Virus diseases - diagnosis
Indicators and reagents
Viruses - isolation and purification
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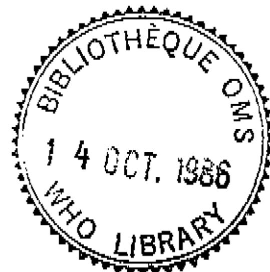

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WHO PROGRAMME FOR PRODUCTION AND DISTRIBUTION OF REAGENTS FOR RAPID VIRAL DIAGNOSIS

Report of a Meeting of Directors of
WHO Collaborating Centres for Virus Reference and Research,
Geneva, 25-28 November 1985*

1. INTRODUCTION

The meeting of Directors of the WHO Collaborating Centres for Virus Reference and Research took place in Geneva from 25 to 28 November 1985. On behalf of the Director-General of WHO, Dr F. Assaad, Director of the Division of Communicable Diseases, emphasized the importance of international cooperation in the field of production and distribution of reagents for viral diagnosis.

One of the priorities of the WHO Virus Diseases programme is to encourage viral diagnosis by use of rapid and simplified techniques. Imperative to the implementation of these techniques is the availablity of high quality reagents which, to some extent, has been facilitated by cooperation with the European Group for Rapid Viral Diagnosis. It is expected that the Pan American and Asian Groups for Rapid Viral Diagnosis will also take an active part in the WHO programme.

The importance of a WHO programme on viral reagents was recognized by the WHO Scientific Group on Rapid Virus Diagnosis[1] and by a previous meeting of the Directors of the WHO Collaborating Centres.[2] In 1982, a meeting was held in Stockholm on the 'Review of Rapid Laboratory Viral Diagnosis with Special Emphasis on Coordination of Production, Quality Control and Supply of Reagents'.[3] In 1983, WHO arranged a meeting in Geneva on Simplified Techniques for Virus Diagnosis.[4]

The aim of the present (1985) meeting is to monitor the progress made since previous meetings in the field of viral reagents and to outline practical programmes for the production and distribution of reagents for viral diagnosis.

As previous meetings have discussed extensively the modern techniques for viral diagnosis and requirements for reagents, the present report only briefly defines the viral infections to be diagnosed and the techniques recommended, and focuses more extensively on the type and quality of reagents and the principles for arranging a reliable supply on a larger scale.

Although the report is structured along the lines of aetiological agents, the meeting felt that the eventual aim would be to develop rapid diagnosis schemes by disease syndromes. This would require future integration among the disciplines of virology, bacteriology and parasitology.

2. RESPIRATORY VIRUSES

2.1 The agents which can be diagnosed by rapid techniques are respiratory syncytial (RS) virus, influenza A and B, parainfluenza 1-3 viruses, adenoviruses, rhinoviruses as well as other viruses.

* Participants are given in Annex 1.

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2.2 Rapid diagnostic methods include immunofluorescence (IF) and enzyme-linked immunosorbent assay (ELISA) for the detection of viral antigens in clinical specimens. Virus isolation should be performed to monitor the appearance of antigenic variation in the case of influenza viruses and to confirm results obtained with rapid diagnostic techniques.

Serological methods are complementary to rapid techniques for diagnosis and are important in epidemiological surveillance. Enzyme immunoassay for the detection of an IgG antibody titre rise in paired serum specimens is a highly sensitive method for the diagnosis of respiratory infections. However, rises in IgG antibody titres may be delayed in infants. HI tests are important in demonstrating the prevalence of antibodies to particular antigenic subtypes or variants of influenza viruses.

Estimations of IgM or IgA antibody estimations as single antibody classes in the diagnosis of acute respiratory virus infections appear to have only a limited value.

2.3.1 Reagents available for virus and antigen detection

(a) Commercial reagents include:

Wellcome Diagnostics products for IF which are controlled for quality by the European Group for Rapid Viral Diagnosis. Other commercial reagents may not always be quality controlled externally.

Pools of monoclonal antibodies for RS virus diagnosis are produced by Boots-Celltech and Ortho Diagnostics Systems as FITC conjugates.

(b) Reference strains of parainfluenza 1, 2 and 3, RS virus, most adenoviruses and the corresponding antisera are available from WHO Collaborating Centres in London and Atlanta, USA.

(c) Non-commercial-locally produced reagents include:

Reagents	Supplier*
The WHO kit for Influenza A and B, which also contains two MAb pools for typing of tissue culture isolates by the IF method.	1
Reagents for parainfluenza 2 (which are not available from commercial sources).	2
Adenovirus reagents including an adenovirus hexon antibody purified by affinity chromatography.	3
MAbs against RS virus and the influenza A nucleocapsid for use in the IF test.	4

Reagents for ELISA for RS virus, parainfluenza 1-3, influenza A and B viruses and adenovirus. 1,3,5

* Suppliers:

1. WHO Collaborating Centre for Reference and Research on Influenza, Centers for Disease Control, Atlanta, USA.
2. Virus Department, University of Newcastle, England.
3. Virus Department, National Bacteriological Laboratory, Stockholm, Sweden.
4. Virology Department, University of Dijon, Dijon, France.
5. Virus Department, University of Turku, Turku, Finland.

2.3.2 Serological diagnosis

(a) Commercial reagents. Many producers supply complement-fixing (CF) and haemagglutinating antigens (HA). So far antigens for ELISA are not commercially available.

(b) Reference HA antigens for influenza A and B are included in the WHO Influenza kit. CF and ELISA reference antigens are not available.

(c) Locally produced antigens are available from many laboratories for all the viruses defined in section 2.1.

ELISA reagents are being developed in a number of laboratories.

2.4 Minimal quality control criteria.

Stringent testing of all reagents is necessary, especially reagents for IF, and this must include testing on clinical specimens as well as on tissue culture material to ensure that the reagents are free from all non-specific reactions. The criteria for this testing are well described in the WHO manual for Rapid Laboratory Viral Diagnosis[5].

2.5 Main problems

These are associated with either (a) reagent production, (b) distribution or (c) use of reagents.

The reagent production problem is essentially to make available good quality reagents at reasonable cost. Distribution problems can be minimized by the lyophilization of reagents which greatly enhances their stability. Optimal use of reagents requires precise information as to methodology, appropriate choice and treatment of specimens, together with accurate information on the interpretation of results.

2.6 Quality control laboratories

For detection of respiratory virus antigens by IF and ELISA the following laboratories are suggested:

Europe: Virus Department, National Bacteriological Laboratory, Stockholm
Virus Department, University of Turku, Finland
Virus Department, University of Newcastle, England
Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, London, England

Asia: Department of Microbiology, University of Malaya, Kuala Lumpur, Malaysia

Americas: Centers for Disease Control, Atlanta, USA

Australia: Fairfield Hospital, Victoria, Australia

South America: Dept. de Laboratorios de Higiene Publica, Ministerio de Salud Publico, Montevideo, Uruguay
Institute Oswaldo Cruz, Rio de Janeiro, Brazil

In addition to the above laboratories there are the WHO Collaborating Centres for Reference and Research on Influenza in London, UK and CDC, Atlanta, USA.

2.7 Possible centres for reagent production

Reagent production centres in addition to commercial sources should be nominated and supported by WHO. The following laboratories are suggested for the production of limited amounts of reagents for rapid diagnosis:

Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden
Central Public Health Laboratory, Colindale, London, England
Institute of Hygiene and Epidemiology, Prague, Czechoslovakia
National Institute of Virology, Pune, India
National Institute of Health, Islamabad, Pakistan
Virus Research Institute, Bangkok, Thailand
Department of Microbiology, University of Malaya, Kuala Lumpur
Department of Biologics, CDC, Atlanta, USA
National Institute of Health, Tokyo, Japan

Monoclonal antibodies have been produced by many laboratories and commercial companies including:

Karolinska Institute and National Bacteriological Laboratory, Stockholm, Sweden
Department of Virology, University of Turku, Turku, Finland
INSERM, Strasbourg, France
Central Public Health Laboratory, London, England
Centers for Disease Control, Atlanta, USA
National Institute of Health, Tokyo, Japan,

Additional laboratories may have this capability and be interested in reagent production. WHO should announce its intention to support local production of reagents and invite such laboratories to contact the Organization.

3. ENTEROVIRUSES

3.1 Until now, no method has proved useful for the rapid diagnosis of diseases caused by enteroviruses. The role of electron microscopy as well as that of rapid detection of viral antigens in clinical specimens by immunofluorescence microscopy in enteroviral diagnosis is limited. Taking into account the large range of clinical presentations which involve enteroviruses, their diagnosis is necessary in a great number of syndromes.

The listing of the clinical syndromes associated with infection by various species is given in Table 1.

3.2. The method of choice for diagnosis of an enterovirus infection is the isolation and identification of the virus in cell cultures, or in mice.

(a) Isolation of viruses

The growth of viruses can be carried out on:

- cell cultures (all enteroviruses except most Coxsackie A types);
- suckling mice (Coxsackie A)

(b) Determination of virus species type should be done by:

- cross neutralization with intersecting pools of either monospecific sera or, in the future, of type-specific MAbs;
- solid-phase immunoassay with monospecific sera or, in the future, with type-specific MAb.

A special issue of importance in the enterovirus field is the intratypic serodifferentiation of poliovirus. The method recommended to be used is the neutralization index with strain specific MAbs. (See list of MAbs selected for this aim in WHO collaborative study on the use of MAbs for the intratypic serodifferentiation of poliovirus strains (Annex 2).

For the replacement of enterovirus type-specific intersecting pools and monospecific sera with MAb, the following is now proposed:

1. to list the enterovirus type-specific neutralizing MAb already existing in the world;
2. to list laboratories involved in enterovirus research having facilities to prepare the new hybridoma cell lines secreting MAbs with desired specificities.
3. to test the specificity of all these MAb so as to be able to make intersecting pools for the enterovirus type identification.

(c) Serodiagnosis

The demonstration of serum antibody increase in enterovirus infection is laborious and heterophil rises are frequently encountered. The usefulness of type-specific IgM antibody detection in the diagnosis of enterovirus has to be further evaluated.

3.3 Available reagents for identification of enteroviruses

Intersecting LBM pools (A through H and J through P) are available for distribution according to already agreed rules set down by WHO.

3.4 Because of their elaborate preparation and expensive quality control, reagents for enterovirus type identification are available only in limited quantities. The use of MAb may provide advantages with respect to both quality and quantity.

TABLE 1. CLINICAL SYNDROMES ASSOCIATED WITH INFECTIONS BY ENTEROVIRUSES

Polioviruses, types 1–3

- Paralysis (complete to slight muscle weakness)
- Aseptic meningitis
- Undifferentiated febrile illness, particularly during the summer

Coxsackieviruses, group A, types 1–24[a]

- Herpangina (types 2–6, 8, 10)
- Acute lymphatic or nodular pharyngitis (type 10)
- Aseptic meningitis (types 2, 4, 7, 9, 10)
- Paralysis (infrequently) (types 7, 9)
- Exanthem (types 4–6, 9, 16)
- Hand-foot-and-mouth disease (types 5, 10, 16)
- Pneumonitis of infants (types 9, 16)
- "Common cold" (types 21, 24)
- Hepatitis (types 4, 9)
- Infantile diarrhea (types 18, 20–22, 24)
- Acute hemorrhagic conjunctivitis (type 24)

Coxsackieviruses, group B, types 1–6

- Pleurodynia (types 1–5)
- Aseptic meningitis (types 1–6)
- Paralysis (infrequently) (types 2–5)
- Severe systemic infection in infants, meningoencephalitis and myocarditis (types 1–5)
- Pericarditis, myocarditis (types 1–5)
- Upper respiratory illness and pneumonia (types 4, 5)
- Rash (type 5)
- Hepatitis (type 5)
- Undifferentiated febrile illness (types 1–6)

Echoviruses, types 1–34[b]

- Aseptic meningitis (all serotypes except 12, 24, 26, 29, 32–34)
- Paralysis (types 2, 4, 6, 9, 11, 30; possibly 1, 7, 13, 14, 16, 18, 31)
- Encephalitis, ataxia, or Guillain-Barré syndrome (types 2, 6, 9, 19; possibly types 3, 4, 7, 11, 14, 18, 22)
- Exanthem (types 2, 4, 6, 9, 11, 16, 18; possibly 1, 3, 5, 7, 12, 14, 19, 20)
- Respiratory disease (types 4, 9, 11, 20, 25; probably 1–3, 6–8, 16, 19, 22)
- Others: Diarrhea (different types have been recovered; a consistent association has not been established)
  - Epidemic myalgia (types 1, 6, 9)
  - Pericarditis and myocarditis (types 1, 6, 9, 19)
  - Hepatic disturbances (types 4, 9)

Enterovirus, types 68–72[c]

- Pneumonia and bronchiolitis (type 68)
- Acute hemorrhagic conjunctivitis (type 70)
- Paralysis (types 70, 71)
- Meningoencephalitis (types 70, 71)
- Hepatitis (type 72)
- Hand-foot-and-mouth disease (type 71)

[a] Coxsackievirus A23 was never formally accepted as a new type, since it was found to be identical with the previously described echovirus 9.

[b] Echovirus type 10 was excluded from this group. It turned out to be a larger RNA virus and has been reclassified as the prototypic reovirus. Type 28 was reclassified as rhinovirus type 1; type 34 is related to coxsackievirus A24 as a prime strain.

[c] Since 1969, new enterovirus types have been assigned enterovirus type numbers rather than being subclassified as coxsackieviruses or echoviruses. The vernacular names of the previously identified enteroviruses have been retained.

* From: J. L. Melnick. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. pp 739-794 In: Virology; Edited by B.N.Fields, et al. Raven Press, New York, 1985.

3.5 Laboratories suggested for quality control of the reagents are:

1. Baylor College of Medicine, Houston, Texas, USA
2. Institute of Poliomyelitis and Viral Encephalitides, Moscow, USSR
3. Fairfield Hospital, Melbourne, Victoria, Australia
4. Department of Enteroviruses, National Institute of Health, Tokyo, Japan
5. Institute of Medical Biology Chinese Academy of Medical Sciences, Kunming, People's Republic of China
6. Statens Seruminstitut, Copenhagen, Denmark

The supply of available interesecting pools and monospecific sera is estimated to be enough for 15 years.

4. ARTHROPOD-BORNE VIRUSES

4.1 Arboviral diseases of high priority to WHO for application of rapid viral diagnostic techniques order of priority are:

(a) dengue (DEN)
(b) yellow fever (YF)
(c) Japanese encephalitis (JE)
(d) chikungunya (CHIK) and Igbo Ora
(e) tick-borne encephalitis (TBE)
(f) West Nile encephalitis (WN)
(g) Venezuelan encepalitis (VE)
(h) Ross River fever (RR)
(i) Phlebotomus fever
(j) Sindbis fever

Other diseases of lesser priority for development of reagents include California group encephalitis, Western encephalitis, Sao Paulo encephalitis, (Rocio virus), St Louis encephalitis, eastern encephalitis, group C fevers, group Guama fevers, Oropouche fever, Colorado tick fever, Murray Valley encephalitis, Bwawba fever, Germiston fever, Bunyamwera fever, Orungo fever, Zika fever, Ilesha fever, Tataguine fever and about 50 other diseases caused by arboviruses. Development of reagents for the haemorrhagic fever viruses is described in the report of another WHO meeting dealing with these viruses.[6]

4.2 Test procedures are recommended for rapid or early recognition of virus or antigen. In cases when such a reference procedure is not available, a technique for antibody detection is recommended.

(a) Dengue. Virus is isolated from acute phase serum in C6/36 clone of *Aedes albopictus* cells and identified by immunofluorescence or ELISA using MAbs for dengue types 1, 2, 3 and 4. Intrathoracic inoculation of mosquitos is an acceptable alternative to cell culture, as is inoculation of other cell lines such as AP61 or *Toxorhynchites* cells: IgM may be detected in acute phase sera using antibody capture ELISA (anti-mu chain).

(b) Yellow fever. Antigen is detected in blood and in liver using antigen capture ELISA with monoclonal or polyclonal antibody. This test is positive during the early phase of illness before the appearance of IgM. IgM antibody is detected in acute phase serum by antibody capture ELISA (anti-mu chain). Virus is isolated from acute phase serum and/or liver in C6/36 clone of *A. albopictus* cells, and identified by immunofluorescence or ELISA using polyclonal or MAb for yellow fever and other flaviviruses known in the

area. Isolation can be done at the bedside using whole blood as inoculum. Vero cells, $LLCMK_2$, C17 clone of A. aegypti cells, AP61 cells, baby mice and mosquitos are acceptable alternatives.

(c) Japanese encephalitis/West Nile. Antibodies are identified by IgM antibody capture ELISA of acute phase serum and CSF, followed in three to six days with second test of convalescent serum if first is negative. It should be noted that use of paired sera with detection of seroconversion by HI, PRNT or ELISA is still useful to confirm the diagnosis in the small proportion which may be missed with the IgM antibody capture technique.

(d) Chikungunya/Ross River/Sindbis. Virus is isolated from acute phase serum in baby mice in C6/36 mosquito cells, or Vero cells, then identified with polyclonal antibody by HI, CF, neutralization tests or ELISA.

(e) TBE. Antibody is identified by IgM antibody capture, ELISA of acute phase serum and CSF. ELISA kits from Immuno AG, Vienna are commercially available. MAbs have been developed for specific identification of TBE virus strains.

(f) Venezuelan encephalitis. Virus is isolated from acute phase serum in baby mice or Vero, duck embryo, or chicken embryo cell culture, then identified by ELISA, CF, HI or neutralization test using polyclonal mouse ascitic fluid.

(g) Phlebotomus fever. Antigen capture ELISA has been successfully applied to detect antigen in acute phase sera of patients infected with the Sicilian type. The IgM antibody capture ELISA is also applicable. These techniques have not yet been tried with other serotypes of phleboviruses but presumably will be found effective.

(h) Other viruses. The techniques for rapid diagnosis have not been developed for most of the other arboviruses.

4.3.3 The available reagents and their suppliers are:

Virus*	Antigen/serum	Supplier**
dengue	monoclonal dengue 1-4 antibodies	1,3
	antigen	1,2,3,5
	human hyperimmune serum	2
yellow fever	hyperimmune mouse ascitic fluid	1,3,4,10
	MAb	1
	YF mouse brain antigen	1,3,4,10
JE	human flavivirus hyperimmune serum (conjugated)	5
	alternate mouse ascitic fluid conjugated)	1,3,12
	hyperimmune mouse ascitic fluid	3,6
	JE mouse brain antigen or commercial vaccine	1.3.5.11.12
VEE	Hyperimmune mouse ascitic fluid	1,3,6
West Nile	Human flavivirus hyperimmune serum (conjugated)	5
	WN mouse brain antigen	3,12,13

TBE	IgG and IgM detection kit	14
	TBE monoclonal antibody	8
CHIK/RR/Sindbis	Polyclonal mouse ascitic fluid	3,12 (CHIK, Sindbis)
Phlebotomus fever	Sicilian mouse brain antigen	3
	Hyperimmune mouse ascitic fluid	3

Cell line, mosquito, human sera	Supplier
Mosquitos	locally available
C6/36 *A. albopictus*	1,2,3,4,9
Vero (E6 clone)	1,3,4,9
AP61 *A. pseudoscutellaris*	1,4,9,10
C17 *A. aegypti*	4
human flavivirus antibody negative serum	7
human flavivirus positive serum	5

* These viruses are available from the ATCC and from WHO Collaborating Centres.

** Suppliers:
1. Division of Vector-borne Viral Diseases, CDC, Fort Collins, Colorado, USA
2. Virus Research Institute, Bangkok, Thailand
3. Yale Arbovirus Research unit, New Haven, USA
4. Institut Pasteur, Dakar, Senegal
5. AFRIMS Laboratory, Bangkok, Thailand
6. NIH Research Reference Reagents Program, Washington, DC, USA
7. WRAIR, Washington, DC, USA
8. Ivanovsky Institute, Moscow, USSR.
9. ATCC, Rockville, MD, USA
10. Caribbean Epidemiology Centre (CAREC), Port-of-Spain, Trinidad
11. National Institute of Health, Tokyo, Japan
12. National Institute of Virology, Pune, India
13. National Institute of Health, Islamabad, Pakistan
14. IMMUNO AG, Vienna, Austria

4.4 Main problems

4.4.1 Positive and negative control sera for IgM and IgG. Positive control human sera are available only in geographically local areas and only in small amounts because of the increasing use of filter paper collection. WHO should solicit cooperation of national laboratories, collaborating centres and other organizations to collect large quantities of sera and deposit them in WHO collaborating laboratories for lyophilization, storage and distribution.

4.4.2 Positive sera for high titered flavivirus antibody to be conjugated with horseradish peroxidase. These sera are not readily available in large quantities. Sera from persons recovered from dengue haemorrhagic fever have been found ideal for this purpose. The meeting encourages WHO to help in finding donors.

4.4.3 Distribution of reagents. The reagents for rapid diagnosis are often held for prolonged periods in local customs and quarantine facilities. WHO, through its Programme Coordinators, should obtain special regulations to permit free and rapid passage of reagents through local customs.

4.5 Minimal requirements for reagents. The supplying institution will provide positive and negative controls and will be responsible for internal quality control of reagents. The supplier will provide information on the limits of the test procedure and reagents including, where available, potency and specificity, absence of non-specific reactions, and freedom from adventitious agents. The reagent will be tested independently in one or more WHO collaborating centres for reference and research or other suitable laboratories. The recipient will assume responsibility for retesting to ensure absence of specific viral infectivity in the case of inactivated antigens.

Handling of acute phase sera and other infected patient tissues should be in accordance with specified guidelines.[7] The recipient should provide adequate feed-back and, where discrepancies are encountered, should submit aliquots to the reference laboratory for further study.

The minimal titre for an antibody preparation will be at least four-fold higher than its non-specific reactivity.

Cell lines will be free of mycoplasma and specific contaminant viruses such as alphaviruses and lymphocytic choriomeningitis virus.

4.6 Suggested quality control laboratories are:

1. Division of Vector-borne Viral Diseases, CDC, Fort Collins, Colorado, USA.
2. Virus Research Institute, Bangkok, Thailand.
3. Yale Arbovirus Research Unit, New Haven, Connecticut, USA.
4. Institut Pasteur, Dakar, Senegal.
5. AFRIMS Laboratory, Bangkok, Thailand.
6. USAMRIID, Frederick, Maryland, USA.
7. Walter Reed Army Medical Research Institute for Infectious Diseases, Washington, USA
8. Ivanovsky Institute, Moscow, USSR.
9. Caribbean Epidemiology Centre, Port-of-Spain, Trinidad.
10. National Institute of Health, Tokyo, Japan.
11. National Institute of Virology, Pune, India.
12. National Institute of Health, Islamabad, Pakistan
13. Department of Virology, University of Vienna, Vienna, Austria.

4.7 Possible centres for reagent production include:

(a) Centres already producing on a regular basis: Vector-borne Viral Diseases Division, Centers for Disease Control, Fort Collins, Colorado, USA; Yale Arbovirus Research unit, New Haven, Connecticut, USA.

(b) Producing centres which need expansion and strengthening:

1. Virus Research Institute, Bangkok, Thailand.
2. Institut Pasteur, Dakar, Senegal.
3. AFRIMS Laboratory, Bangkok, Thailand.
4. Ivanovsky Institute, Moscow, USSR.
5. Caribbean Epidemiology Centre, Port-of-Spain, Trinidad.
6. National Institute of Health, Tokyo, Japan
7. National Institute of Virology, Pune, India.
8. National Institute of Health, Islamabad, Pakistan
9. Department of Virology, University of Vienna, Vienna, Austria.
10. Biofarma, Bandung, Indonesia.
11. Institute of Poliomyelitis and Viral Encephalitides, Moscow, USSR

(c) Centres with potential for production: University of Malaya, Kuala Lumpur, Malaysia; University of Ibadan, Ibadan, Nigeria; Virus Research Institute, Entebbe, Uganda; Institute of Virology, Beijing, China; Instituto Evandro Chagas, Belem, Brazil; Institut Pasteur, Abidjan, Ivory Coast.

Not all laboratories interested in and capable of production of reagents are listed here. WHO should announce its intention to support local reagents production and invite laboratories to contact the Organization. Then WHO should assess the laboratories' capability to produce reagents and provide training.

5. HERPESVIRUSES, RUBELLA, MEASLES, AND MUMPS

5.1 The herpes group of viruses (herpes simplex viruses, cytomegalovirus, varicella zoster virus and Epstein Barr virus), and rubella, measles and mumps viruses were discussed.

5.2 Definition of working techniques to be used:

	HSV	CMV	VZV	EBV	Rubella[a]	Measles	Mumps[b]
Antigen/virus detection							
IF	2		2				
EIA	2		2				
isolation	3	3	1		1		1
EM	1	1	1				
Antibody detection							
IF		1	1	3(IgM)			
EIA	2	3(IgM)	2		3(IgM)	3(IgM)	3(IgM)
SPIT[c]					2(IgM)		
CF	1	1	1			2	
HI					1		
RPHA[d]		2			1		

[a] Congenital infections
[b] Cerebrospinal fluid specimens
[c] Solid phase immune haemagglutination test
[d] Passive haemagglutination

3 = highest priority technique
2 = medium
1 = lowest

5.3 Available reagents

(a) Commercially available.

Various firms produce diagnostic reagents some of them include:

1. For antigen detection

	Producer	Method
HSV	MA Bioproducts, USA	IF
	Biosoft, France	IF, EIA
	Dakopatts A/S, Denmark	EIA, EIA KIT
	Ortho Diagnostics Systems, USA	IF
	Wellcome Diagnostics, UK	IF
V-Z	Flow Laboratories, USA	IF

2. For antibody detection

HSV, IgM and IgG	Behringwerke AG, FRG	EIA
IgM and IgG	Gull Laboratory, USA	IF
CMV IgM	Biosoft, France	IF
	Behringwerke AG, FRG	EIA
	Gull Laboratory USA	IF
	Merck, FRG	EIA
VZV IgM	Biosoft, France	IF
	Gull Laboratory, USA	IF
BV IgM	- do -	IF
Rubella IgM	Behringwerke AG, FRG	EIA
	Abbott, USA	EIA
	Northumbria, UK	EIA
Measles IgM	Behringwerke AG, FRG	EIA
Mumps IgM	- do -	EIA

* It should be noted that this list is not complete and is provided to give examples only.

(b) Reference reagents:

	Antigen	Antiserum
CMV	n.a.	IgG, IgM positive human pools*
VZV	n.a.	IgM**
Rubella	n.a.	IgG***

* IgM reference preparation is available in limited supply from the Paul Ehrlich Institute, Frankfurt, FRG. A larger supply is in preparation under the auspices of the European Group for Rapid Viral Diagnosis.

** National Bacteriological Laboratory, Stockholm, Sweden.

*** IgG reference preparation available from Central Public Health Laboratory, Colindale, London, UK.

(c) Non-commercial locally produced working reagents are not available for large-scale distribution for any of the viruses discussed in this group. Limited supply may be available through direct contact with producing laboratories.

5.4 Main problems concerning reagents for diagnosis

Commercially produced reagents for antigen and antibody detection of HSV are satisfactory. However, immunoreagents, preferably MAbs, for EIA detection of CMV and VZV are highly desirable. Also, enzyme-labelled viral antibody or antigen for the detection of IgM antibody to CMV, rubella, measles, and mumps by EIA u-capture techniques would be very useful.

5.5 List of suggested quality control laboratories

Quality control of commercially and locally produced reagents is required for each reagent. Control should preferably be carried out in two independent laboratories. The following laboratories have been identified as possible control laboratories for the viral reagents discussed in this group:

HSV - Statens Seruminstitut, Copenhagen, Denmark.
Emory University, Atlanta, Georgia, USA.
CMV - Stockholm County Council Microbiological Laboratory, Stockholm, Sweden.
VZV - Dept of Virology, University of Turku, Turku, Finland.
EBV - Virus Department, National Bacteriological Laboratory, Stockholm, Sweden.
Rubella - Virus Diagnostic Institute, Stuttgart, FRG.
Viral Diseases Division, CDC, Atlanta, Georgia, USA.
Measles - National Bacteriological Laboratory, Stockholm, Sweden.
Dept of Virology, University of Turku, Turku, Finland.
Mumps - Statens Seruminstitut, Copenhagen, Denmark.
National Bacteriological Laboratory, Stockholm, Sweden.

5.6 Possible centres for reagents production

Laboratories interested in, and capable of production of reagents cannot be identified here. Therefore, WHO should announce its intention to support local reagent production and invite laboratories to contact the Organization. The next step would be to assess the laboratory's capability to produce reagents and provide training.

6. SUGGESTED STRATEGY FOR THE PRODUCTION AND DISTRIBUTION OF WORKING REAGENTS

It is proposed that WHO continues the provision of reagents for the time being and also when the anticipated benefits of new techniques including techniques based on the use of MAbs, are achieved. The arrangements for the distribution of reagents will depend on the structure of laboratory services in different countries but, as a rule, the central national laboratories would be responsible for local distribution. Further, it is suggested that WHO, when making definite plans for production and quality control of reagents, should consider:

(a) The promotion and educational support of laboratories producing certain reagents for the whole region.

(b) The promotion of laboratories working independently for quality control of reagents.

(c) Agreement on reference preparations for monitoring regionally produced reagents (possibly in collaboration with the European, Pan American and Asian Groups for Rapid Viral Diagnosis and their commercial sources).

(d) Funds should be made available for this scheme.

Because of the limited amount of reagents available and in order to obtain comparable results from different laboratories, the distribution of reagents should be done under the following conditions:

- a detailed technical protocol of the use, the interpretation of results and possible pitfalls should accompany each working reagent;
- the working reagent has to be provided but only to laboratories making a written application, including a justification for its specific use (particular epidemiological conditions, field trials, etc.);
- the quantity of reagents to be sent should correspond to the real need of the laboratory;
- a report on the results obtained should be recommended so as to document the usefulness of the reagent, the standardization of its use and any resulting interesting epidemiological information.

7. Support for laboratories to produce working reagents for virus rapid diagnosis

Centres for reagent production must have a reasonable level of equipment, appropriate laboratory accommodation and trained personnel. WHO funding can then be directed towards defined objectives with maximum efficiency.

The group recommends that WHO implements a plan to train personnel in the production and distribution of working reagents for rapid viral diagnosis. Training may be accomplished by:

(a) Individual training for a period of at least 3-6 months of supervisory professional scientists in one or more of the centres listed above as quality control laboratories.

(b) This training should be followed within six months by the participation of the trainee in a collaborative study with coded unknown diagnostic specimens.

(c) If indicated, a WHO short-term consultant should visit the trainee's local laboratory to reorient and retrain where deficiencies are found. In selected cases, training in the local laboratory may be preferred to training in a central laboratory.

(d) WHO should organize training courses for the production of reagents. Short-term regional training courses to introduce improved and new techniques for production should be given periodically to former trainees. The person who is going to produce the reagent should be the individual accepted for the training course. Emphasis in the training course should preferably be on the production of working reagents and not on a single technique.

(e) Training should be followed by supplying reference and working reagents. In regions where the disease is endemic or epidemic, the scientists should be trained to establish adequate facilities and to prepare their own working reagents.

(f) In each case, adequate financial support should be provided to the trainee on return to the local laboratory to purchase equipment and supplies to enable him to implement the plan.

8. RECOMMENDATIONS TO WHO

1. WHO should continue to direct resources in the rapid diagnosis of viral infections. This will require a major effort to fund the production of high quality reagents.

2. WHO should promote the training required for the production of working reagents. Training in an accredited laboratory should be supported by funding to permit cooperation between the two laboratories. The training should be directed towards the production of a specified reagent rather than towards training in a particular laboratory technique.

3. Collaborative centres should be available to interested scientists from other laboratories for training in production and standardization of reagents for rapid viral diagnosis. The working reagents produced should be available for the scientists to take back to their laboratory.

4. IgM detection is based principally on the recapture technique on a solid phase. WHO should develop a plan for quality control of available commercial anti-μ chain preparations in order to identify good products for reference purposes. WHO should negotiate with the producer the lowest possible price for purchase of the selected anti-μ preparations to use as working reagents. WHO should develop a similar plan for evaluation and designation of reference plastic microtitre plates or strips.

5. WHO should encourage production and distribution of enzyme labelled viral antibody/antigen for the anti-μ capture assays for major virus infections.

6. WHO should encourage collection of sera from convalescent patients with e.g. dengue, for use as high titre antibody for conjugation with enzyme.

7. Hybridomas secreting antibody used in recommended techniques for rapid diagnosis of viral infections should, where possible, be maintained in WHO collaborating centres and made available to requesting laboratories.

8. WHO should support the establishment of reference preparations. These preparations should include:

(a) stable preparations of known viral antigen concentration;

(b) a sufficient quantity (approximately 200-300 ml) of human serum containing high titre IgM by ELISA representing each of the major virus pathogens should be collected, lyophilized and made available through the collaborating centres. Similar sera containing IgG should also be collected.

9. The reagents for rapid diagnosis are often held for prolonged periods in local customs and quarantine facilities. WHO, through its respective WPCs, should obtain special regulations to permit free passage of reagents through local customs and quarantine.

10. WHO and its collaborating centres should assume responsibility for receiving requests for reagents or viruses and act as the intermediary for forwarding the reagents to requesting laboratories.

11. Diagnostic IgM antibody capture ELISA kits for flavivirus encephalitis, especially Japanese encephalitis (JE), should be assembled under WHO auspices. The Collaborating Centres for Arbovirus Reference and Research should be enlisted to field-test the kits. The JE kits should then be distributed to sub-regional health facilities in the South-East Asian and Western Pacific Regions.

12. WHO should assist in the development of rapid diagnosis schemes of similar clinical syndromes to improve epidemiological and clinical studies.

13. WHO should help to establish minimum requirements for viral diagnostic reagents and control procedures.

9. RESEARCH RECOMMENDATIONS

1. Support the development of non invasive rapid techniques for diagnosis of HSV encephalitis rhinovirus infections.

2. Improve the sensitivity of EIA detection of HSV, particularly HSV-2 in genital specimens.

3. Support the development of EIA and DNA and RNA and hybridization techniques for e.g. CMV and enterovirus detection.

4. Support the development of immunoreagents for VZV antigen detection by EIA.

5. Efforts should also be directed towards the selection of appropriate hybridomas in the case of those viruses where reagents are not yet widely available.

6. Efforts should be also be directed towards the selection of appropriate hybridomas in the case of those viruses where reagents are not yet widely available or where replacements would be beneficial, e.g. respiratory viruses and enteroviruses.

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ANNEX 1.

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ANNEX 2

WORKING GROUP ON RESEARCH ON DEVELOPMENT OF
POLIOMYELITIS VACCINE USING MODERN BIOTECHNOLOGIES

Geneva, 17-19 April 1984

WHO COLLABORATIVE STUDY ON THE USE OF MONOCLONAL ANTIBODIES FOR THE INTRATYPIC DIFFERENTIATION OF POLIOVIRUS STRAINS

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At a meeting of the WHO informal group on the characterization of poliovirus strains held at NIBSC in March 1982, it was agreed that a panel of monoclonal antibodies should be evaluated for the intratypic differentiation of poliovirus strains. The aim of the study was to identify monoclonal antibodies which could reliably differentiate strains with T_1-oligonucleotide maps similar or identical to Sabin vaccine virus from unrelated wild strains in neutralization assays.

Six laboratories submitted 31 monoclonal antibodies. After preliminary studies at NIBSC,18 antibodies were selected for further study. In addition, absorbed polyclonal antisera provided by Dr van Wezel were used in the studies. For each poliovirus type, the virus strains included Sabin vaccine virus, at least two strains which gave T_1 maps closely similar to that of Sabin vaccine virus, strongly suggesting they are vaccine derivatives, and two strains unrelated to vaccine virus.

These studies indicated that for poliovirus types 2 and 3 monoclonal antibodies have been obtained which can differentiate poliovirus strains with T_1-oligonucleotide maps similar or identical to that of Sabin vaccine virus from wild strains with unrelated maps. In addition, for poliovirus type 2, monoclonal antibodies have been obtained which react only with strains of poliovirus with maps identical to that of Sabin vaccine virus.

For the type 1 viruses, monoclonal antibodies which neutralized Sabin vaccine virus failed to react with two virus strains whose T_1-oligonucleotide map differed from the Sabin map by only 2 spots. Thus lack of reactivity of viruses with the Sabin specific monoclonal antibodies in the study does not necessarily mean that a type 1 strain was not derived from Sabin vaccine. In this instance, the absorbed polyclonal sera were a more reliable indicator of a Sabin relationship since the Sabin-like serum reacted with all the strains having Sabin related T_1-oligonucleotide maps and the non-Sabin-like serum failed to neutralize only the Sabin vaccine strain.

Recommendations

The results of the present collaborative study indicated that monoclonal antibodies are of potential use in the characterization of poliovirus isolates as likely to have been derived from Sabin vaccine virus. Based on the limited collection of strains examined, it was concluded that

Poliovirus type 1: (i) Monoclonal antibodies M1.2 and M1.4 could be used to determine whether a strain is antigenically identical to Sabin vaccine virus.

(ii) Until suitable monoclonal antibodies are identified and evaluated, Sabin specific absorbed sera (SL) should continue to be used to identify poliovirus type 1 strains as being derived from Sabin vaccine virus.

(iii) Monoclonal antibody M1.7 is broadly reactive and could be of use for typing isolates.

Poliovirus type 2: (i) Monoclonal antibody M2.1 could be used to identify strains as antigenically identical to Sabin vaccine virus. It reacted only with strains giving T_1-oligonucleotide maps identical to those of Sabin virus.

(ii) Monoclonal antibodies M2.3 and M2.4 could be used to identify strains which are derived from Sabin vaccine virus.

(iii) Monoclonal antibodies M2.5, M2.6 and M2.7 are broadly reactive and could be of use for the typing of isolates.

Poliovirus type 3: (i) Monoclonal antibody M3.1 could be used to identify strains which are derived from Sabin vaccine virus. Antibodies M3.2 and M3.3 could also be used although some participants reported low titres against the wild strains.

(ii) Monoclonal antibodies M3.4 and M3.5 are broadly reactive and could be used for typing isolates.

The above mentioned monoclonal antibodies should be subjected to more extensive field evaluation with a wide range of well characterized poliovirus strains. In addition collaborating laboratories may wish, for a limited period, to continue to compare data obtained with monoclonal antibodies to poliovirus 2 and 3 with results obtained with absorbed sera for above serotypes. Where unexpected or ambiguous results are obtained the strains in question should be submitted to a specialist laboratory for T_1-oligonucleotide mapping.

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