

Guidelines on Establishment of Virology Laboratory in Developing Countries

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Preface

Emerging infectious diseases caused by viruses have assumed great public health significance in the recent past. During the last three decades, almost 20 new viral pathogens have been detected. Some of these—e.g. human immunodeficiency virus (HIV) and hepatitis viruses—have already caused substantial mortality, morbidity and economic loss all over the world. The pandemic of serious acute respiratory syndrome (SARS) unequivocally demonstrated the rapidity with which new viruses can travel across the world and inflict misery. The threat of a pandemic with one of the subtypes of influenza virus is considered the greatest public health challenge in the current millennium so far.

Laboratories play a critical role in surveillance, diagnosis and monitoring of viral disease as well as in the understanding of the genetic changes in the viral genome. Laboratory tools are also essential for the diagnosis of dengue fever and chikungunya fever—two of the important vector-borne viral diseases—and for differentiating from other fevers for institution of rational and specific therapy and control measures.

Establishment of a reliable viral laboratory is a prerequisite for a strong public health response to emerging viral diseases. Unfortunately, several developing countries lack adequate capacity to diagnose viral infections. These guidelines are therefore intended to provide the minimum requirements for establishing a national virology laboratory, keeping in view the emergence of new viral pathogens. It is not meant to serve as a comprehensive laboratory manual for diagnosis of viral infections.

These guidelines cover key aspects of establishing a virology laboratory in a developing country and address issues pertaining to policy and programme, infrastructure, human resources, technologies available, and creating high-quality systems.

We hope that these guidelines provide fundamental information to the policy-makers and laboratory directors in developing countries to aid them in establishing virology laboratories in their respective countries, thereby enhancing national self-reliance in this area.

Abbreviations

ACD	Anticoagulant citrate dextrose
BSC	Biological safety cabinet
BSL	Biosafety level
CMV	Cytomegalovirus
CPE	Cytopathic effect
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ECHO	Enteric cytopathic human orphan
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
FA	Fluorescent antibody
FBC	Full blood count
GMT	Good microbiological techniques
HEPA	High efficiency particulate antigen
HHV6	Human herpes virus
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
IHR	International Health Regulations
JE	Japanese encephalitis
PCR	Polymerase chain reaction
QC	Quality control
RNA	Ribonucleic acid
SARS	Severe acute respiratory syndrome
SOP	Standard operating procedures
VTM	Viral transport medium
VZV	Varicella zoster virus

Chapter 1

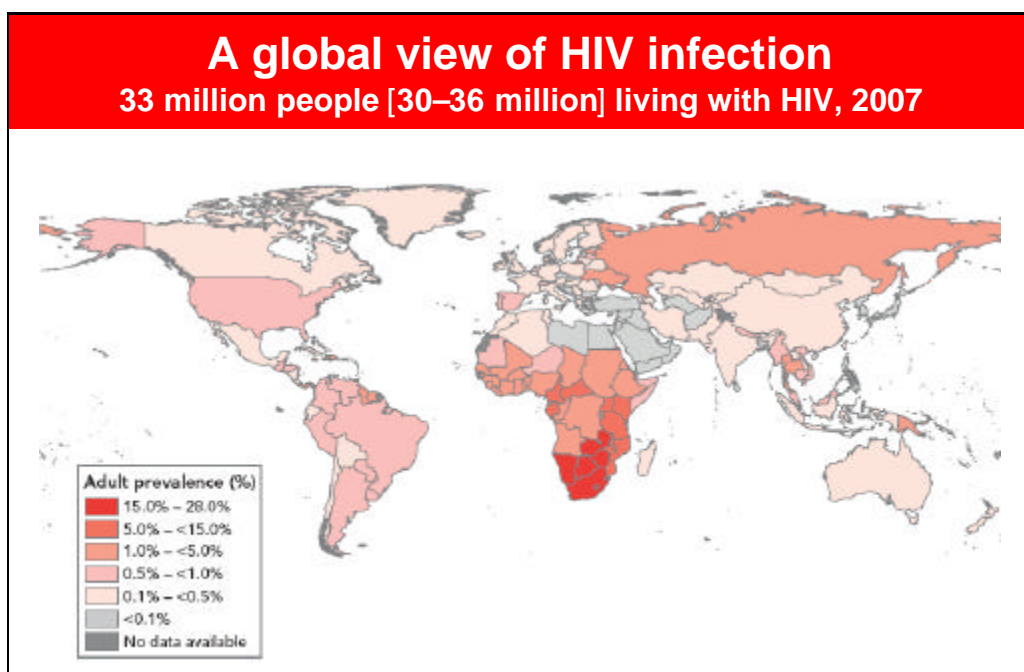
Introduction

Diseases caused by viruses have assumed great public health significance in the recent past and an increase in the frequency and spread of such diseases observed globally. Several new viruses have been isolated. During the past three decades, of the 30 new pathogens discovered, 16 are viruses (Table 1.1). These emerging viruses have added a new paradigm to public health concepts. Not only the health but the economic and social fabric of global communities have been affected by viruses such as HIV, hepatitis B and C, severe acute respiratory syndrome (SARS) and avian influenza.

Table 1.1: Newly discovered viruses of public health importance

Year	Virus
1973	Rotavirus
1975	Parvovirus B-19
1977	Ebola virus
1977	Hantaan virus
1980	Human T-lymphotropic virus I (HTLV-I)
1982	HTLV-II
1983	Human immunodeficiency virus
1988	Hepatitis E virus
1989	Hepatitis C virus
1991	Guanarito virus
1993	Sin nombre virus
1994	Sabia virus
1995	Human Herpes virus (HHV-8)
1999	Nipah virus
2002	SARS virus
2003	Influenza virus H5N1

Ever since its discovery, the human immunodeficiency virus (HIV) has emerged as a global disaster. Around the world AIDS has led to more than 20 million deaths. Over 33 million people are living with HIV today and by 2010 it is estimated that more than 40 million children will have one or both parents dead from AIDS. People in productive age groups are predominantly affected by AIDS and hence in some countries the impact of AIDS has led to a major decrease in their respective gross national products. Till date the only tool available to ascertain the presence of HIV in an otherwise healthy looking individual is a laboratory assay.



Source: 2008 Report on the global AIDS epidemic

The beginning of the millennium saw the appearance of Nipah virus in Malaysia which caused the deaths of 100 people in the country as well as significant economic losses due to the slaughter of pigs. It is estimated that the outbreak cost was nearly USD 500 million. Subsequently, Nipah virus outbreaks were identified in India and Bangladesh.

In 2003, SARS caused by a coronavirus inflicted a major impact on health, tourism and travel in many countries. In a very short period this virus caused 7761 probable cases and 623 deaths in 28 countries. SARS demonstrated the potential of a new virus for a rapid spread, high infectivity, huge mortality and the inability of health systems to mount rapid and effective response because of nonavailability of

diagnostic services. The isolation of the virus and understanding its genetic characteristics were major contributors in curtailing the spread of this disease.

Outbreaks of highly pathogenic influenza A (H5N1) occurred in Asia beginning in early 2004. As on October 2008, 387 cases with 245 deaths have been reported from 15 countries (Table 1.2). In Indonesia, the case fatality ratio is as high as 85%. More than 250 million birds have either died from the disease or were culled in efforts to contain the outbreaks. The world continues to live under the threat of a pandemic of influenza. Laboratory support is critical not only to detect the presence of the virus in clinical material and birds but, more importantly, to understand the genetic changes in the viral genome which shall be the harbinger of the pandemic.

Table 1.2: Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO

Country	2003		2004		2005		2006		2007		2008		Total	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D
Azerbaijan	0	0	0	0	0	0	8	5	0	0	0	0	8	5
Bangladesh	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Cambodia	0	0	0	0	4	4	2	2	1	1	0	0	7	7
China	1	1	0	0	8	5	13	8	5	3	3	3	30	20
Djibouti	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Egypt	0	0	0	0	0	0	18	10	25	9	7	3	50	22
Indonesia	0	0	0	0	20	13	55	45	42	37	20	17	137	112
Iraq	0	0	0	0	0	0	3	2	0	0	0	0	3	2
Lao PDR	0	0	0	0	0	0	0	0	2	2	0	0	2	2
Myanmar	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Nigeria	0	0	0	0	0	0	0	0	1	1	0	0	1	1
Pakistan	0	0	0	0	0	0	0	0	3	1	0	0	3	1
Thailand	0	0	17	12	5	2	3	3	0	0	0	0	25	17
Turkey	0	0	0	0	0	0	12	4	0	0	0	0	12	4
Viet Nam	3	3	29	20	61	19	0	0	8	5	5	5	106	52
Total	4	4	46	32	98	43	115	79	88	59	40	30	391	247

Note: Total number of cases includes number of deaths.
WHO reports only laboratory-confirmed cases.
All dates refer to onset of illness.

Apart from new viruses, several other viruses—especially those that cause dengue fever and chikungunya fever—have emerged as major public health issues. Both these diseases are vector-borne and have a complex epidemiology. Laboratory tools are essential for their diagnosis and for differentiating from other fevers for institution of rational and specific therapy.

It is in this context that the establishment and strengthening of existing national virology laboratories gains paramount importance. A national viral surveillance system needs to be established. The epidemiology of virus diseases needs to be studied in depth. The need to be able to easily, cheaply and quickly diagnose these and other potential outbreaks of viral infections as public health problems in specific geographic regions, in addition to travel and bio-terrorism concerns, is crucial. Apart from development of diagnostic reagents and their supply to investigating centres, a central serum bank, and a virus repository are important factors. Research on viruses, as regards the epidemiology, diagnosis, pathogenesis and vaccinology of virus infections, needs to be strengthened. An international network of databases of virus infections, needs to be instituted. A global network for the diagnosis and containment of emerging viral diseases is the need of the hour. This document has therefore been conceived and developed to provide to policy makers, administrators and public health professionals in developing countries, an overview of the requirements for establishing a national virology laboratory.

Diagnostic virology is also rapidly moving into the mainstream of clinical medicine as a result of the convergence of several independent developments. First, dramatic progress in antiviral therapeutics has increased the need for specific viral diagnosis. Second, technological developments, particularly in the area of nucleic acid chemistry, have provided important new tools for viral diagnosis. Third, the number of patients at risk accruing opportunistic viral infections has expanded greatly as a result of the HIV/AIDS epidemic. Finally, modern management of HIV infection and hepatitis C is providing a new paradigm for the integration of molecular techniques into management of chronic viral infections. All these developments are not only increasing the use of diagnostic virology techniques but have enabled the detection of a variety of emerging pathogens that pose a threat to mankind. These guidelines are therefore intended to provide the minimum requirements for establishing a national virology laboratory keeping in view the emergence of new viral pathogens. It is not meant to serve as a comprehensive laboratory manual for diagnosis of viral infections.

Scope

These guidelines cover key aspects of establishing a virology laboratory in a developing country and address issues pertaining to policy and programme, infrastructure, human resources, technologies available, and high-quality systems. The guidelines do not describe the technique for processing the specimens because every laboratory has to develop its own standard operating procedures (SOP). Also, various standard textbooks are available for these techniques.

These guidelines shall principally assist the national laboratory programmes in expanding their diagnostic profile and should be used in conjunction with any other national directives on International Health Regulations (IHR 2005) or disease surveillance programmes.

Guideline development process

The WHO Regional Office for South-East Asia commissioned the Department of Virology, National Institute of Mental Health and Neurosciences, Bangalore, India (NIMHANS) to develop the first draft of the guidelines. The objectives were to assist developing countries in establishing practical diagnostic facilities for viral diseases based upon a pragmatic approach and available scientific evidence.

The draft guidelines were first reviewed by WHO and subsequently peer reviewed by two eminent virologists from Sri Lanka and Thailand.

The guidelines were revised by NIMHANS as per the observations made by the peer reviewers.

The guidelines also provide information for the procurement of products that can be used in laboratories. However, the information is only suggestive and WHO does not specifically endorse these products.

Guidelines development team

WHO wishes to acknowledge the support provided by various experts in drafting, reviewing and finalization of the guidelines (please see list in Annex 2).

Chapter 2

Key elements of a virology laboratory

The key elements for the establishment of a virology laboratory and diagnostic services are:

- (1) Physical infrastructure
- (2) Human resources
- (3) Equipment and supplies

Physical infrastructure

Virus isolation and a number of methods for detection of viral antigens, nucleic acids, and antibodies (serology) are the core repertoire of techniques used in a diagnostic virology laboratory. Virus isolation using cell culture is always performed in designated virology laboratories although the other methods may be performed in diverse laboratory settings such as clinical microbiology, serology, blood bank, clinical chemistry, pathology or molecular biology. In future, the likelihood of viral diagnostic testing being conducted outside the traditional virology laboratory setting is likely to increase as rapid diagnostic techniques based on immunologic and nucleic acid detection methods gain greater acceptance.

A diagnostic virology laboratory should ideally be located in a separate, multi-storied building. If this is not possible, it must be separated from other areas and facilities that are open to unrestricted staff movement within the building. It is therefore ideal to have the virology laboratory situated at the end of a corridor in a building where other laboratories are located. This would restrict entry of visitors, prevent contamination and facilitate maintaining biosafety standards.

Biosafety requirements

Micro organisms have been divided into four categories (Box 1) according to the risk posed to individuals and communities.

Box 1: Classification of infective micro organisms by risk group

Risk Group 1 (no or low individual and community risk)

- A microorganism that is unlikely to cause human or animal disease.
- Example - Adeno-associated virus (AAV) types 1-4

Risk Group 2 (moderate individual risk, low community risk)

- A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment.
- Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
- Examples include herpes viruses, foot-and- mouth disease viruses, adenoviruses and unconventional slow viruses.

Risk Group 3 (high individual risk, low community risk)

- A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
- Examples include human immunodeficiency virus (HIV), hepatitis B virus (HBV), hantaviruses, Japanese encephalitis virus (JEV), rabies, rift valley fever and yellow fever virus

Risk Group 4 (high individual and community risk)

- A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.
- Examples include Lassa fever, filoviruses, smallpox, Crimean-Congo haemorrhagic fever, avian influenza viruses, Nipah virus, Russian spring-summer encephalitis and Kyasanur forest disease viruses.

The biosafety infrastructure must be designed on the basis of risk assessment for handling specific pathogens. An agent that is assigned to Risk Group 2 may generally require Biosafety Level 2 facilities, equipment, practices and procedures for safe conduct of work. The desired biosafety levels are established on the basis of professional judgement based on risk assessment.

The general relation of risk groups to biosafety levels, practices and equipment is shown in Table 2.1.

Table 2.1: Relation of risk groups to biosafety levels, practices and equipment (WHO Manual on Biosafety, 2004)

Risk group	Biosafety level	Laboratory type	Laboratory practices	Safety equipment
1.	Basic-in Biosafety Level 1	Basic teaching, research	Good microbiological techniques (GMT)	None; open bench work
2.	Basic-in Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus biological safety cabinet (BSC) for potential aerosols
3.	Containment-in Biosafety Level 3	Special diagnostic services, research	Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4.	Maximum containment-in Biosafety Level 4	Dangerous pathogen units	Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

Diagnostic virology laboratories must be designed for Biosafety level 2 (BSL 2) or above and special attention should be paid to conditions that are known to pose biosafety problems. It is desirable that every country have at least one national laboratory that is equipped with Biosafety level 3 (BSL 3) facility especially because most of the viral pathogens that have emerged in the recent past have been agents that require BSL 3 facilities for handling specimens and cultures. However, if resources are limited, a BSL 2 laboratory with negative pressure (BSL 2) is essential to handle most emerging pathogens.

Table 2.2 provides a comparative summary of the essential infrastructure requirements for laboratories with respect to various biosafety levels.

Table 2.2: Summary of biosafety level requirements

	Biosafety level			
	1	2	3	4
Isolation ^a of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
• inward airflow	No	Desirable	Yes	Yes
• controlled ventilating system	No	Desirable	Yes	Yes
• HEPA-filtered air exhaust	No	No	Yes/No ^b	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	Yes	Yes
Airlock with shower	No	No	Optional	Yes
Anteroom	No	No	Yes	-
Anteroom with shower	No	No	Yes/No ^c	No
Effluent treatment	No	No	Yes/No ^c	Yes
Autoclave:				
• on site	No	Desirable	Yes	Yes
• in laboratory room	No	No	Desirable	Yes
• double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personal safety monitoring capability ^d	No	No	Desirable	Yes
Viruses that can be handled		herpes viruses (HSV, VZV, EBV, CMV and HHV6), measles, mumps, HTLV, rubella, hepatitis A-E viruses, dengue virus, enteroviruses (polio, Coxsackie, ECHO and entero)	Arboviruses (JE, West Nile, Chikungunya), Rabies, Nipah, Chandipura, HIV and Avian influenza.	Marburg, Ebola, Lassa, Kyasanur Forest disease, Small pox virus

^a Environmental and functional isolation from general traffic

^b Dependent on location of exhaust

^c Dependent on agent(s) used in the laboratory.

^d For example, window, closed-circuit television, two-way communication.

A typical biosafety level 3 laboratory

The laboratory is separated from general traffic flow and accessed through an anteroom (double door entry or basic laboratory – Biosafety Level 2) or an airlock. An autoclave is available within the facility for decontamination of wastes prior to disposal. A sink with hands-free operation is available. Inward directional airflow is established and all work with infectious materials is conducted within a biological safety cabinet.

In principle, unless the recommended biosafety level facilities are available the laboratory should not handle a particular virus. In situations where there is an outbreak of a viral illness and the required biosafety levels are not available, it is advisable to collect and refer the specimens to the nearest laboratory in the Region that has the required BSL laboratory. If specimens are collected from patients who are suspected to be suffering from viral infections caused by agents which need BSL 3 facilities (e.g. avian influenza) it is advisable that they be directly forwarded to the reference laboratory which has facilities for handling such agents.

Apart from the biosafety requirements mentioned above, the following are some of the essential features that need to be incorporated in the design of a virology laboratory.

- Adequate space must be provided for the safe conduct of laboratory work and for cleaning and maintenance. Designated cubicles, rooms or areas should be available to carry out different activities, e.g. office rooms, specimen collection cubicle, specimen reception and processing room, serology laboratory, cell culture cubicle, molecular diagnostic laboratory comprising three cubicles, cold room, dark room for fluorescent microscopy, common equipment room, media preparation room, washing and sterilization section, store room, toilets and lunch room.
- Each laboratory room should have space for housing a BSL 2 cabinet, one workbench, a sink, discard bins, wall cabinets to store consumables, a centrifuge, an incubator and a refrigerator.
- Ideally the entire laboratory should be air conditioned to maintain a dust-free environment and an ambient temperature of 22–25 °C. If this is not possible, at least cell culture cubicles, virus handling cubicles, the serology lab and the molecular biology labs should be air conditioned.

- Walls, ceilings and floors should be smooth, easy to clean, impermeable to liquids and resistant to chemicals and disinfectants normally used in the laboratory. Floors should be slip-resistant.
- Bench-tops should be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat.
- Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided.
- Laboratory furniture should be sturdy. Open spaces between and under benches, cabinets and equipment should be accessible to cleaning.
- Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on bench tops and in aisles. Additional long-term storage space conveniently located within or outside the lab area should also be provided.
- All doors should have vision panels, appropriate fire ratings and preferably be self-closing.
- Facilities for storing outer garments, personal items as well as having tea and lunch and restrooms should be provided outside the laboratory working area.
- Hand-washing basins with running tap water should be provided in each laboratory room, preferably near the exit door. A dependable supply of good quality water is essential. There should be no cross-connections between sources of laboratory water supply and drinking water supplies.
- There should be reliable and adequate electricity supply and emergency lighting for safe exits. A stand-by generator is essential at least for some equipment such as incubators, biosafety cabinets, freezers etc.
- Safety systems should cover fire, electrical emergencies, emergency shower and eyewash facilities. These include:
 - Avoiding overcrowding and too much equipment
 - Preventing infestation with rodents and arthropods
 - Preventing unauthorized entry into the laboratory areas

Human resources

A diagnostic virology laboratory should be adequately staffed. The minimum staff requirements for a diagnostic virology laboratory should include:

- *A qualified virologist* possessing a postgraduate qualification in virology with three to five years experience in diagnostic virology who will be the chief of the laboratory. This officer will have overall responsibility for activities of the laboratory and will supervise all the staff working at the centre. In addition, this officer will be directly responsible for reporting results of the various diagnostic assays performed in the laboratory.
- *Two junior microbiologists* possessing a Master's degree in Medical Microbiology with one to two years experience in diagnostic virology. These microbiologists will be responsible for the day-to-day functioning of the diagnostic laboratory such as supervision of technical staff who carry out the various diagnostic tests, stock management and procurement of lab supplies and diagnostic kits, quality control and quality assurance.
- *Two laboratory technologists* possessing a graduate degree in science with a diploma in Medical Laboratory Technology (one to be trained in cell culture and virus isolation methods and the other to be trained in serology). The technicians will be responsible for specimen processing, testing, laboratory safety, maintenance of laboratory records and media preparation.
- *One or two laboratory supportive staff:* With this minimum staff, routine diagnostic assays for several viral diseases can be performed. However, this would depend upon the load of clinical specimens and the range of diagnostic assays that the laboratory wishes to engage in. The most important aspect of human resource management in a virology laboratory is to ensure that roles and responsibilities of all the staff are clearly outlined and provide periodic training to upgrade the knowledge and skills of the laboratory personnel. This would ensure that the laboratory is abreast with contemporary diagnostic methodology as well as maintain quality in the services rendered.

Equipment and supplies

The virology laboratory should be provided with adequate equipment which should take into account certain general principles, i.e. it should be:

- Designed to prevent or limit contact between the operator and the infectious material (e.g. biosafety cabinets, electronic pipetting aids, etc.)
- Constructed of materials that are impermeable to liquids, resistant to corrosion and meet structural requirements.
- Should be free of sharp edges, burrs and unguarded moving parts.
- Designed, constructed and installed to facilitate simple operation and provide for ease of maintenance, cleaning, decontamination and certification testing. Glassware and other breakable materials should be avoided wherever possible.

A list of essential and desirable equipment required for a virology laboratory is listed below. Details of equipment specifications and sources of laboratory equipment required for a virology laboratory can be obtained from a variety of sources and some of these are appended to this report as Annex 1.

Essential equipment

- Biosafety cabinets Class II – Three (one for handling cell cultures, one for handling stock viruses and one for processing clinical specimens)
- One incubator and two CO₂ incubators (one for uninfected cell cultures and the other for infected cell cultures)
- 20 °C and –70 °C freezers
- Inverted light microscope
- Fluorescent microscope with photography attachments
- Filtration apparatus for preparation of tissue/cell culture media
- Refrigerate centrifuge
- water bath
- pH meter

- Magnetic stirrer
- Vortex mixer
- Electronic balance for weighing chemicals
- Elisa Reader and washer
- Micropipettes (100ul, 200 ul, 20 ul)
- Multi-channel pipettes – 8 and 12 channel pipettes (20-200 ul and 50-300 ul)
- Autoclave – Two (one for decontamination and one for sterilization)
- Hot air oven for sterilizing glassware
- One personal computer with printer, photocopier, fax machine and telephone lines
- PCR machine (conventional and real-time)
- Gel electrophoresis apparatus
- UV transilluminator
- Ice-making machine
- Liquid nitrogen containers
- Water purification/distillation system, which provides high-grade water suitable for tissue culture work
- Glassware such as volumetric flasks, measuring cylinders, pipettes (1 ml, 2 ml, 5 ml and 10 ml), conical flasks, reagent storage bottles (50 ml, 100 ml, 250 ml, 500 ml and 1000 ml)
- Electric brushing machine and automatic pipette washer

Desirable equipment

- Shaker water bath
- Rocking platform
- Ultracentrifuge

Reagents and supplies

A diagnostic virology laboratory requires a whole range of reagents and supplies. Most of the reagents and supplies listed below are generic and would suffice for the diagnosis of a variety of viral infections.

- Diagnostic kits as per requirements of the laboratory
- Tissue culture media
- Foetal bovine serum
- Fluorescent conjugates
- ELISA plates, antibodies and conjugates
- Analytical-grade fine chemicals for preparation of buffers
- Sterile tissue culture plastic ware (25 cm² and 75cm² flasks, 24 and 96 well plates, petridishes, tissue culture tubes centrifuge tubes and pipettes)
- V-bottom polystyrene microtitre plates for haemagglutination
- Serum storage cryovials and boxes
- Micropipette tips
- PCR tubes
- PCR reagents (*Taq* polymerase, reverse transcriptase, primers, probes and agarose)
- DNA and RNA extraction kit

It is critical to procure high-quality reagents and also confirm the same before use in routine diagnostic work.

Functions

The National Virology Laboratory should be the principal laboratory of the country in providing quality virology laboratory investigations and technical coordination of the entire network of the laboratories. The laboratory may perform some or all of the following functions:

- (1) To provide technical advice to national authorities on the progress, needs and aspirations of the virology laboratory network in the country.
- (2) To assist national authorities in planning, organization and supervision of the virology laboratory network.
- (3) To undertake referral services for providing efficient virology laboratory services.
- (4) To develop technical/training material for use in laboratories in the network to enhance their quality.
- (5) To assess needs and impart training to staff of laboratories in provincial and country laboratories in quality testing for viral pathogens.
- (6) To validate reagents and kits that may be used nationally.
- (7) To validate new technologies that may become available and recommend their implementation in the country.
- (8) To develop minimum standards for virology laboratories and assist laboratories in their implementation.
- (9) To develop national database on the laboratory results.
- (10) To organize external quality assessment schemes to periodically assess the quality of testing in networks and suggest remedial measures to those laboratories that show poor performance.
- (11) To undertake research to improve the quality and cost-effectiveness of virology laboratory services in the country.
- (12) To collaborate with WHO and other international agencies on technical matters pertaining to improvement of laboratories.

Chapter 3

Viral diagnostic tests

Virtually all diagnostic techniques used in virology are based on the following principles:

- (1) Isolation and identification of viruses
- (2) Detection of virus antigens
- (3) Detection of viral nucleic acid
- (4) Detection of virus-specific antibodies

The choice of the system used in the laboratory for confirmation or ruling out of a viral infection would primarily depend on the facilities and resources available and linkages with national and international laboratories. A broad outline of the suggested methods available for common viral infections is presented in Tables 3.1–3.6. However, the choice of the methods to be used should be determined by the laboratory depending on the available infrastructure, resources and trained manpower.

Isolation and identification of viruses

The relative importance of viral isolation as a diagnostic method is rapidly diminishing; however, it still remains necessary because it is the only technique capable of providing a viable isolate that can be used for antiviral susceptibility testing. An additional advantage is that in contrast to most antigen and nucleic acid detection methods, viral culture allows detection of multiple viruses, not all of which may have been suspected at the time the culture was requested.

No one cell culture type can support the growth of all medically relevant viruses. Hence, virology laboratories must maintain several different cell culture types. The minimum requirements are HeLa and/or RD cells, L₂₀B used for the isolation of enteroviruses, Vero and BHK 21 cell lines used for the isolation of herpes simplex viruses, MDCK and embryonated eggs for isolation of influenza viruses, Hep2 for the isolation of RSV, and an insect cell line such as *Aedes albopictus* C6/36 which is useful for isolation of a number of arboviruses. The type

of cell lines that are required for a specific specimen is determined by the information communicated from the ordering physician to the laboratory and by knowledge of the range of viruses that can usually be isolated from a given specimen type.

Growth of viruses in cell culture is usually detected by visualizing morphological changes in the cells, known as cytopathic effect (CPE). Cell cultures are typically viewed microscopically to detect CPE every one to two days for the first week of incubation. When necessary, confirmation can be achieved by scraping the infected cells from the walls of the tube or vessel in which they are growing and preparing a fluorescent antibody stain with use of monoclonal antibodies specific for the virus, or a neutralization test using specific antisera.

The shell vial culture method, first developed for cytomegalovirus (CMV), dramatically decreases the time required for detection of viruses in cell culture. The method involves centrifugation of the specimen onto the cell culture monolayer grown on a cover slip, which is incubated for one or two days, followed by fluorescent antibody staining of the cell culture, regardless of whether CPE is visible or not. In addition to detection of CMV, shell vial cultures have also been used to speed the detection of herpes simplex virus (HSV), varicella zoster virus (VZV), respiratory viruses, and the enteroviruses.

A recent modification of the traditional cell culture involves the use of genetically engineered cell lines such as the L₂₀B, which is used for the isolation of polio viruses, and Vero cells for isolation of measles and rubella viruses. Another example of genetically engineered cell line uses genes that are transfected into indicator cell lines so that the cell line responds to a specific viral protein present in the specimen. Activation of the promoter triggers a reporter enzyme, such as β-galactosidase, which acts on a substrate to indicate the presence of the virus being sought. This approach has been most widely used for HSV and HIV.

Detection of virus antigens

Methods of antigen detection include fluorescent antibody (FA) staining, immunoperoxidase staining and EIA. Of these, FA staining is the most widely used in diagnostic virology. This method was widely adopted by clinical laboratories during the 1980s, particularly for detection of respiratory viruses. The commercial availability of specific monoclonal antibodies and simultaneous staining with

multiple different antibodies labeled with different fluorescent labels has enabled rapid detection of a variety of viruses.

Antigen detection methods are particularly useful for viruses that grow slowly or are labile, making recovery in culture difficult. The most important targets have been detection of viruses in respiratory specimens; cutaneous specimens; stool specimens; and cerebrospinal fluid (CSF). Viruses such as the enteroviruses and the rhinoviruses that have extensive antigenic heterogeneity and cross-reacting antigens are not suitable for antigen-detection techniques. The advantages of antigen-detection techniques are rapidity (results can be available within hours of receipt of the specimen in the laboratory) and lack of requirement for viral viability in the specimen, allowing greater flexibility in the handling and transport of specimens.

Detection of viral nucleic acid

The development of PCR analysis in 1985 made possible the diagnosis of viral infection through sensitive detection of specific viral nucleic acids. Any virus can potentially be detected using this technology. By inclusion of a step employing the enzyme reverse transcriptase (RT), PCR analysis can be adapted to detect viral RNA, especially for rapid diagnosis during outbreaks of diseases such as avian influenza and Nipah virus infections.

Viral load assays for HIV and hepatitis C virus (HCV) are examples of quantitative nucleic acid detection techniques. Multiplex techniques permit the simultaneous detection of more than one virus or even of a virus and a different class of pathogen. For example, a multiplex PCR analysis that detects the DNA of Epstein-Barr virus (EBV) and *Toxoplasma gondii* has been used for the diagnosis of mass lesions in the brains of patients with AIDS.

Recent modifications of PCR analysis called "real-time" PCR are a dramatic development that may potentially greatly expand the applicability of PCR analysis for diagnosis of viral infections. In these assays, a fluorescent signal is generated as PCR takes place. The assays are run on highly specialized automated instruments that include optical systems to excite the fluorescent dyes and detect fluorescent emissions. The combination of amplification and signal detection markedly reduces the time required for nucleic acid detection and greatly reduces the likelihood of contamination.

Many laboratories have developed their own "home-brew" PCR assays. The quality and performance of these assays vary widely. Inter-laboratory certification programmes to help standardize these assays are only beginning to become available.

Detection of virus specific antibodies

The diagnosis of viral infections by detection of more than a four-fold increase in specific antiviral antibodies is a traditional method whose clinical utility is limited by the need for comparison of acute and convalescent antibody titres. However, detection of virus-specific IgM antibodies allows a diagnosis to be made from a single specimen. Viruses for which detection of virus-specific IgM antibodies are useful include flaviviruses (JEV, dengue, West Nile), hepatitis A virus; hepatitis B virus (IgM antibodies to the hepatitis B core antigen), measles, rubella and mumps viruses.

Serological methods are also useful for diagnosis of certain chronic infections such as with HIV or HCV, in which the presence of any antiviral antibodies is always (HIV) or usually (HCV) indicative of current infection. Finally, serology is uniquely useful for defining specific antiviral immunity. Viruses for which definition of immune status by serology is useful include arboviruses (Japanese encephalitis, dengue, West Nile and chikungunya viruses) VZV, CMV, EBV, HSV, measles and rubella viruses, parvovirus B19, hepatitis A (total antibodies), and hepatitis B (antibodies to the hepatitis B surface antigen) and rabies (presence of neutralizing antibodies after vaccination).

The successful exploitation of diagnostic virology laboratory in clinical practice depends on the adherence to the following principles; which are important to both the treating clinician as well as the clinical virologist.

- The clinician should be aware that three components that can contribute to any diagnostic result are the information provided by the physician, the type of assay used and the competence of laboratory personnel. It is vital that these three must communicate and perform well to ensure that proper results are obtained.
- False positive and false negative results are a possibility with most assays that are currently in use, and therefore it should be noted that no assay is perfect; hence it may be dangerous to base all patient management procedures on a single result.
- It is the responsibility of the clinician to ensure that adequate and relevant information pertaining to the illness is provided to the

laboratory. A sample of a virology request form is appended as Annex 2. Collection of specimens, storage and transport require constant dialogue between clinicians and diagnostic laboratory personnel in order to obtain rapid and accurate results.

Diagnostic assays that are currently used for several syndromes are summarized in tables 3.1 to 3.5.

Table 3.1: Diagnostic assays for central nervous system viral infections

No.	Organism	Specimen(s)	Suggested methodology	Comments
1.	<i>Herpesviridae</i> (HSV 1 and 2, CMV, VZV, EBV, HHV 6)	CSF and paired serum specimens	Isolation using cell culture, antigen detection and PCR, antibody detection	PCR has replaced culture as the "Gold standard"
2.	<i>Enteroviruses</i>	CSF and paired serum specimens	Isolation using cell culture, detection of rising antibody titres and PCR	PCR using genus-specific conserved primers has proved most sensitive
3.	<i>Flaviviridae</i> (JEV, West Nile, dengue)	CSF and paired serum specimens	Detection of IgM antibodies in CSF and sera, virus isolation	Utility of PCR has not been comprehensively evaluated
4.	<i>Paramyxoviridae</i> (mumps, measles, Nipah)	CSF and paired serum specimens	Detection of IgM antibodies in CSF and sera, virus isolation	Utility of PCR has not been comprehensively evaluated
5.	<i>Retroviridae</i> (HIV and HTLV-1)	CSF and paired serum specimens	Antibody detection, PCR	PCR is more sensitive
6.	<i>Papovaviridae</i> (JC virus)	CSF and paired serum specimens	Virus isolation and PCR	PCR has proved more sensitive
7.	<i>Rhabdoviridae</i> (rabies, chandipura)	Brain(post-mortem) CSF and paired serum specimens	Virus isolation, immuno-fluorescence for antigen detection, antibody detection and PCR	PCR has not been extensively evaluated

Table 3.2: Diagnostic assays for respiratory viruses

No.	Organism	Specimens(s)	Suggested methodology	Comments
1.	Influenza viruses (Influenza virutyypes A, B and C, avian influenza virus)	Nasopharyngeal aspirates, throat swab, paired serum samples collected at 7 and 14 days after onset of symptoms	Isolation using cell culture, antigen detection by immuno-fluorescence, antibody detection by neutralization, and nucleic acid detection by PCR	PCR using genus-specific conserved primers has proved most sensitive
2.	Para-influenza viruses (PI types 1-3)	Nasopharyngeal aspirates, throat swab, paired serum samples collected at 7 and 14 days after onset of symptoms	Isolation using cell culture, antigen detection by immuno-fluorescence, and nucleic acid detection by PCR	PCR using genus specific conserved primers has proved most sensitive
3.	Respiratory syncytial virus (RSV)	Nasopharyngeal aspirates, throat swab, paired serum samples collected at 7 and 14 days after onset of symptoms	Isolation using cell culture, antigen detection by immuno-fluorescence, and nucleic acid detection by PCR	Utility of PCR has not been comprehensively evaluated
4.	Paramyxoviridae (mumps, measles, Nipah)	Nasopharyngeal aspirates, throat swab, single serum samples collected at 430 days after onset of symptoms	Isolation using cell culture, antigen detection by immuno-fluorescence, antibody detection by ELISA IgM, and nucleic acid detection by PCR	Utility of PCR has not been comprehensively evaluated

Table 3.3: Diagnostic assays for gastroenteritis viruses

No.	Organism	Specimens(s)	Suggested methodology
1.	Reoviruses (Rotaviruses)	Stool	Antigen detection by ELISA, electron microscopy, and PCR
2.	Enteroviruses	Stool	Antigen detection by ELISA, electron microscopy, and PCR
3.	Astroviruses	Stool	Electron microscopy, virus antigen detection and PCR
4.	Caliciviruses	Stool	Electron microscopy, virus antigen detection and PCR

Table 3.4: Diagnostic assays for hepatitis viruses

No.	Organism	Specimen	Suggested methodology	Comments
1.	Hepatitis A virus	Serum	IgM/IgG antibody detection by ELISA and nucleic acid detection by PCR	IgM antibody detection is most sensitive
2.	Hepatitis B Virus	Serum	HbsAg antigen detection by ELISA and nucleic acid detection by PCR	HbsAg detection is most sensitive
3.	Hepatitis C virus	Serum	Antibody detection by ELISA and nucleic acid detection by PCR	Detection antibodies most useful
4.	Hepatitis D virus	Serum	Delta antigen by ELISA and Nucleic acid detection by PCR	PCR is the test of choice
5.	Hepatitis E virus	Serum	IgM antibody detection by ELISA and nucleic acid detection by PCR	IgM antibody detection is very useful
6.	Other hepatitis viruse: hepatitis G virus (HGV) & transfusion transmitted virus (TTV)	Serum	Nucleic acid detection by PCR	PCR is the method of choice

Table 3.5: Diagnostic assays for viruses that cause fever with rash or haemorrhage

No.	Organism	Specimens(s)	Suggested methodology
1.	Measles virus	Nasal aspirates, throat washings urine whole blood, single serum samples	Virus isolation, IgM antibody detection by ELISA and nucleic acid detection by PCR
2.	Rubella	Nasal aspirates, throat washings, single serum samples	
3.	Flaviviruses (dengue virus serotypes 1-4, West Nile virus)	Paired serum samples	
4.	Alphaviruses (chikungunya)	Paired serum samples	
5.	Enteroviruses (coxsackie and ECHO viruses)	Stool specimens, throat washings, paired serum samples	
6.	Filoviridae (Marburg, Ebola)	Paired serum samples	

Chapter 4

Specimens management

Laboratory diagnosis of viral infections requires an understanding of the pathogenesis of the suspected agent and knowledge regarding the type and duration of illness, the stage of infection, age of the patient, immunization status, and whether outbreaks of similar illness have been recorded in the community. This information facilitates selection of the appropriate specimen, careful collection to optimize recovery of the agent, and transport of specimens in a manner that maintains viability and minimizes overgrowth with contaminating organisms.

Detailed guidelines for collection, transport and processing of specimens are available for a variety of viral diseases. However, every laboratory has to prepare its own standard operating procedures (SOPs) depending on the local conditions.

Selection of specimens

For best correlation with a particular disease, the specimen should reflect the target organ whenever possible, which is usually based on the clinical symptoms. A brief summary of the type of specimens to be collected in common viral infections has been presented in Tables 3.1 to 3.6.

Materials required

Virus culture

Viral transport medium (VTM): A number of different VTM formulations are used, quite often prepared in-house. VTM typically consists of a protein such as gelatin and anti-microbial agents in a buffered salt solution. VTM prevents specimen from drying, helps maintain viral viability and retards the growth of microbial contaminants. VTM tubes usually contain 2 ml to 3 ml of medium; a few sources provide kits that include VTM tubes and collection swabs. Larger volumes of VTM (5 ml to 7 ml) are usually prepared for transport of tissue samples; these volumes should not be used for swabs because of the dilution effect. The following supplies are required for collection of specimens for virus culture:

- Sterile, leak-proof, externally threaded screw-cap containers including urine cups, disposable centrifuge tubes (15 ml and 50 ml), and smaller tubes (e.g., 4-ml Nunc vials, 13x100 tubes), suitable for holding 1 ml to 2 ml of VTM.
- Sterile cotton swabs with plastic or aluminium shafts; small-tip flexible (fine-shafted aluminium) swabs are used for certain samples such as urethral swabs. Calcium alginate swabs and wooden-shafted swabs are not recommended.
- Tuberculin syringe with 26-gauge or 27-gauge needle for aspirating vesicular fluid.
- Blood collection tubes containing anticoagulant (ACD).

Specimen collection

For virus isolation, specimens should be collected as soon after onset of symptoms as possible. The chance of viral recovery is best during the first three days after onset and is greatly reduced beyond 5 days with many viruses.

For serological tests, two blood specimens need to be collected—one during the acute phase of the illness and the second sample seven to ten days after the first specimen. In some diseases only one sample is needed, which should not be collected earlier than four to seven days or later than one month after onset of disease. A minimum of 5 ml of clotted blood collected into sterile containers needs to be sent to the laboratory as early as possible; if not, serum should be separated aseptically within four to six hrs of collection and transported in screw-capped vials to the laboratory under cold conditions.

As a general rule, specimens collected for PCR should be obtained during the early part of the illness. Cotton swabs with wooden sticks should not be used for collection specimens that are processed for PCR. Similarly, heparin should not be used as anticoagulant for the PCR test on blood as it interferes with Taq polymerase enzyme. Instead, EDTA may be used as an anticoagulant.

Optimal times for collection of specimens

Collect specimens as aseptically as possible. Place each specimen into a separate container labeled with the patient's name and identification number, the collection site, the date of collection and the time of the collection. In general, place swabs

into a tube containing a small volume of VTM, and scrapings and small pieces of tissue into a tube containing a small volume of VTM or saline. Place fluid and bulk specimens (e.g. tissue) into a sterile leak-proof container; add a volume of VTM sufficient to prevent drying of tissue. Collect up to 1 ml – 5 ml of serum or CSF.

Table 4.1: *Optimal time for collection of specimens*

Specimen	Optimal time after onset of symptoms in days within which sample should be collected (days)
CSF and plasma	7
Stool, Urine	14
Nasopharyngeal swab, nasal washings	5
Throat washings, buffy coat for CMV	As early as possible
Vesicular fluid (fluid/scraping), genital swab	
Post-mortem specimens	

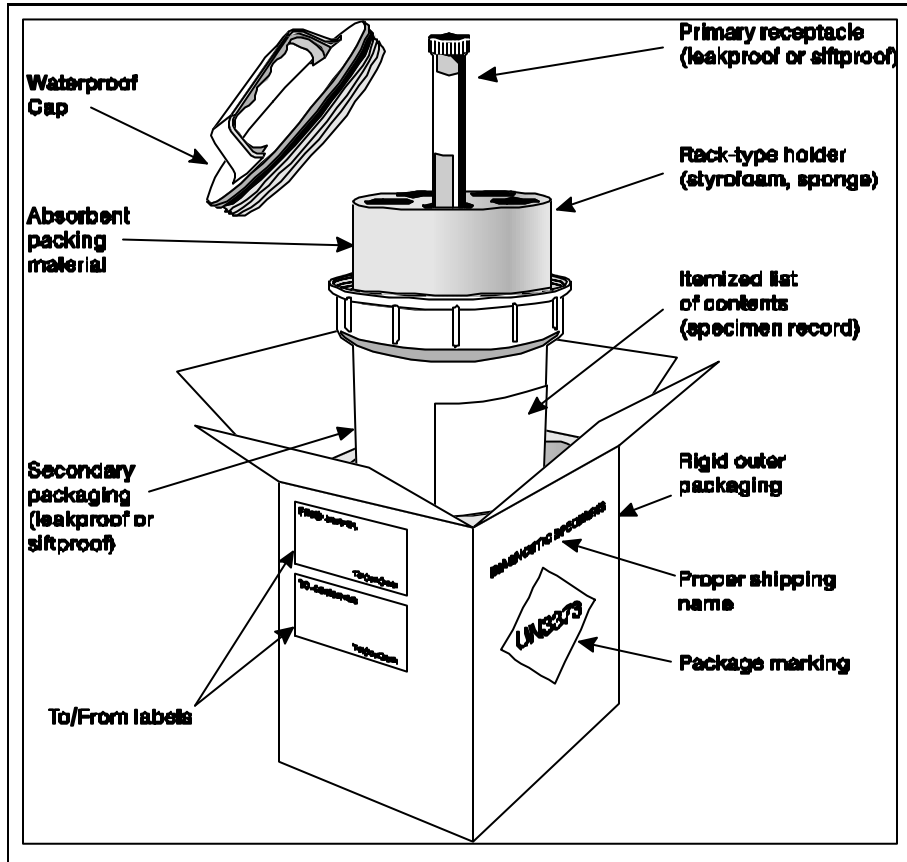
The health worker who is collecting the material should be trained to obtain a complete patient history, including the date of onset of symptoms, clinical findings, recent exposure history, animal or arthropod contacts or bites, recent travel to areas of endemic infections, and recent vaccination.

Specimen transport

Place the tightly capped specimen container and the appropriate laboratory requisition form into separate compartments of a plastic specimen transport bag (Annex 3). *All specimens should be delivered to the laboratory as soon after collection as possible, since a loss of infectivity occurs over time; samples containing labile viruses at low titres are those most likely to show loss of infectivity with delayed transport.*

If immediate delivery is not possible, refrigerate specimens (2 °C to 8 °C), or place them on wet ice or a cold pack. Loss of viability is slower at refrigeration temperature. But as far as possible, do not freeze samples. If samples must be frozen, freeze them rapidly at –20 °C or –70 °C.

Figure 4.1: Packing of specimens for transport to laboratories



Biosafety

Safety in a virology laboratory is part and parcel of laboratory quality control to obviate laboratory-acquired infections. Each laboratory should have the WHO Laboratory Biosafety Manual/Document WHO/CDS/CSR/LYO/2004.11, available at: <http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf>).

The main sources of laboratory-acquired viral infections are accidents, working with infected animals, clinical specimens and aerosols. The types of accidents involved consist mainly of spillage and splashes, needle and syringe, sharp objects and broken glass, animal scratches or bites.

Where pathogens that cause severe human disease are known to infect by the airborne route, primary containment using microbiological safety cabinets and the provision of secondary containment using appropriate ventilation have been recommended. For work with blood-borne organisms such as HIV and HBV, the use of "sharps" should be avoided. Where available, vaccination is recommended for people working with known organisms. Vaccination is essential for work with HBV, polio, rabies, Japanese encephalitis, and Kyasanur Forest disease virus. Vaccination is also recommended for measles, mumps and rubella.

Personal protective equipment

Over the years, three kinds of protective clothing came into general use: gowns, front-buttoned white coats, and specially designed overalls, which afforded protection up to the neck. The latter is considered the best protective clothing available since they are easy to remove, aesthetically pleasing, and afford much better protection than front-buttoned white coats. It is advisable to wear disposable plastic aprons over ordinary protective clothing when working with blood that might be infected. Gloves should be worn when handling the specimens and all hazardous agents/materials.

Amenities

Hand basins should be provided in each laboratory, lockers for staff clothing should be available near to (but not in) laboratory rooms, and rooms for eating and drinking should be provided close to laboratory rooms.

Health of staff

It is advisable to have a pre-employment medical examination for all staff. The form of the examination may include a medical history, physical examination, full blood count (FBC), clinical chemistry, urinalysis, ECG and chest X-ray. It is desirable to have some sort of medical monitoring for workers in BSL 3 and BSL 4 laboratories. The scope of the monitoring depends on the agent. Women who work with viruses should tell their supervisors if they become pregnant, and as soon as possible. There should be an individual evaluation of the risks involved in continued employment and it may be necessary for them to change their work. Limited information is available about laboratory exposure to CMV.

Medical surveillance

A system that records and follows up all cases of illness in the laboratory staff to ascertain the source of infection and initiate any follow-up action should be developed.

Accidents

There should be a rule in all microbiological laboratories that all accidents causing personal injury with exposure to infectious agents or not should be reported to the supervisor or safety officer. Records should be kept of all staff sicknesses, injuries and accidents, and of X-rays and immunization. Accident records are particularly important for legal reasons.

It is highly desirable to have a qualified first-aid provider in the laboratory, but he/she should receive additional training in dealing with exposure to and ingestion of infectious, toxic and corrosive chemicals. All staff should know whom to contact if an accident occurs. The standard first aid kit for minor injuries should be in a prominent position and well sign-posted. Care should be taken with eye irrigation since the bottles of "sterile" water are frequently contaminated. Standard operating procedures for management of post-exposure prophylaxis should be in place.

Minimizing equipment and technique-related hazards

Pipettes

Mouth pipetting is banned at all times. Pipetting devices should be inspected routinely for leakage and replaced as necessary. When discharging infectious material from a pipette, it is important to minimize the formation of aerosols. Contaminated pipettes should always be discarded into disinfectant fluid that is prepared daily and placed in a flat, covered metal tray. Broken and chipped pipettes should be promptly discarded into sharps containers.

Hypodermic needles and syringes

These are the most hazardous pieces of equipment in common use. Surveys have shown that at least one-quarter of overt accidents which result in infection were

caused by these instruments. Many inoculations result during experiments with animals, others occur during transfer of infected material by syringes. Many needlestick accidents occur when the needle is being recapped. It is important that used needles are promptly disposed of properly into harden "sharps containers" which are then autoclaved before disposal. These sharps containers should be available wherever needed.

Centrifugation

Infectious material may be dispersed by a centrifuge either through broken tubes or other means, such as through the threads of the tubes and caps. Sealed centrifuge buckets should be used when centrifuging infectious material. Care should be taken to ensure that the centrifuge tubes are not cracked or flawed. Tubes should not be more than three-quarters full, especially if angle centrifuges are used. Tubes should be capped and they and the buckets should be balanced carefully to avoid vibration, which may lead to breakage.

Pouring infectious material

Pouring of the supernatant after centrifuging cultures or viruses should be avoided. All infectious material should be transferred from one container to another using mechanical/electrical pipetting devices.

Management of laboratory waste

Laboratory waste should ideally be separated into colour-coded containers. The recommended colours are listed below; however, the laboratories should follow and adhere to the national guidelines:

- *Yellow*–for incineration
- *Light blue or transparent with blue inscription*–for autoclaving (but may be incinerated subsequently)
- *Black*–normal household waste: local authority refuse collection
- *White or clear plastic*–soiled linen

There are three practical methods of treating contaminated laboratory waste:

- Sterilization by autoclaving
- Chemical disinfection
- Incineration

The first two are laboratory processes; incineration involves the transport of material off-site. Sterilization and disinfection are not synonymous: sterilization implies the killing of all microorganisms; disinfection kills most microorganisms but depends greatly on the chemical used.

Autoclaves

Autoclaving involves the timed exposure of materials to steam above atmospheric pressure and hence at temperatures above 100 °C. Autoclaves operate at high pressures and temperatures and their manufacture, installation, and use are regulated. Sterilization is achieved by exposure of material to 121 °C for 15–20 minutes. Chemical and biological indicators are also widely used, the most well-known of which is the "autoclave" tape. It was customary to place material for autoclaving in wire baskets and cylindrical buckets. Autoclavable plastic bags are now in general use but they must be permeable to steam. The mouths of plastic bags should not be closed before autoclaving since this will reduce steam penetration.

Chemical disinfection

Chemical disinfection should be regarded as a first line defence, especially in the case of discarded bench equipment, which should be followed by autoclaving or incineration. There is a rough spectrum of susceptibility of microorganisms to disinfectants. The most susceptible are vegetative bacteria, fungi, and enveloped viruses. Mycobacteria and non-enveloped viruses are less susceptible and spores are generally resistant. The most commonly used disinfectants in laboratory work are clear sodium hypochlorite, clear phenolics, and alcohols and aldehydes. Some disinfectants have undesirable effects on the skin, eyes and respiratory tract. Disposable gloves and safety spectacles, goggles, or a visor should be worn by anyone handling strong disinfectants. Full-face respirators should be worn when rooms are being fumigated with formaldehyde.

Discard jars

Discard jars should be robust and autoclavable. Glass jars should not be used because they are easily broken. Polypropylene beakers or jars are probably the most serviceable items. They are left open on the bench but are closed at the end of the working session. It is important that discard jars are filled frequently with known active dilutions of disinfectant, not overloaded with protein or floating articles. Laboratory supervisors should ensure that inappropriate articles are not placed in discard jars (e.g., a 25 ml pipette has no place in a small 1 litre jar). Funnels may be used to prevent splashing and aerosol dispersal. Discard jars should be washed with hot water before reuse. It is better still to autoclave the jar before washing. The contents of the discard jars should then be autoclaved and/or incinerated.

Reusable pipettes

After use, reusable pipettes should be completely immersed in disinfectant placed in metal/plastic trays that have lids so that no air remains within the pipette. They should remain in disinfectant for at least 18 hours before washing and/or autoclaving.

The key to effective disposal of wastes is segregation and decontamination at the point of generation. Generators of wastes are responsible for ensuring that wastes are placed in the correct receptacle. In the clinical virology laboratory this is crucial, because the generator may often be the only person who fully appreciates the risks the waste poses. The generator has a legal duty to take reasonable care for the health and safety of persons at the place of work who may be affected by acts or omissions.

Wastes should be segregated on the basis of the major or primary hazard they pose. Generators need to make this assessment and decide if further segregation is necessary because of secondary hazards associated with the waste.

Infectious waste

These must be segregated from general wastes because they need to be autoclaved or incinerated before being removed from the area in which they are generated.

Routine contaminated wastes

Generators must also take care to segregate these wastes from general wastes because they, too, may need to be decontaminated prior to removal from the area in which they are generated. Alternative means of decontamination to autoclaving or incineration may be used, provided they are effective.

Sharps

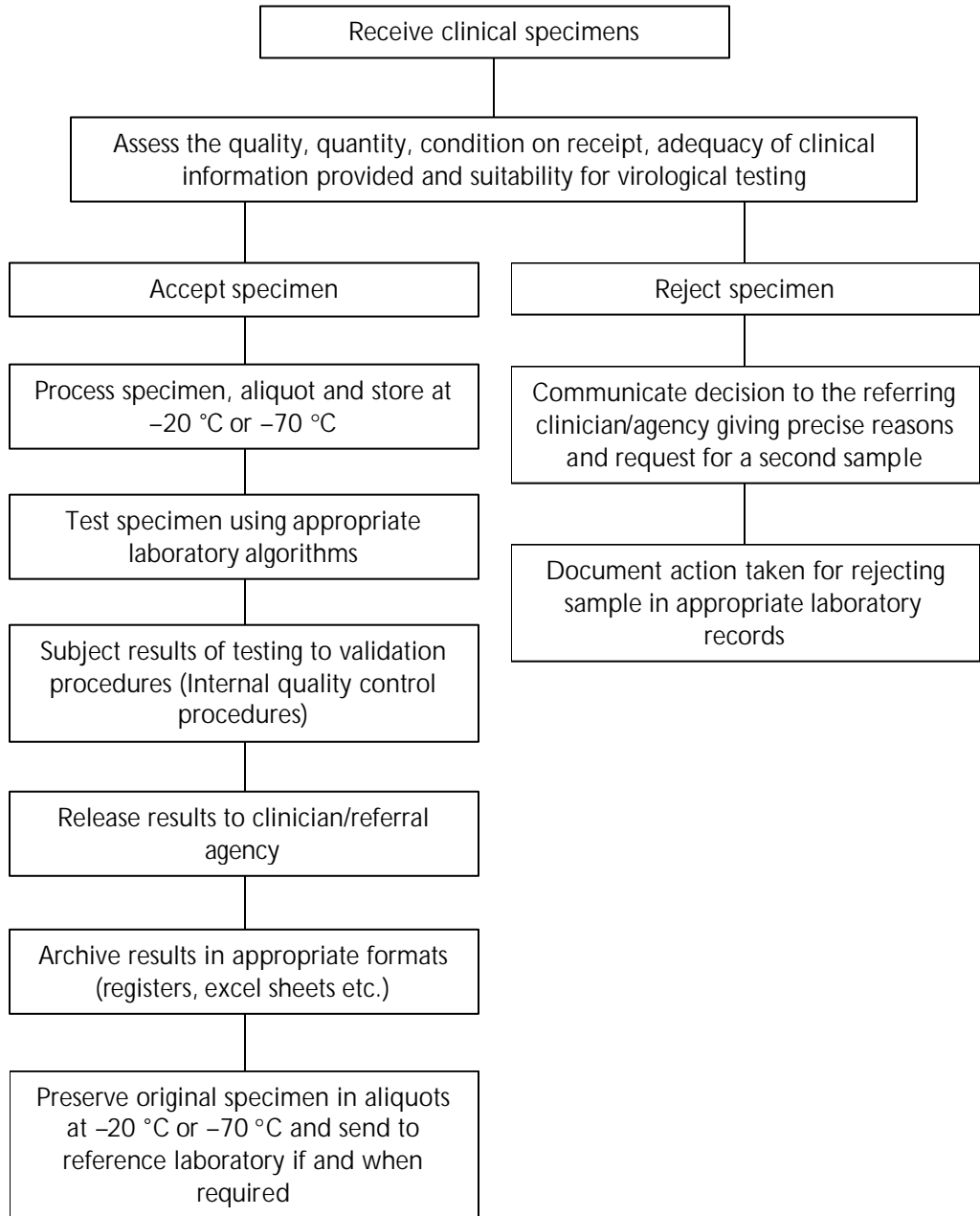
All sharps must be segregated from other wastes and placed in dedicated and labelled single-use rigid containers immediately after use. Sharps containers must not be compacted or emptied into ordinary waste streams.

Labelling of wastes

Standard labels to identify major waste categories are available commercially. All wastes must be identified with an appropriate type of label which will remain attached to the waste. The label must state the nature of waste material, the department, date, and the name of the person responsible for the waste.

In transporting wastes through buildings and across campus, the main consideration is that no one is exposed to risks from the contaminated material. Containers should be leak-proof. Wastes should be decontaminated as far as is practical before removal from the area of generation. All laboratories should have access to local autoclave and other appropriate decontamination facilities so as to minimize the need to transport contaminated wastes around laboratories. Sharps should only be placed in rigid containers. All contaminated waste should be so identified.

Flow of clinical specimens in a virology laboratory



Chapter 5

Quality systems

Quality systems in virology laboratories are of paramount importance. A system should be comprehensive and must cover all aspects from the decision to collect the specimen to interpretation of the results. Errors at any stage in the investigation can affect the outcome of the result. Further, any break in the chain can result in the generation of a faulty report. Quality does not just happen on its own.

Systematic efforts through organizational structure and efficient utilization of resources are needed to implement all the steps that will assure generation of quality reports by the laboratory. Accordingly, a quality system is a part of overall quality management that aims at consistency, reproducibility, traceability and efficaciousness of the laboratory services. A quality system therefore comprises of five essential elements:

- Organizational management structure
- Quality standards
- Documentation
- Training
- Monitoring and evaluation

A well-structured clinical virology service should include a full-fledged serology setup, cell culture facility and the capacity for molecular diagnosis. The quality of results from the laboratory depends on the quality control (QC) of the testing laboratory. The outcome is significantly influenced by many pre-analytical, analytical and post-analytical factors. A good quality system addresses these concerns, with objectives such as improvement in diagnostic quality; production of reliable and reproducible results; establishment of inter-laboratory testing comparability; promotion of credibility of the laboratory among patients, doctors, other institutions, collaborators and accrediting/regulatory bodies; motivation of staff for further improvement; and preclusion of poor results which could cause legal complications. Quality control in the clinical virology laboratory consists of a set of procedures designed to help ensure delivery to the medical staff of laboratory results that are consistent and accurate. These results must be supplied in a timely

fashion while the data is still clinically relevant. A clinical virology laboratory should be designed in a manner so that biohazard risks to the laboratory personnel and the general public are minimized and that cultures are protected from environmental contamination.

Documentation

Routine procedures must be described in written Standard Operating Procedures (SOPs). They must be reviewed regularly and modified, if necessary. The modified versions should be signed and dated by the laboratory director. The recent most version of SOPs should be available directly at the work place. The old versions should be achieved in the laboratory and should be archived if required.

The records must be stored for long periods of time but be available for prompt retrieval, safe archiving of all records must be ensured. Archiving of the source documents and other essential documents must be such that data are kept in an integer state and can neither be lost nor altered to achieve this goal.

Records of usage, maintenance and calibration should be kept in the laboratory and should be routinely monitored. Reports of the tests should be released only after proper scrutiny and documentation of the scrutiny with the signature and date by the lab-supervisor.

Standard operating procedure (SOP)

SOP is one of the most important documents in a diagnostic laboratory. Apart from providing the complete details of how exactly a test or a procedure is carried out in a laboratory, the SOP also provides information on specimen collection, laboratory safety instructions, purpose and limitations of the procedure, turnaround times, interpretation of results, and above all, the line of authority.

The procedure manual used in the laboratory should be complete enough in detail so that an inexperienced technologist can perform the procedure without additional information. One copy of the manual should be readily available to bench personnel, and another copy should be stored separately in case of accidents. The document control system is required to ensure that the up-to-date document is used.

Some of the areas where quality control checks are critical are briefly described below:

Specimen transport

Specimens for viral isolation are often held for long periods of time before they reach the laboratory. Enveloped viruses such as RSV and CMV are extremely labile at room-temperature and freeze-thaw cycles, whereas non-enveloped viruses such as enteroviruses tolerate these conditions well. As a general rule, viral specimens held for short periods should be refrigerated, while those for longer periods may be frozen at -20°C or -70°C .

Transport media

The composition and type of viral transport media can affect viral isolation rates. In general, the media should be a balanced isotonic solution at physiological pH. It should contain a substance that will stabilize the virus such as gelatin, fetal calf serum or bovine serum albumin, and antibiotics against bacteria and fungi. The swab should be made of a material that is non-toxic to viruses, such as dacron or rayon.

Smears

Smears are becoming increasingly popular because of the rapidity of staining techniques. The smear should contain a reasonable number of cells, be of a reasonable size, and not mixed with blood or pus, as the latter may lead to nonspecific staining.

Specimens for serology

Excessively haemolyzed, lipaemic, bacterially contaminated, or leaking specimens should be rejected. Sera should be heat-inactivated depending on the tests to be performed. In the event of a specimen being rejected, the sender must be informed, preferably by an oral report followed by a written one. Extenuating circumstances may warrant the acceptance of a substandard specimen.

Tissue culture and media

Tissue/cell culture remains the mainstay of non-serological viral diagnosis. Therefore, adequate quality control for commercially purchased or for in-house preparation of tissue culture cells is of great importance. Within a given cell line, there may be significant variations in sensitivity to virus isolation which may depend on the particular cell line or clone and the passage number. Information on a particular cell line should be recorded accurately including the source, type, passage number, confluency and cell condition. The loss of a cell line routinely passaged for use can lead to a severe disruption of workflow, and therefore provisions must be made for back-up cells in the event of contamination or laboratory accident. These back-up systems include:

- Freezing and storage of low passage cells at $-70\text{ }^{\circ}\text{C}$ liquid nitrogen tanks.
- Use of paired stock flasks. When the flasks reach confluency, only one is passaged, while the second flask is held as a back-up until the new flask displays good growth.
- Carrying of a parallel set of stock flasks using a separate set of tissue culture reagents and glassware.

Cells purchased from a commercial company should be certified to be free from mycoplasma, fungal, and bacterial contamination as well as adventitious viral agents such as SV40. Tissue culture lines carried in-house should be subjected to:

- Daily checks for growth rate and contamination.
- Mycoplasma contamination monitored monthly by Hoechst stain or others as available.
- The sensitivity to viral isolation monitored by periodic TCID_{50} experiments with stocks of reference virus.

Other quality control procedures that may aid in minimizing the risk of contamination include the exclusion of laboratory with infectious diseases from handling tissue culture, and separate laboratory apparel, reagents and glassware for tissue culture. Cell lines should be handled separately and the cabinet be decontaminated in between uses.

Media

Following filter sterilization, aliquots of the media should be taken and checked for bacteriological or fungal contamination. These samples should be examined daily for five days and should be free from contamination. Aliquots of all other medium components such as fetal calf serum and L-glutamine should also be checked. New lots of medium and fetal calf serum that have passed the sterility check should be monitored for their ability to support cell growth.

Reagents and kits

Reagents and kits should be ordered from reputable manufacturers and dealers with reliable transportation systems. Upon receipt, the reagents should be checked for obvious breakage or contamination. The quantity, source, lot number and date of receipt should be entered in a logbook and the reagents stored according to the manufacturer's storage specifications. When new lots of any reagent are opened, the date should be noted on the container. Caution must be exercised in the case of kits as different components of a kit may require different storage conditions and have different expiration dates.

Instruments

Laboratory instruments should be subjected to routine preventive maintenance and checked and calibrated on a regular basis. Some of these checks can be performed by laboratory staff and entered into a logbook. The following are some recommendations for routine laboratory maintenance and performance checks on instruments:

- Incubators: daily temperature, CO₂ and humidity checks. Weekly decontamination of interior.
- Safety cabinets: daily air pressure check and cleaning of UV lamp. Work surface should be decontaminated after each use. Annual checks for air velocity and filter integrity and paraldehyde decontamination, as applicable.
- Microscopes: daily cleaning of objectives and stage, log of lamp usage and annual overhaul.

- Refrigerators and freezers: daily temperature check; annual check of compressor and refrigerant levels.
- Water baths: daily temperature checks, weekly decontamination.
- Refrigerate Centrifuges: weekly decontamination, annual inspection of motor, speed calibration and drive system.
- Autoclaves: daily temperature check and monthly spore strip testing.
- pH meters: single reference buffer check before each use, multiple point check monthly.
- Pipetting devices: gravimetric volume check monthly; annual overhaul.

Assessment of Quality System

A quality system can be assessed either through an onsite inspection (audit) or by sending known but undisclosed material to the laboratories for testing (quality assessment scheme). The latter can be done within an institute by internal staff (internal quality assessment scheme – IQAS) or through an external agency (external quality assessment scheme – EQAS).

Further reading

- (1) Isenberg HD, editor-in-chief. *Clinical Microbiology Procedures Handbook*. Second Edition. ASM Press; 2004
- (2) *Quality Control in a Virology Laboratory* (<http://virology-online.com/general/QualityControl.htm>)
- (3) *Safety in a Clinical Virology Laboratory* (<http://virology-online.com/general/Safety.htm>)
- (4) Smith T, editor. *Laboratory Diagnosis of Viral Infections*. Third edition. Informa HealthCare; 1999
- (5) Storch GA. *Diagnostic Virology*. *Clinical Infectious Diseases* 2000;31:739-51
- (6) Specter SC, Hodinka RL, and Young SA. *Clinical Virology Manual*. Third edition. ASM Press; 2000
- (7) Tibbets MW, Gomez R, Kannangai R and Sridharan G. Total quality management in clinical virology laboratories. *Indian Journal of Medical Microbiology*. 2006;24:258-62
- (8) U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories (BMBL) Fifth Edition*. U. S. Government Printing Office, Washington; 2007 (http://www.cdc.gov/OD/OHS/biosfty/bmb15/BMBL_5th_Edition.pdf)
- (9) World Health Organization. *Laboratory biosafety manual*. Third Edition. WHO; 2004 (<http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf>)

Annex 1

The following websites are listed for information only and are not recommended by WHO.

Suggested sources for laboratory equipment and supplies

Abbott Diagnostics	Lab instrument systems and tests	www.abbottdiagnostics.com
AIDS Medicines and Diagnostics Service (AMDS)	HIV diagnostics	www.who.int/hiv/amds/en/
Axygen	Lab consumables	www.axygen.com
Bio-Rad Laboratories	Lab reagents & kits	www.bio-rad.com
BD (Becton, Dickinson & Company)	Lab instruments, reagents and kits	www.bd.com
Beckman Coulter	Lab instruments, reagents and kits	www.beckmancoulter.com
bioMérieux	Lab instrument systems and tests	www.biomerieux.com
Labindia Instruments Pvt. Ltd.	Lab instruments & reagents	www.labindia.com
Ortho-Clinical Diagnostics	Diagnostic products and services	www.orthoclinical.com
Remel	Lab reagents & tests	www.remelinc.com
Roche	Lab instruments and tests	www.roche.com
Span Diagnostics Ltd	Lab instruments, reagents and kits	www.span.co.in
Thermo Scientific	Lab instruments	www.thermo.com
Transasia Biomedicals Ltd	Lab instruments, reagents and kits	www.transasia.co.in

Sources of EQAS

American Association of Bioanalysts – Proficiency Testing Service	www.aab-pts.org/
CDC – External QA Programs for Clinical (Medical) Laboratories	wwwn.cdc.gov/mlp/EQA_qlist.aspx
WHO – EHT – Quality Assurance	www.who.int/diagnostics_laboratory/quality/en

Bio-specimen Shipping Training (IATA)

www.worldcourier.com

www.saftpak.com

Annex 2

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Laboratories play a critical role in the surveillance, diagnosis and monitoring of viral disease. Establishment of a reliable viral laboratory is a prerequisite for a strong public health response to emerging viral diseases. These guidelines provide the minimum requirements for establishing a national virology laboratory and cover key aspects of physical infrastructure, human resources, technologies and quality systems. The guidelines should be of use to policy-makers and laboratory directors in the developing countries in establishing virology laboratories in their respective countries, thus enhancing national self-reliance in this area.



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