise.

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RECOMMENDATIONS

General

1. WHO should consider forming a committee to implement the standardization and quality control of viral reagents.
2. WHO training programmes in rapid viral diagnosis should be prepared in collaboration with the various expert groups, both as regards courses and individual training.
3. WHO should consider the establishment of centres in developing countries for research, reference, and training in rapid viral diagnosis.
4. Collaborative studies to compare the conventional and the new, simple, rapid techniques should be encouraged between laboratories in developed and developing countries.

Further research

1. The potential of the solid-phase aggregation of coated erythrocytes (SPACE) and thin-layer immunosassay (TIA) techniques should be explored for several viruses, their sensitivity and accuracy being compared with that of other established assays.
2. More research should be performed to test the ability of the enzyme-linked immunosorbent assay (ELISA) to detect viral antigens in a wider range of clinical specimens.
3. The use of protein A conjugates should be investigated for a wider range of viral infections.
4. Comparative studies on the various techniques for the separation and measurement of IgM should be undertaken.

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