

Manual for the laboratory diagnosis of measles virus infection

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Glossary

CF	complement fixation
CPE	cytopathic effect
EIA	enzyme immuno-assay
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
g	gram
HAI	haemagglutination inhibition
IF	immunofluorescence
IgA	immunoglobulin type A
IgG	immunoglobulin type G
IgM	immunoglobulin type M
IU	International Unit
ml	millilitre
nm	nanometre
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PRNT	plaque reduction neutralization test
RIA	radio-immuno-assay
rpm	revolutions per minute
RRL	regional reference laboratory
RT	reverse transcription
TMB	tetramethylbenzidine
µl	microlitre
VN	virus neutralization tests
VTM	virus transport medium
WHO	World Health Organization

1. Introduction

1.1 Purpose and target audience of this guide

This manual aims to assist in effective measles virological surveillance by:

- presenting information on the agent, the disease, the immune response and prevention strategies;
- discussing the role of the laboratory in measles control and prevention and the requirements for laboratory surveillance; and
- presenting detailed descriptions of the laboratory procedures recommended for the diagnosis of measles infection by detecting specific antibody.

It is intended for use by virologists and technologists working in laboratories collaborating in measles control and elimination efforts. It may also be of interest to managers of measles control programmes and field staff, who will be better able to appreciate the role of the laboratory and use it appropriately.

1.2 Progress with measles control

Despite the availability of an effective vaccine, measles continues to be one of the leading causes of childhood morbidity and mortality in many regions of the world. Global immunization coverage increased dramatically between 1983 and 1990 from less than 20% to 80%, and has remained at close to that level. This increase in coverage was accompanied by a decline in reported measles cases from pre-immunization levels of over 4 million cases per year to 0.7 million in 1997. It is estimated, however, that 31 million cases and one million measles-related deaths occurred in 1997⁽¹⁾ and it is known that global figures conceal great disparities between regions and countries. Thus vaccine coverage rates range from less than 50% to greater than 90%, and case fatality rates from 0.1% in industrialized countries to 10–30% in some outbreaks in high-risk populations.

1.3 Feasibility of eradication

Measles is considered an eradicable disease due to the single serotype, effective vaccine, lack of naturally occurring non-human reservoirs and high clinical expression of the disease. The high communicability of measles infection, its resemblance in the prodromal stage to other febrile rash diseases, and the occasional occurrence of asymptomatic and non-classical cases are seen as challenges which can be surmounted. Some efforts are currently being directed towards the elimination of measles, defined as the sustained interruption of transmission in a sizeable geographic area with the continuation of vaccination to guard against reintroduction. Global eradication will be based on successful elimination in all countries.

1.4 WHO targets and goals

The World Health Organization measles control targets have evolved over the past decade. The initial target of 80% infant immunization coverage was achieved in 1990. The 1990 goals set by the World Summit for Children of 95% reduction in deaths and 90% reduction in cases compared with pre-immunization levels were partially met in 1996 by an estimated decrease of 88% in measles-associated mortality and 78% in morbidity.

The Region of the Americas was the first, in 1994, to declare a goal of measles elimination by the year 2000. An innovative strategy was formulated aimed at interrupting transmission of indigenous measles virus from the countries of the western hemisphere. Elimination strategies have been successfully implemented in many countries of Latin America and the Caribbean.

By 1998, regional goals for the elimination of the measles have been established in three of the six regions of WHO: Region of the Americas (AMR) by 2000, the European Region (EUR) by 2007, and the Eastern Mediterranean Region (EMR) by 2010. Globally, 115 countries have set a measles elimination goal. Specific activities aiming at measles elimination have been also implemented in the countries in southern Africa, the Pacific Islands, Australia, New Zealand and Mongolia.

2. Measles infection

2.1 The virus

The measles virus is a member of the *Morbillivirus* genus of the family Paramyxoviridae. Within the morbilliviruses, the measles virus is most closely related to the rinderpest sub-group than to the canine distemper sub-group. The virions are pleomorphic and range in size from 100 to 300 nm.

The measles virus is antigenically stable and genetic differences are few among vaccine strains. However, wild-type viruses are more variable. Several different genotypes of wild measles virus are currently circulating worldwide and this genetic variation provides the basis for the application of molecular epidemiological techniques to study the transmission of measles virus.⁽²⁾

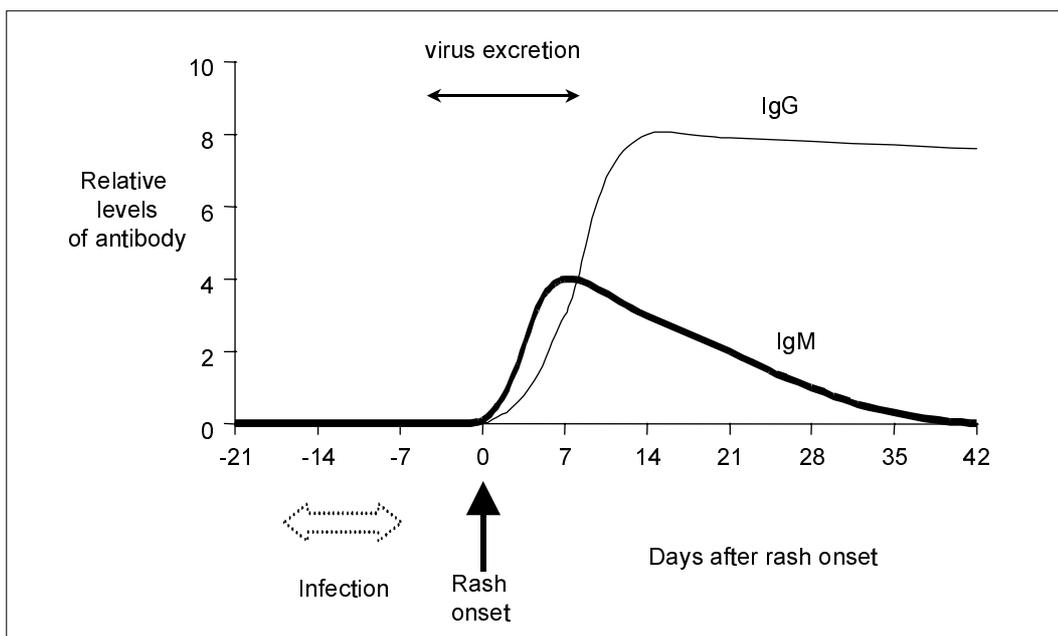
2.2 Pathogenesis

Measles is typically a febrile rash disease with an incubation period of 10 days (range 7–18 days) between infection by the respiratory route and the onset of fever. Virus replication initially takes place in tracheal and bronchial epithelial cells, followed by invasion of local lymph nodes. The disease spreads through blood monocytes to other organs such as spleen, thymus, lung, liver, kidney, conjunctivae and skin. Virus replication occurs in these tissues and measles virus is present in the prodromal stage of the disease in nasal secretions, the conjunctivae, blood and urine.

2.3 Immune response to natural infection

Cell-mediated immune responses appear to be important in both the pathology and recovery from the disease. Measles-specific immune suppression begins with the onset of clinical disease, before the rash, and continues for many weeks after apparent recovery.

Figure 1: Antibody response to measles virus infection



Antibodies are first detectable when the rash appears, and life-long protection results from natural infection. IgM antibodies are produced initially, followed by IgG and IgA in serum and secretions.

Both IgM and IgG are initially produced. However, IgM antibodies peak at 7–10 days after rash onset and fall rapidly, rarely being detected more than 8 weeks after rash onset. The presence of IgM is generally accepted as evidence of primary measles infection (by wild virus or vaccine). However, absence of IgM, particularly in samples drawn within 3 days of rash onset, does not exclude infection, as sensitivity of some of the IgM assays may be low. IgG antibody peaks about 2 weeks following rash onset and subsequently declines, but is detectable for years after infection.

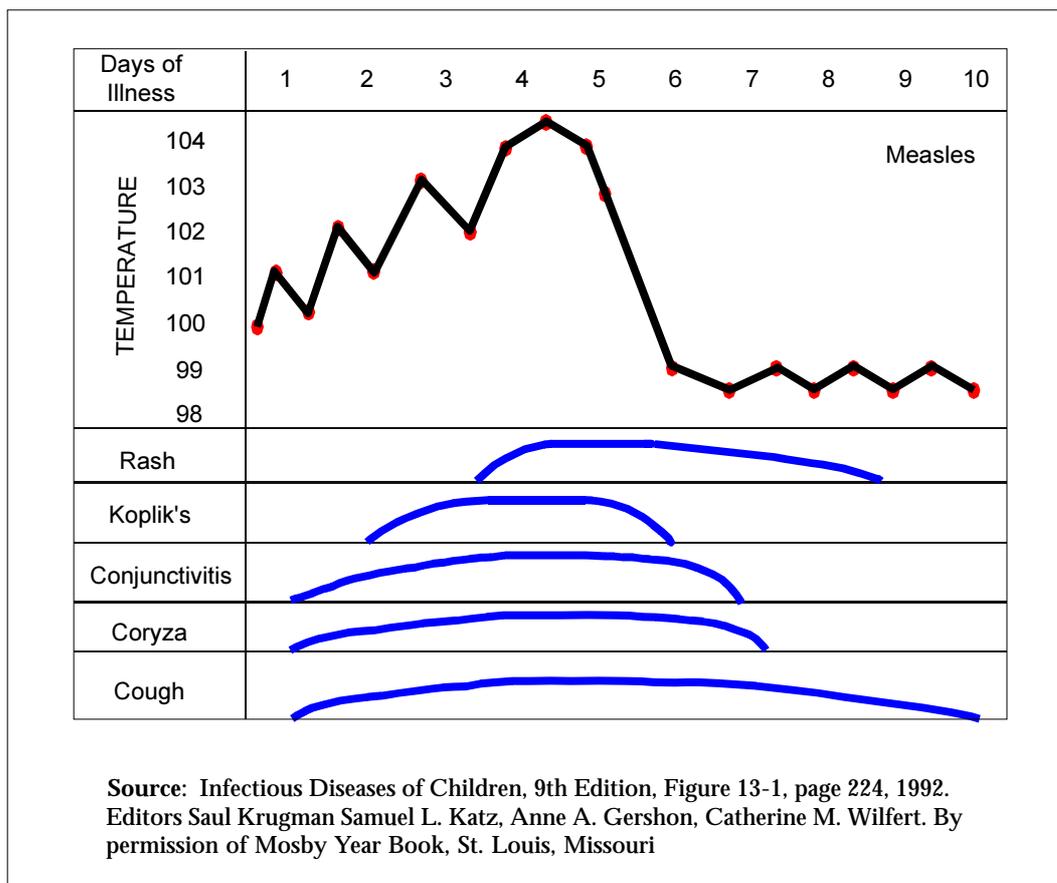
For a more complete review of the virology, pathogenesis and immunology of measles virus, see Bellini & Griffin.⁽³⁾

3. Measles clinical presentation and differential diagnosis

3.1 Clinical presentation

The incubation period is usually 10 days (and can range from 7 to 18 days) from exposure to onset of fever. The disease is characterized by prodromal fever, conjunctivitis, coryza, cough and Koplik spots on the buccal mucosa. This is the period of maximum respiratory transmission to susceptible individuals. A characteristic red rash (maculo-papular erythematous rash) appears on the third to seventh day, beginning on the face, becoming generalized and lasting four to seven days. The period of communicability continues for 4 to 5 days after rash onset, although the patient is then less infectious.

Figure 2: Time course of clinical events in measles disease



Measles standard case definition
Any person with fever of 38°C or more (if not measured “hot to touch”) and maculo-papular rash (i.e. non-vesicular) and at least one of the following: cough, coryza (i.e. runny nose) or conjunctivitis (i.e. red eyes) or any person in whom a health professional suspects measles.

3.2 Differential diagnosis

The non-specific nature of the prodromal signs and the existence of mild cases make clinical signs unreliable as the sole diagnostic criteria of measles disease. As disease prevalence falls many medical practitioners will be inexperienced in recognizing measles and the need will increase for laboratory methods of distinguishing measles from other clinically similar diseases. Misdiagnosis of measles is, for example, more common among young infants, and outbreak associated cases are more likely to be laboratory confirmed than sporadic cases. Measles may resemble infections with rubella, dengue fever, ECHO, coxsackie, parvovirus B19 and herpesvirus 6 viruses, as well as some bacterial and rickettsial diseases. Moreover, there are other conditions that may present in a similar form, including Kawasaki’s disease, toxic shock and drug reactions. Selection of appropriate testing algorithms will depend upon the prevalence of these conditions in countries, and the availability of adequate laboratory services. Countries in the elimination phase with successful measles immunization programmes are finding that a high percentage of suspected measles cases are due to rubella. As measles and rubella may be coincidentally eradicated with use of MMR vaccine, testing negative measles serum samples for rubella will provide useful information for rubella surveillance.

Commercial and research-level tests have been developed, but there is a need for batteries of tests for febrile rash disease, preferably presented in a similar format for easy utilization by diagnostic laboratories.

4. Phases of measles prevention and control

Prevention of measles infection rests on successful immunization with currently available live, attenuated vaccine. The immunity produced by the vaccine lasts many years and is probably life-long. The recommended age of administration in infant immunization programmes is 9 to 15 months, and countries have opted for either one or two-dose schedules. Vaccine efficacy is 85% at 9 months of age and increases to 90–95% at 12–15 months of age. Measles virus is highly transmissible. High routine immunization coverage can reduce measles incidence but will not prevent accumulation of susceptibles, which can lead to outbreaks if virus is introduced into a population where the number of susceptibles is above the critical threshold for that population.

Prevention of measles at the community level requires the simultaneous vaccination of a large proportion of children in an epidemiologically determined age range. The introduction of measles vaccine into routine immunization programmes has resulted in a considerable reduction in the incidence of the disease and its associated morbidity and mortality.

There are two sequential phases for measles immunization programmes.

- measles control phase; and
- measles outbreak prevention and elimination phase.

4.1 Measles control phase

Measles control is defined as a significant reduction in the incidence and mortality from measles. When high levels of vaccine coverage are attained (i.e. vaccine coverage is in the range of 75–80%), measles incidence decreases and the intervals between outbreaks are lengthened (i.e. 4–8 years) when compared to those observed during the pre-vaccine era (i.e. 2–4 years).

Sustained high levels of vaccine coverage result in an increasing proportion of cases among individuals in older age groups. As the vaccine coverage improves there is an expected increment in the proportion of cases with vaccination history.

4.2 Outbreak prevention and elimination phase

Once measles has been drastically and persistently reduced, due to increased immunization coverage, countries may wish to implement strategies aimed at the prevention of periodic measles outbreaks. These strategies include improved surveillance in order to understand the changing epidemiology of the disease (e.g. changes in the age distribution of cases, settings for measles transmission, etc.) and to identify high-risk populations.

It is possible to predict outbreaks and to prevent them by timely immunization of susceptible individuals in high-risk populations and by improving overall population vaccine coverage level. If an outbreak is anticipated supplementary immunization activities should be considered.

A number of developing and industrialized nations have begun to implement innovative measles immunization and surveillance strategies in an effort to eliminate indigenous transmission of measles virus. The development of innovative strategies has been prompted by the ongoing low level transmission and intermittent outbreaks in these countries, despite high coverage with either one or two dose measles-immunization schedules.

Measles-elimination strategies are currently defined on the basis of past experience. A common principle to all measles strategies currently being implemented is the need to maintain the number of susceptible individuals in the population below a certain critical number required to sustain transmission of the virus.

5. Role and function of the laboratory in measles control and elimination

5.1 Role of the laboratory in measles surveillance

The laboratory has two main functions in measles surveillance:

5.1.1 *Monitoring and verifying virus transmission*

- *confirmation of outbreaks*: to confirm the clinical diagnosis in the early stages of an outbreak;
- *confirmation of cases*: to confirm or discard any suspected cases of measles once the laboratory confirmation of cases has been introduced; and
- *identification of measles virus strains* and genetic characterization of viral isolates.

5.1.2 *Monitoring susceptibility profile of the population*

- determination of the age distribution of susceptibility to measles in order that the need for immunization campaigns might be assessed;
- evaluation of the impact of mass campaigns.

For each sequential phase of the measles control programme there are specific surveillance activities (Table 1). In performing its functions, laboratories cannot act in isolation but must be organized into a supportive network which will efficiently provide accurate information to the programme.

Table 1: Role of the laboratory in measles control and elimination

Phase of measles control	Function
Control	<ul style="list-style-type: none">• Confirm initial cases during outbreaks.• Analyse wild virus strains from selected cases to facilitate genetic characterization of circulating measles viruses.
Outbreak prevention and elimination	<ul style="list-style-type: none">• Confirm the clinical diagnosis of suspected cases to help in early detection of virus circulation.• Analyse wild virus strains from selected cases to identify virus strains circulating and to complete global genomic mapping of measles virus• In special circumstances, establish seroprevalence of the population and assist outbreak forecasting.

5.2 The laboratory network for measles surveillance

There are five main objectives in setting up a network of laboratories which support various aspects of measles eradication:

- to develop standards for the laboratory diagnosis of measles and provide the necessary support as the programme evolves;
- to establish mechanisms for reference and support for regional and national laboratories in the diagnosis of measles and other rash illness;
- to provide training resources and facilities for staff of regional and national laboratories;
- to provide a source of reference materials and expertise for the development and quality control of improved diagnostic tests;
- to serve as a bank of measles virus isolates for molecular epidemiology and reference sera for quality control.

Individual laboratories will not be expected to undertake the full range of tasks listed above, but will perform specific duties according to the needs of the national/regional programmes and their level within the network. Laboratories involved in the network will be monitored by proficiency testing in selected techniques and by performance evaluation.

It is essential that the laboratory network is planned in tandem with regional control and elimination programmes, and established with properly trained personnel, suitable equipment and reagents. The measles laboratory network is being organized on four levels. (See Figure 3.)

5.2.1 *Global specialized laboratories*

These are laboratories which have set the technical standards for laboratory diagnosis. Their responsibilities extend to measles laboratories in all regions and countries.

5.2.2 *Regional reference laboratories*

These are centres of excellence in each region able to undertake international responsibilities. They will serve as reference laboratories for national laboratories in neighbouring countries and to serve as national laboratories in their own countries. Each region may have up to 3–4 regional reference laboratories (RRLs).

5.2.3 *National laboratories*

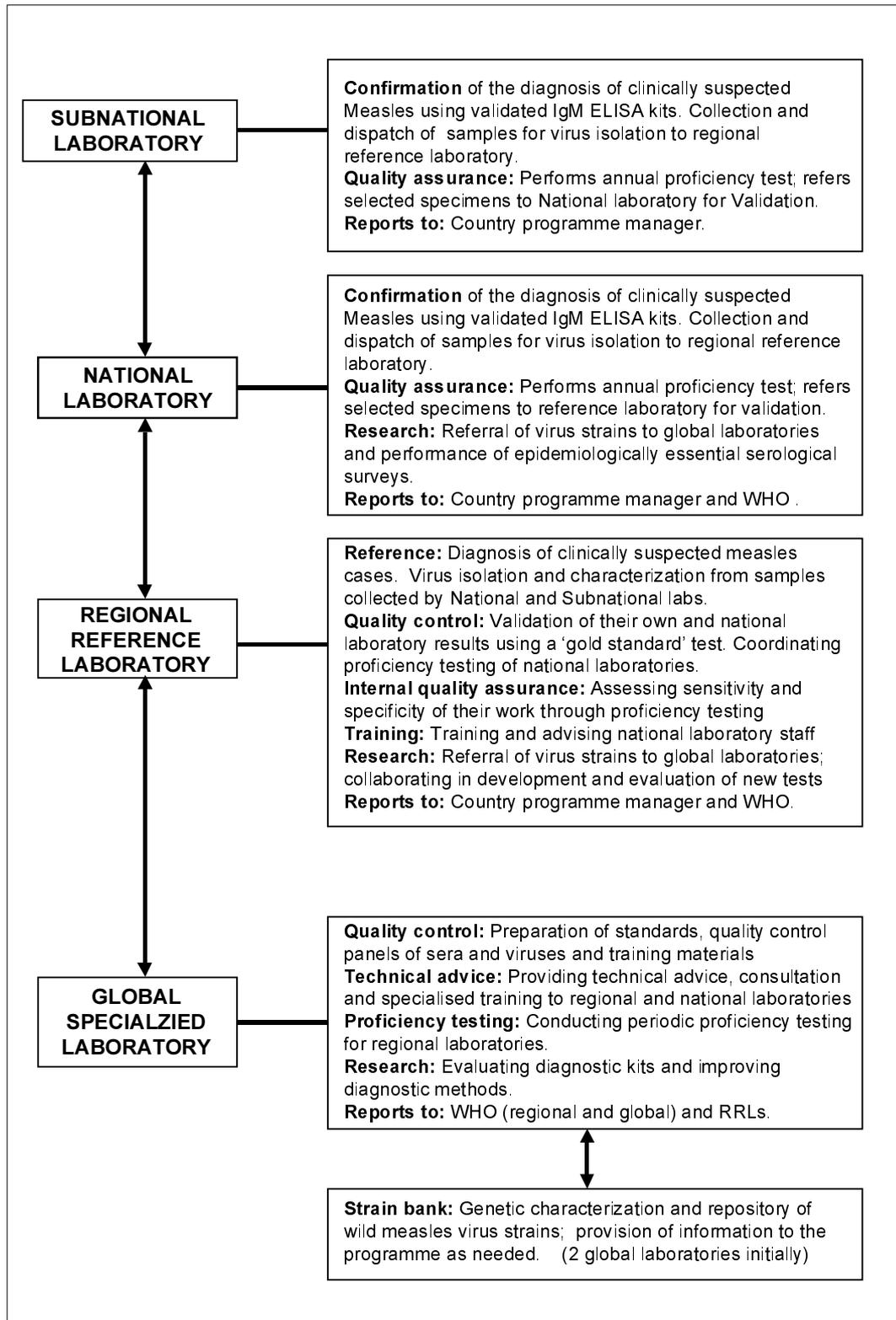
These will have the closest links with national programme managers. They will test specimens from suspected cases by IgM enzyme-linked immunosorbent assay (ELISA) and report directly to the programme manager. The number of national laboratories will depend on the epidemiological priorities and resources available.

Due to the significant population size of some countries, testing of specimens for measles may be beyond the capacity of a single national laboratory. In these countries *subnational laboratories* may also be established at provincial or prefecture levels.

To achieve the objectives outlined in Figure 3, the measles network laboratories should have:

- known links to the immunization and surveillance units at the Ministry of Health;
- proven capability to perform testing;
- appropriately trained scientists and technicians;
- adequate laboratory facilities and resources to cover running costs; and
- suitable equipment to conduct routine serological assays.

Figure 3: Laboratory network for measles surveillance activities at different levels



5.3 Laboratory network communication

The smooth functioning of the laboratories will depend on the establishment of a system of communication within the network and with the programme.

Standard referral and reporting forms will be developed to ensure that all essential patient information is transmitted (see Annex 1 for sample form). The format and timing of result reporting will be agreed upon in consultation with programme managers.

Monitoring indicators of field and laboratory performance will be evaluated and will include:

- the proportion of samples received in good condition;
- the proportion with properly completed laboratory forms; and
- the proportion of results reported within seven days of receipt of specimen in the laboratory.

Virologists and epidemiologists at all levels must establish mechanisms to exchange information on a regular basis and evaluate performance indicators of the surveillance system.

For example, the global specialized laboratories should meet at least once a year, the regional reference laboratories should meet one or two times a year and the national laboratories should hold meetings with the epidemiologist at least once a month.

5.4 Tests available for laboratory diagnosis of measles infection

It is recommended that measles be diagnosed using serological methods which measure virus-specific antibody in single or paired sera. However measles virus can be also be detected from various clinical samples by using cell culture techniques or molecular techniques. Assays based on detection of the measles virus are not suitable as diagnostic tests but are useful for detection of virus or genome for molecular epidemiological studies. A summary of measles identification methods follows.⁽⁴⁾

5.4.1 Serological assays

Measles infection is diagnosed serologically by 1), detecting measles specific IgM antibodies; or 2), quantifying measles specific immunoglobulins in order to demonstrate a significant rise in IgG between paired acute and convalescent sera.

1) Measles specific IgM antibodies

Measles-specific IgM antibodies appear within the first few days of the rash and decline rapidly after one month (Figure 1). Their presence provides strong evidence of current or recent measles infection. IgM is also produced on primary vaccination, and, although it may decline more rapidly than IgM produced in response to the wild virus, vaccine and wild virus IgM cannot be distinguished by serological tests. A vaccination history is therefore essential for interpretation of test results.

The following methods are commonly used to detect measles-specific IgM.

- *IgM capture ELISA*, requires only one blood sample for case confirmation. Assays show 97% sensitivity compared with the plaque reduction neutralization test (PRNT) in detecting infection in vaccinated infants.⁽⁵⁾ In clinically confirmed cases, the sensitivity and specificity of capture assays were 91.8 and 98.2% respectively, while the positive and negative predictive values were 98.2 and 92.0% respectively.⁽⁶⁾ The test can be done with minimal training and results may be available within 2–2.5 hours of starting the assay.

Capture ELISA assays are considered superior to indirect assays, since they do not require the removal of IgG antibodies. Several capture IgM ELISA kits are commercially available, though not all have the same sensitivity and specificity as the assays reported above.

- *IgM indirect ELISA*, requires only one blood sample for case confirmation. In clinically confirmed cases, the sensitivity and specificity of indirect assays were 90.3 and 98.2% respectively, while the positive and negative predictive values were 98.2 and 90.5 respectively.⁽⁶⁾ The test can be done with minimal training and results can be available within 3–3.5 hours of starting the assay.

Indirect ELISA assays are the most widely used. However, this type of assay requires a specific step to remove IgG antibodies. Problems with the incomplete removal of IgG can lead to inaccurate results.

2) Quantification of measles-specific immunoglobulins by:

- *Virus neutralization*, the plaque reduction neutralization test (PRNT), requires two serum samples, acute and convalescent, and shows 100% sensitivity in confirming clinical measles. Single titers of greater than 120 are consistent with 100% protection against clinical measles.⁽⁷⁾ The test is not easy since it requires trained technicians with expertise in tissue culture. Results are available 10 days after the receipt of the convalescent serum.
- *Haemagglutination inhibition (HAI)* requires two serum samples, acute and convalescent, and shows 98% sensitivity in detecting antibody increase in vaccinated students and 100% sensitivity in vaccinated infants.⁽⁸⁾ The test is not easy since it requires technicians trained in viral serology. Results are available 2 days after receipt of the convalescent serum.

5.4.2 Virus isolation

Measles virus can be cultured with difficulty from urine, nasopharyngeal specimens or peripheral blood lymphocytes during the prodrome and rash stages of the disease. Thereafter virus excretion declines rapidly. Detection and identification of the virus in cell culture may take several weeks. Possession of a measles virus isolate permits genomic analysis and comparison with other strains from different locations and years, providing information on its origin and transmission history. This molecular epidemiological analysis requires a collection of indigenous strains from endemic countries.

Virus isolation is costly, time-consuming and requires a sophisticated virology laboratory with cell culture facilities and virus isolation capabilities. Measles virus is extremely temperature labile and specimens for virus isolation must be transported to the laboratory rapidly under reverse cold chain conditions. For the above reasons it has been recommended that virus isolation not be used for primary diagnosis and be limited to regional reference and global specialized laboratories for purposes of genetic analysis only.

5.4.3 Reverse transcription polymerase chain reaction (RT-PCR)

Amplification of measles RNA after reverse transcription (RT-PCR) is done in specialized laboratories at the global level for specific purposes and is not appropriate for routine use in measles surveillance programmes.

RT-PCR has several technical problems related to the sensitivity and variability of the results when tested in duplicate with a different portion of the same sample. In addition, amplified DNA can cause cross-contamination unless stringent standards are maintained.

The cost of PCR methods and the requirements of equipment and technical skills make this method less suitable than other methods available.

The salient features of the above tests are summarized in Table 2.

Table 2: Laboratory diagnosis for measles in clinical materials

Type of test	Purpose of test/ source material	Method	Remarks
Serological assays	Demonstration of measles specific IgM antibody	Indirect IgM ELISA IgM-capture ELISA Enzyme immuno-assay (EIA) for detection of IgM in oral fluids	<ul style="list-style-type: none"> • IgM ELISA requires only one blood sample and kits are available commercially. • Capture assays are generally superior to indirect assays. • Detection of IgM in oral fluids by EIA usually requires an amplification step to reach the sensitivity and specificity of detecting IgM antibody in serum by ELISA.
	Demonstration of measles specific IgG antibody	IgG ELISA	<ul style="list-style-type: none"> • IgG ELISA requires paired sera taken 10–14 days apart. Kits are available commercially.
	Demonstration of total measles specific immunoglobulins in sera	Virus neutralization tests (VN) HAI	<ul style="list-style-type: none"> • VN is useful for detecting immunity. It requires tissue culture facilities and paired sera. Results are available 1 month after onset. • HAI requires paired sera and a source of appropriate monkey red blood cells. Results are available 3 weeks after onset. <p>All these tests are time consuming.</p>
Virus isolation	From urine, nasopharyngeal swabs, or peripheral blood lymphocytes	Cell culture and immuno-fluorescence	<ul style="list-style-type: none"> • Requires carefully collected and transported specimens. • Tissue culture and immunofluorescence facilities needed.
Detection and characterization of genomic viral RNA	<u>On clinical material</u> Nasopharyngeal swab, urine or peripheral blood lymphocytes <u>On wild virus isolates</u>	RT/nested PCR and nucleotide sequence analysis	<ul style="list-style-type: none"> • Special equipment and technical skills. Danger of cross-contamination. • Sequence analysis is needed for genotyping of wild viruses.

5.4.4 Selection of the most appropriate laboratory test for measles diagnosis

The ideal test for measles diagnosis is one that:

- requires a non-invasive sample;
- requires only one sample;
- can use a sample collected at first contact with the patient;
- is highly sensitive (one which detects a high proportion of true measles cases) and specific (has a low level of false positivity);
- has a high positive predictive value (the proportion of cases diagnosed as measles which are truly measles); and
- is easy to perform at local level and provides quick accurate results, upon which control measures can be implemented.

The assay recommended for the WHO measles laboratory network is the ELISA test for the detection of measles-specific IgM antibodies. These tests are commercially available and have been evaluated with the virus neutralization and the haemagglutination inhibition assays.

Reasons for selection of the IgM ELISA

Number of samples required: One serum specimen, preferably collected between 3 and 28 days after onset of illness, but for practical reasons, collected as soon as the case is seen.

Sensitivity: Compared to PRNT in vaccinated infants, 97%; in confirming clinical measles, 91.8%

Specificity: In confirming clinical measles, 98.2%

Predictive value: Positive, 98.2%; Negative, 92.0%

Ease of performance: Technically, measles IgM assays resemble the ELISAs utilized for HIV screening now being performed in laboratories worldwide. Minimal training is therefore needed for the performance of this test. A capable laboratory may be able to provide results within twenty-four hours (even within two hours in some laboratories) after the sample reaches the laboratory.

Given the need for differential diagnosis of febrile rash illness, a battery of tests for three of the most frequently occurring rash diseases in a given area (e.g. measles, rubella and dengue) would be desirable.

6. Collection, storage and shipment of specimens for measles diagnosis and outbreak investigation

Samples for measles diagnosis and virus isolation should be collected, depending on which of the phases of measles control and elimination a particular country is classified (Table 3).

Table 3: Samples to collect for measles serology and measles virus isolation according to the different phases of measles control and elimination

Phase	Function of the lab	Epidemiological situation serology	Sample: blood for measles	Sample: specimen for virus isolation
Control	Confirm initial cases during outbreaks	Isolated case	NO	NO
	Analyse wild virus strains from selected cases to facilitate genetic characterization of circulating measles viruses	Outbreak	YES From initial 5–10 cases to confirm outbreak and suspected spread cases	YES Approximately 10 specimens or until one or two isolates have been made
Outbreak prevention and elimination	Confirm the clinical diagnosis of all suspected cases to help in early detection of virus circulation	Isolated case	YES from all suspected measles cases	YES from suspected measles cases
	Analyse wild virus strains and monitor their distribution and circulation to help assess the impact of immunization strategies	Outbreak	YES From initial 10 cases to confirm outbreak and suspected spread cases	YES Approximately 10 specimens. More may be collected from newly infected districts

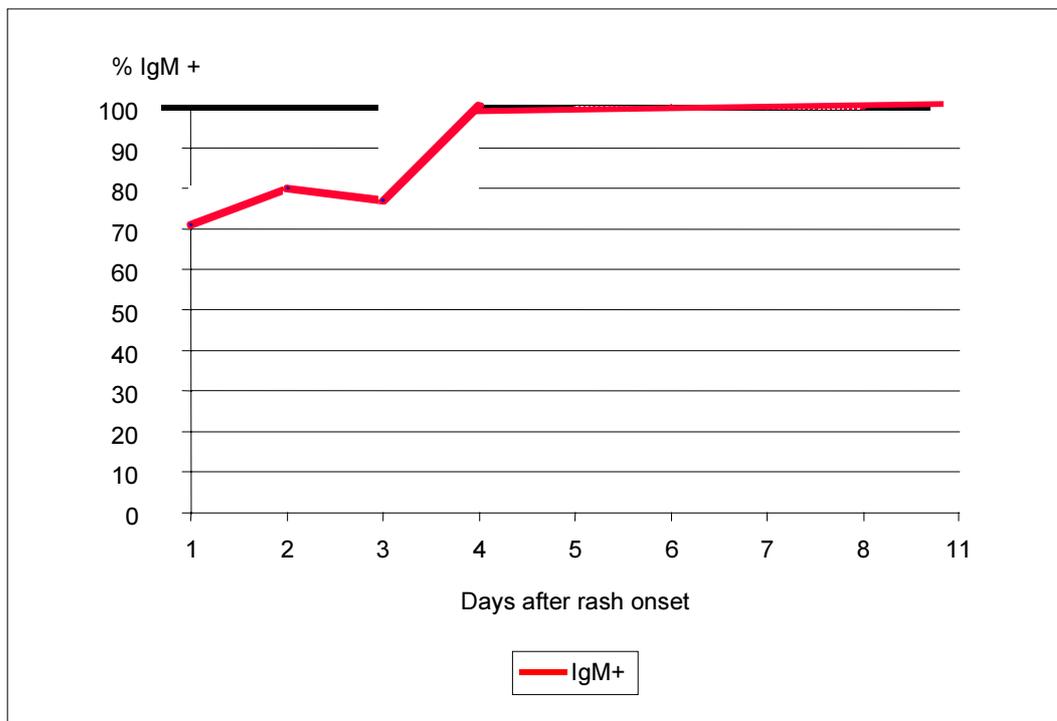
6.1 Serological specimens for measles diagnosis

6.1.1 Timing of single blood specimens for IgM serology

The correct timing of specimens with respect to the clinical signs is important for interpreting results and arriving at an accurate conclusion.

While IgM ELISA tests are more sensitive between day 4 and 28 after rash onset (Figure 4), a single serum obtained at the first contact with the health care system, regardless of which day following the rash onset this occurs, is considered adequate for measles surveillance.

Figure 4: IgM results of 153 specimens tested using antibody capture IgM ELISA by day of collection after rash onset.⁽⁹⁾



IgM capture tests for measles are often positive on the day of rash onset. However, in the first 72 hours after rash onset, up to 30% of tests for IgM may give false-negative results. Tests which are negative in the first 72 hours after rash onset should be repeated; serum should be obtained for repeat testing *more than 72 hours after rash onset*. IgM is detectable for at least 28 days after rash onset and frequently longer.

6.1.2 Second blood samples

These may occasionally be required under the following circumstances:

- the first blood sample submitted for IgM was collected within four days of rash onset and is negative by ELISA. The laboratory may request a second sample for repeat IgM testing given the probability of false negatives on early samples. (See 6.1.1);
- the measles IgM capture ELISA gives an equivocal result;
- the clinician needs to make a definitive diagnosis on an individual patient with an initial negative result.

A second sample for IgM testing may be collected anytime between 4 and 28 days after rash onset. Collection of a second sample 10–20 days after the first will permit the laboratory to retest for IgM or, if a quantitative method is available, test for an increase in IgG antibody level. But this is not recommended on a regular basis since additional information obtained will be limited.

6.1.3 Collection procedure

- collect 5 ml blood by venipuncture into a sterile tube labelled with patient identification and collection date;
- whole blood should be centrifuged at 1000 x g for 10 minutes to separate the serum;
- blood can be stored at 4–8°C for up to 24 hours before the serum is separated;
- do not freeze whole blood;
- if there is no centrifuge, blood should be kept in the refrigerator until there is complete retraction of the clot from the serum;
- carefully remove the serum, avoiding extracting red cells, and transfer aseptically to a sterile labelled vial;
- label vial with patient's name or identifier, date of collection and specimen type;
- store serum at 4–8 °C until it is ready for shipment;
- fill in case investigation forms completely (Annex 1). Three dates are very important:
 - date of last measles vaccination
 - date of rash onset
 - date of collection of sample.

6.1.4 Storage of blood specimens :

a) Outside the laboratory

- whole blood may be held at refrigerator temperatures (4–8°C) if it can be transported to arrive at the testing laboratory within 24 hours;
- if the above step is not possible, the tube must be centrifuged to separate the serum, which is transferred to a sterile, labelled screw-capped tube for transport to the laboratory;
- if no centrifuge is available, the blood is held in a refrigerator for 24 hours for clot retraction. The serum is then carefully removed with a fine-bore pipette and transferred to a sterile tube;
- sterile serum should be shipped on wet ice within 48 hours, or stored at 4–8°C for a maximum period of seven days;
- sera must be frozen at -20°C for longer periods of storage and transported to the testing laboratory on frozen ice packs. Repeated freezing and thawing can have detrimental effects on the stability of IgM antibodies.

b) Inside the laboratory

At the testing laboratory long-term storage of sera should be stored frozen at -20°C.

6.1.5 Shipment of blood specimens

- specimens should be shipped to the laboratory as soon as possible. Do not wait to collect additional specimens before shipping;
- place specimens in ziplock or plastic bags;
- use Styrofoam boxes or a thermos flask;
- place specimen form and investigation form in plastic bag and tape to inner top of Styrofoam box;
- if using ice packs (these should be frozen), place ice packs at the bottom of the box, and along the sides, place samples in the centre, then place more ice packs on top;
- arrange shipping date;
- when arrangements are finalized, inform the receiver of time and manner of transport.

6.2 Urine for measles virus isolation

Ten to fifty millilitre samples are adequate. First passed, morning specimens are preferable. Most of the measles virus excreted in urine samples is located in epithelial cells in the urine. Concentration of the virus is achieved by centrifugation of the urine and resuspension of the pelleted cells in a suitable viral transport medium. Urine should NOT be frozen before the concentration procedure is carried out.

6.2.1 Timing

Measles virus isolation is most successful on specimens collected as soon after rash onset as possible and at least within 5 days of rash onset.

6.2.2 Collection procedure

- urine should be collected into a sterile container;
- it should be placed at 4–8°C prior to centrifugation;
- centrifugation should be done within a few hours (see below).

6.2.3 Storage and shipment of urine samples

- whole urine samples may be shipped in well-sealed containers at 4°C but centrifugation within 24 hours after collection is preferable;
- centrifugation should be done at 500 x g (approximately 1500 rpm) for 5 minutes at 4°C;
- the supernatant should be discarded and the sediment resuspended in 1 ml of viral transport medium (Annex 2) or tissue culture medium;
- *DO NOT FREEZE sediment if shipment is possible within 48 hours and DO NOT FREEZE urine before concentration procedure is carried out;*
- the resuspended pellet may be stored at 4°C and shipped within 48 hours to the appropriate measles reference laboratory. Alternatively, it may be frozen at -70°C in VTM (Annex 2) and shipped on dry ice in a well-sealed screw capped vial to protect against carbon dioxide contamination of the pellet.

6.3 Nasopharyngeal specimens for measles virus isolation

6.3.1 Timing

Nasopharyngeal specimens for virus isolation must be collected as soon as possible after onset and not longer than seven days after the appearance of the rash, when the virus is present in high concentration.

6.3.2 Collection procedures

Nasopharyngeal specimens can be taken as follows (in order of higher yield of virus):

- aspiration;
- lavage; and
- swabbing the mucous membranes.
- **nasal aspirates** are collected by introducing a few ml of sterile saline into the nose with a syringe fitted with fine rubber tubing and collecting the fluid into a screw-capped centrifuge tube containing viral transport medium*(Annex 2);

* If viral transport medium is not available, isotonic saline solution, tissue culture medium or phosphate-buffered saline may be used.

-
- **throat washes** are obtained by gargling with a small volume of sterile saline and collecting the fluid into a viral transport medium;
 - **nasopharyngeal swabs** are obtained by firmly rubbing the nasopharyngeal passage and throat with sterile cotton swabs to dislodge epithelial cells. The swabs are then placed in sterile viral transport medium* in labelled screw capped tubes, refrigerated and transported to the laboratory on wet ice (4–8°C) within 48 hours. (see section 6.2.3)

6.3.3 Storage and transport of nasopharyngeal specimens

- nasopharyngeal specimens should be transported in viral transport medium* (Annex 2), and should be shipped on wet ice (4–8 °C) to arrive at the testing laboratory within 48 hours;
- if arrangements cannot be made for rapid shipment, swabs should be shaken in the medium to elute the cells and then removed;
- the medium or nasal aspirate should be centrifuged at 500 x g (approximately 1500 rpm) for 5 minutes at 4°C and the resulting pellet resuspended in cell culture medium;
- the suspended pellet and the supernatant are stored separately at -70°C and shipped to the testing laboratory on dry ice in well-sealed screw-capped vials to protect against carbon dioxide contamination of the specimens.

NOTE: Samples for virus isolation

The laboratory should agree in advance with the epidemiologists on the number, type and locations that are most appropriate for collection of samples for virus isolation. Ideally, samples for virus isolation should be collected simultaneously with the blood samples for serological diagnosis and confirmation of measles virus as the cause of the outbreak. Since each type of sample has different requirements, the decision on type of samples will depend on the local resources and facilities for transport and storage.

Because virus is more likely to be isolated when specimens are collected within 3 days of rash onset, collection of specimens for virus isolation should not be delayed until laboratory confirmation of a suspected case of measles is obtained.

6.4 Safe transport of specimens and infectious materials

The safe shipment of diagnostic specimens and infectious materials is the concern of all who are involved in the process. There are correct procedures to be followed depending on the material to be transported. Most of the samples expected to be received by, or sent from, a Measles laboratory fit the following definitions which are excerpted from the WHO *Guidelines for the safe transport of infectious substances and diagnostic specimens (1997)*. This document is also available on the Internet at <http://www.who.int/emc/biosafety.html>.

-
- **diagnostic specimens** are any human material including blood, tissue and tissue fluids being shipped for purposes of diagnosis;
 - **infectious substances** are substances containing viable organisms that are known or reasonably believed to cause disease in animals or humans.

Refer to Annex 3 for detailed instructions for the safe transport of specimens fitting the above criteria and to WHO *Guidelines for the safe transport of infectious substances and diagnostic specimens (1997)* for comprehensive guidelines for safe transport of samples.

6.5 Specimen kit for measles diagnosis

Components of a specimen collection kit for measles diagnosis have been specified and are suitable for distributing to facilities collecting samples from suspected measles cases in countries in the measles elimination phase.

The basic kit for blood collection consists of:

- 5 ml vacutainer tube (non-heparinized) with 23 g needle;
- tourniquet;
- sterilizing swabs;
- serum storage vials;
- specimen labels;
- band aid;
- ziplock plastic bags;
- specimen referral form; and
- cold box with ice packs.

7. Specimen data management

A case investigation form needs to be completed for each suspected measles case investigated. A separate laboratory request form should be completed at the time of specimen collection and should accompany all specimens sent to the laboratory.

7.1 Recording receipt of specimens

The following information should be included on the laboratory request form (see example Annex 1) accompanying the specimen:

- unique Identifying Number (in an agreed format);
- in-house laboratory number (optional, but often important);
- patient name (in English script);
- age;
- province (or region);
- town/district;
- country code;
- date of last measles vaccination;
- number of doses of vaccine;
- date of onset of rash;
- does the patient fit the case definition?
- specimen type;
- date of specimen collection;
- date specimen sent to laboratory;
- date specimen received in laboratory; and
- condition of specimen on receipt.

7.2 Recording laboratory results

The information to be collected and recorded on specimen processing and results should include the following:

- case ID;
- date of assay;
- type of assay (IgM direct or indirect);
- result of assay;
- date result reported to the EPI manager; and
- was a sample sent to the RRL (yes or no)?

If yes:

- name of regional reference laboratory;
- laboratory identification of sample sent (local laboratory specimen number);
- date of sending isolate to RRL;
- date of receiving result back from RRL;
- RRL result; and
- date result reported to EPI manager.

7.2.1 Reporting laboratory activity and results

Laboratory results must be reported in a timely and accurate manner for several reasons. Reporting of laboratory results has a direct effect on the measles control and elimination programme through:

- feedback to national EPI teams for case follow up and planning supplementary immunization activities;
- coordination of the control and elimination programme through WHO and other international agencies and bodies; and
- monitoring of laboratory results and performance to identify possible problems and constraints.

Regular reporting of results will provide a continuous record demonstrating that recommended and acceptable procedures have been followed and laboratory accuracy has been at an acceptable level.

7.2.2 Feedback to EPI teams

Details of how and when laboratories report to EPI managers should be arranged locally. In general, however, all results should be reported within a week of receipt of serum sample and positive cases (in the absence of recent cases) should be reported within 24 hours. All other results should be available to the EPI managers on request. It is also helpful to the programme if a formal presentation of laboratory results is made to the EPI manager on a monthly basis.

Details of inadequate specimens and inadequate transport of specimens should be reported to EPI managers as soon as possible so that field staff can be informed and improvements made.

7.2.3 Monthly reports to WHO

All national laboratories are requested to provide a monthly report of results to WHO. This information is used to update country summaries, monitor laboratory performance and coordinate international agency activity. Data provided in the monthly reports is essential to the coordination of the programme as a whole, and it must be a priority activity of all laboratories in the network to send monthly reports in a timely and accurate manner.

Because of the amount of data involved and the time required to analyse the information it is essential that laboratories handling more than 100 specimens a year provide their monthly reports in computer database format, on computer diskettes or sent by e-mail. WHO can now provide a set of laboratory data management programs suitable for most of the measles laboratories in the global laboratory network.

8. ELISA tests for measles antibodies: principles and protocols

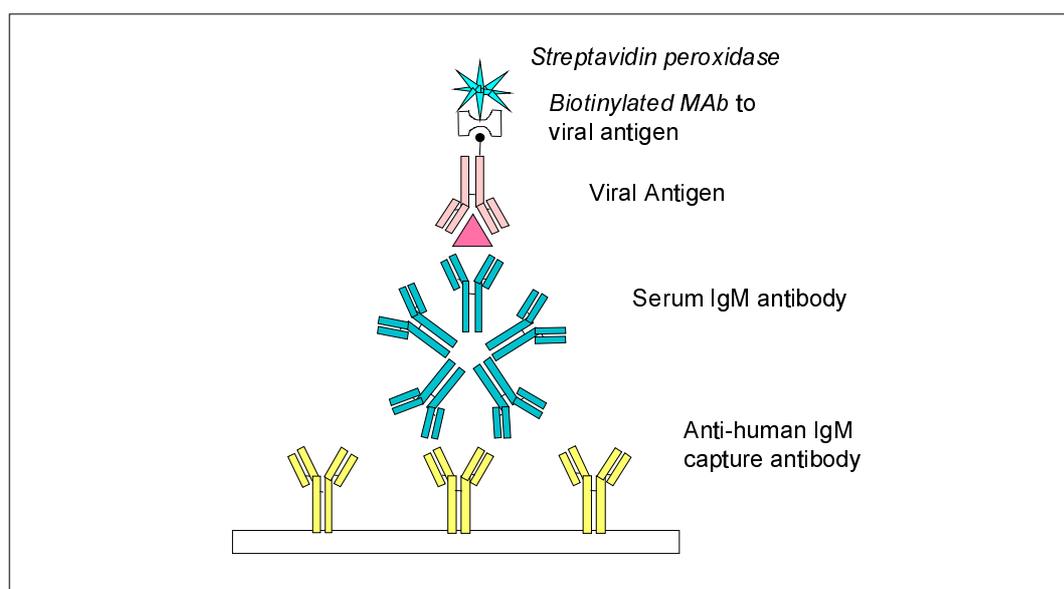
This section outlines the principles of ELISA tests for measles-specific IgM and IgG, the requirements in terms of equipment, reagents and procedure time, and the advantages/disadvantages of their use for surveillance in a measles elimination programme.

8.1 Capture ELISA for measles IgM

8.1.1 Test principle

- in the antibody-capture ELISA technique (Figure 5), IgM antibody in the patient's serum is bound to anti-human IgM antibody adsorbed onto a solid phase. This step is non virus-specific;
- the plate is then washed, removing other immunoglobulins and serum proteins;
- measles antigen is then added and allowed to bind to any measles-specific IgM present;
- after washing, bound measles antigen is detected using anti-measles monoclonal antibody, following which a detector system with chromogen substrate reveals the presence or absence of measles IgM in the test sample.

Figure 5: Measles capture IgM ELISA schematic



8.1.2 Test requirements

This test is available commercially in kit form, such as the Chemicon measles IgM EIA Procedure. The Chemicon kits contain:

- anti-human IgM coated microtitre plates;
- positive and negative control sera;
- measles recombinant nucleoprotein antigen (developed at CDC, Atlanta) and control antigen;
- enzyme-conjugated anti-nucleoprotein; and
- substrate, buffers and diluent.

Materials required but not provided

- piston-type pipettes 20, 50, 100, 150, 200, 400 and 1000 µl;
- ELISA Washer M or comparable washing devices,
- ELISA reader with wavelength 450nm (450/470nm), and
- a pocket calculator with exponential and logarithmic functions is required for the quantitative evaluation of the test.

8.1.3 Advantages/disadvantages

Table 4: Advantages and disadvantages of the currently available commercial capture ELISA for measles IgM test

Advantages	Disadvantages
<ul style="list-style-type: none">• Specimen collection and referral is simplified by the need for only one serum specimen in most cases.• The IgM capture ELISA is very sensitive since virus specific IgG antibodies in the test sera are removed by washing in the first step and cannot compete with IgM antibodies when the antigen is added.	<ul style="list-style-type: none">• Care must be exercised in evaluating results, since blood drawn within the first three days of rash onset may give a negative result in up to 20–30% of the cases.

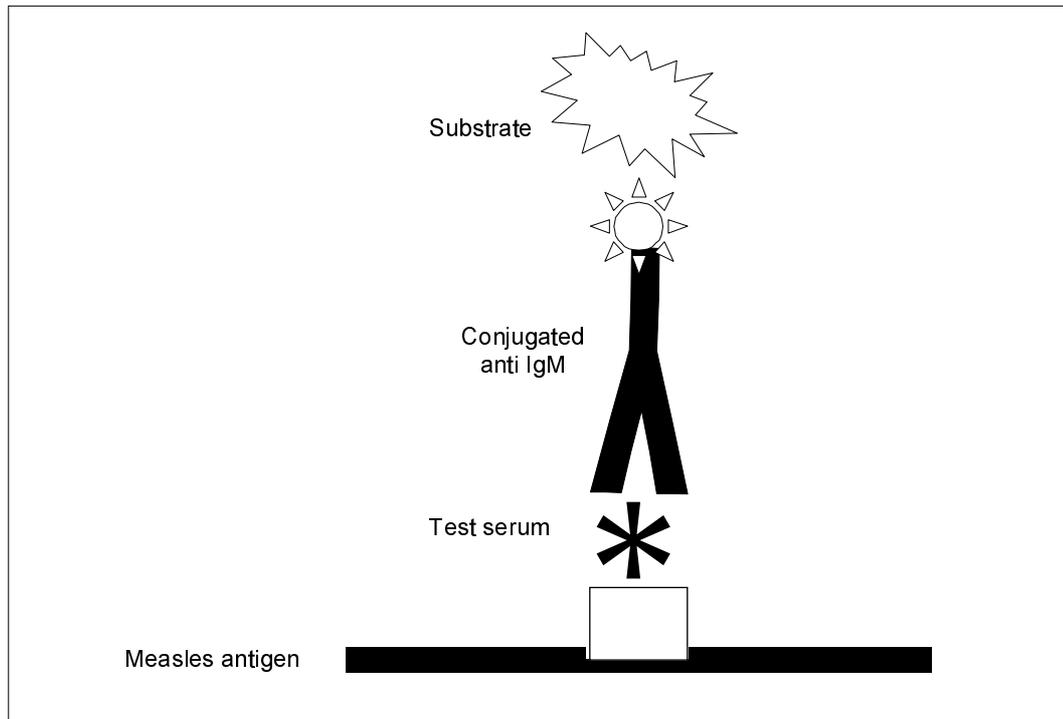
8.2 Indirect ELISA for measles IgM

8.2.1 Test principle

- in the indirect ELISA for IgM (Figure 6), a rheumatoid factor absorbent is used for the removal of IgG antibodies from test sera in a pre-treatment step;
- the first step is the absorption of measles antigen onto the solid phase;
- the patient's serum is then added and any measles-specific antibody (IgM and non-removed IgG) binds to the antigen;

- IgM antibody is detected either directly, by means of an enzyme-labelled anti-human IgM monoclonal antibody *or* indirectly by means of anti-human IgM monoclonal antibody plus enzyme-labelled anti-mouse antibody. A chromogen substrate is added to reveal the presence of specific measles IgM in the test sample.

Figure 6: Measles IgM indirect ELISA schematic



8.2.2 Test requirements

Several commercial kits are available – the Behring Enzygnost Anti-Measles Virus IgM test has been validated against clinically confirmed cases.⁽⁷⁾ This assay kit contains:

- test plates coated with measles antigen and control antigen;
- positive and negative control sera;
- anti-human IgM conjugate, buffers and diluent;
- the chromogenic detector reagents are separately supplied;
- an RF absorbent is supplied for the removal of IgG antibodies from test sera in a pre-treatment step.

Materials required but not provided

- piston -type pipettes 20, 50, 100, 150, 200, 400 and 1000 µl;
- ELISA Washer M or comparable washing devices;
- ELISA reader or spectrophotometer with wavelength 450nm (450/470nm; and

- a pocket calculator with exponential and logarithmic functions (required for the quantitative evaluation of the test).

8.2.3 Advantages/disadvantages

Table 6: Advantages and disadvantages of the Indirect ELISA for measles IgM test

Advantages	Disadvantages
<ul style="list-style-type: none"> • It enjoys the same programmatic advantage of a single serum specimen. • Some commercial kits avoid the potential disadvantage of IgG interference by pre-absorption of the test sera with anti-human IgG. • Sensitivity and specificity have been shown to be equivalent to the capture antibody methods. 	<ul style="list-style-type: none"> • Caution must be exercised in interpreting negative results on early specimens, since blood drawn within the first 3 days of rash onset may give a false negative result in up to 20–30% of the cases. • Potential for inaccurate results due to incomplete removal of IgG.

8.3 Indirect ELISA for measles IgG

8.3.1 Test principle

- measles antigen is adsorbed onto a solid phase;
- the patient's serum is added and any measles-specific antibody binds to the antigen;
- measles-specific IgG is detected using anti-human IgG;
- the reaction is revealed by a direct or indirect detector system using a chromogen substrate.

8.3.2 Advantages/disadvantages

Table 8: Advantages and disadvantages of the indirect ELISA IgG test

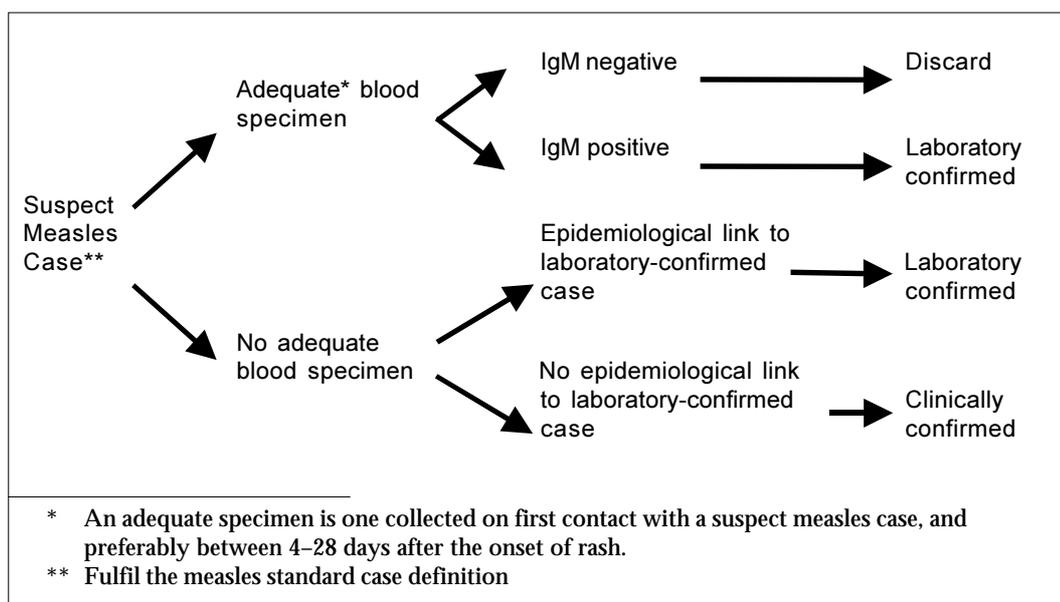
Advantages	Disadvantages
<ul style="list-style-type: none"> • Measles IgG in a single serum indicates past infection or vaccination. • Can be used for serological surveys • May add diagnostic information in conjunction with the IgM test. • Quantitative test (in IU/ml; i.e. single point quantification). 	<ul style="list-style-type: none"> • Diagnosis of recent measles infection using IgG detection requires paired sera, taken at acute and convalescent stages of illness.

8.4 Interpreting laboratory results

8.4.1 Final classification of suspected measles cases for countries in the measles outbreak prevention and elimination phase

- only patients that have a positive result with a validated IgM ELISA assay or an epidemiological link to such a case, are considered to be *laboratory confirmed measles cases*;
- patients with assay results obtained by other methods are considered as **suspected pending final laboratory testing**;
- if for any reason, an approved IgM ELISA is not performed on samples positive by other methods, these cases, for surveillance purposes, are considered as “*clinically confirmed*” measles cases.

Figure 7: Laboratory confirmation flow-chart for countries in the measles elimination phase



For countries in the control phase, laboratory confirmation is only required for the first few cases in an outbreak. Thereafter the clinical classification is recommended (i.e. cases that fulfil the measles standard case definition).

However, in the outbreak prevention and elimination phase, the goal is to test every isolated case to obtain laboratory confirmation. This will decrease the number of clinically-confirmed cases. Such cases will be regarded as failures of the surveillance system.

8.4.2 Interpretation of results in patients with a history of recent vaccination

Natural measles infection and measles vaccine can stimulate an IgM response in the host. If the suspect measles case has a history of measles vaccination *within 6 weeks of rash onset* the interpretation of results may become a surveillance dilemma, because:

- measles vaccine can cause fever in 5% and rash in approximately 20% of vaccinees;
- first time vaccinees are expected to have detectable measles IgM after vaccination;
- a mild rash lasting one to three days may occur approximately one week after vaccination;
- serological techniques cannot distinguish between the immune response to natural infection ,versus immunization. This can only be accomplished by viral isolation and characterization;
- other medical conditions such as rubella, dengue, etc. can cause rash and fever illness in persons who have recently received measles vaccine.

Bearing the above in mind, an operational definition is required to approach the final classification of these measles suspected cases with an IgM positive result:

Table 10: Classification of cases with IgM positive result and recent history of measles vaccination

Final classification	Vaccination history	Epidemiological findings
Discarded	History of measles vaccination within 6 weeks prior to rash onset.	Active search in community does not find evidence of measles transmission. No history of travelling to areas where measles virus is known to be circulating.
Confirmed	History of measles vaccination within 6 weeks prior to rash onset.	Active search in community does find other laboratory confirmed measles cases.

References

- (1) **World Health Organization**, *EPI information system global summary*, September 1998 (document WHO/EPI/GEN/98.10).
- (2) **World Health Organization**. Standardization of the nomenclature for describing the genetic characteristics of wild-type measles viruses. *Weekly Epidemiological Record*, 1998, 73:265–272.
- (3) **Bellini WJ, Griffin D**. Measles virus. In *Virology*, BN Fields, DN Knipe, PM Howley, et al., eds, 3rd ed. Philadelphia, Lippencott-Raven Publishers, 1996.
- (4) **Bellini WJ and Rota PA**. Diagnosis of measles virus. In: Lennette EH, Lennette DA, Lennette ET, eds, *Diagnostic procedures for viral, rickettsial and chlamydial infections*. 7th ed. Washington, D.C., American Public Health Association, 1995:447.
- (5) **Erdman DD; Anderson LJ et al**. Evaluation of monoclonal antibody-based capture enzyme immunoassays for detection of specific antibodies to measles virus. *J. Clin. Microbiol.*, 1991, 29:1466–1471.
- (7) **Arista S et al**. Detection of IgM antibodies specific for measles virus by capture and indirect enzyme immunoassays. *Res. Virol.*, 1995, 146(3):225–232.
- (8) **Chen RT, Markowitz, LE, Albrecht P, et al**. Measles antibody: Reevaluation of protective titres. *J. Inf. Dis.*, 1990, 162:1036–1042.
- (9) **Kalter SS, Herberling RL, Barry JD**. Detection and titration of measles virus antibody by haemagglutination inhibition and by dot immunobinding. *J. Clin. Microbio.*, 1991, 29:202–204.
- (10) **Helfand RF, Heath JL, Anderson LJ, Maes ER, Guris D, and Bellini WJ**. Diagnosis of measles virus with an IgM capture EIA: the optimal timing of specimen collection after rash onset. *J. Inf. Dis.*, 1997, 175:195–199.

Further reading

- **Bellini WJ, Rota PA.** Genetic diversity of wild-type measles viruses: implications for global measles elimination programs. *Emerging Infectious Diseases*, 1998, 4:1–7.
- **Cremer NE, Cossen CK et al.** Enzyme immunoassay vs plaque neutralization and other methods for determination of immune status to measles and varicella-zoster viruses and vs complement fixation for serodiagnosis of infections with those viruses. *J.Clin. Microbiol.*, 1985, 21:869–873.
- **Hummel KB, Erdman DD, Heath J, Bellini WJ.** Baculovirus expression of the nucleoprotein gene of measles virus and utility of the recombinant protein in diagnostic enzyme immunoassays. *J. Clin. Microbiol.*, 1992, 30:2874–2880.
- **Kleimann MB, Blackburn CKL, Zimmermann SE, French MLV (1981).** Comparison of enzyme-linked immunosorbent assay for acute measles with haemagglutination inhibition, complement fixation, and fluorescent-antibody methods. *J. Clin Microbiol.*, 14:147–152.
- **Kobune, F, Sakata H, Sugiura A.** Marmoset Lymphoblastoid Cells as a sensitive host for isolation of Measles virus. *J. Virol.*, 1990, 64(2):700–705.
- **Pan American Health Organisation.** *Measles Eradication: Field Guide.* PAHO 1999. Technical Paper 41.
- **Perry KR. et al.** Detection of measles, mumps and rubella antibodies in saliva using antibody capture radioimmunoassay. *J. Med. Virol.*, 1993: 40(3):235–240.
- **Rossier E, Miller H, McCulloch B, Sullivan L, Ward K.** Comparison of immunofluorescence and enzyme immunoassay for detection of measles-specific immunoglobulin M antibody. *J. Clin. Microbiol.*, 1991, 29:1069–1071.
- **World Health Organization,** *The immunological basis for immunization, measles* (document WHO/EPI/GEN/93.16).
- **World Health Organization.** *Measles surveillance guide.* (document WHO/GPV/GEN/98—)
- **World Health Organization.** *Weekly Epidemiological Record*, 1996, 71(41):305–312.

Annex 1: Laboratory Form

Measles laboratory request and result form					
Country:		Patient number:		Date / /	
Patient name:				M	F
Date of birth / /			Age in months:		
Name of parent or guardian:					
Address:					
Number of doses of measles vaccine:			Date of last dose / /		
Date of onset of fever / /			Date of onset of rash / /		
Type of rash:					
Provisional clinical diagnosis:					
SPECIMEN	DATE OF COLLECTION		DATE OF SHIPMENT		
(1)	/ /		/ /		
(2)	/ /		/ /		
(3)	/ /		/ /		
Name of person to whom laboratory results should be sent:					
Address:					
Telephone number:			Fax number:		
For use by the receiving laboratory:					
Name of laboratory:					
Name of person receiving the specimen:					
Specimen condition:					
SPECIMEN	DATE RECD IN LAB	DATE RESULT	TYPE OF TEST	TEST RESULT	COMMENT
	/ /	/ /			
	/ /	/ /			
	/ /	/ /			
	/ /	/ /			

Annex 2:

Composition of media and reagents

1. Phosphate buffered saline, pH 7.2 (PBS)

NaCl	8.00 g
KCl	0.20 g
NaHPO ₄	1.15 g
KHPO ₄	0.20 g

Dissolve in distilled water. Make up to 800ml. Adjust to pH 7.2 with HCl. Autoclave at 10 PSI for 15 minutes. This gives a working solution of PBS without calcium or magnesium ions.

(PBS is also commercially available in powder, tablet or liquid form)

2. PBS-Tween wash solution

PBS (No. 1 above)

Tween 20 (Commercially available)

Add 0.05ml Tween 20 per 100 ml PBS. Prepare sufficient volume for one test.

3. PBS-gelatin-Tween

PBS (above) 1 litre

Tween 20 1.5 ml

Gelatin 5.0 g

Mix 5.0 g gelatin in 1 litre PBS. Heat to dissolve gelatin and add 1.5 ml Tween 20. Store at 4° C.

4. Citrate-acetate buffer, 0.1M pH 5.5

Sodium acetate, anhydrous 8.2 g

1 M citric acid 4.0 ml

Dissolve sodium acetate in 800 ml distilled H₂O. Add citric acid and adjust to pH 5.5 with additional citric acid. Add distilled H₂O to 1 litre.

5. Anti-human IgG peroxidase

Peroxidase-labelled goat antibody to human IgG(y)

Dilute vial in 50% glycerol/PBS to stock conc. 1:3

Titer new lot for optimum dilution. Store at -20° C.

6. TMB substrate

(3,3',5,5'-tetramethylbenzidine (TMB)-H₂O₂ Chromogen Substrate Reagent)

Stock TMB Solution, 50X:

TMB 5.0 mg

Dimethyl sulfoxide (DMSO) 1.0 ml

Dissolve fresh TMB in fresh dimethyl sulfoxide avoiding contact with skin.
Dispense 1 ml volumes and store at -20°C. Stable 1+ years.

Working TMB Solution:

50X TMB 200 ml

0.1 M Citrate-acetate buffer 10.0 ml

30% H₂O₂ 2.0 ml

Make working substrate just prior to use in CLEAN container.

TMB: Sigma Chemical Co.
PO Box 14508
St. Louis, MO 63178
800-325-3010

Cat. No. T-2885 powder

7. 2M phosphoric acid (H₃PO₄)

Phosphoric acid 85% 135 ml

Distilled water 865 ml

Carefully add phosphoric acid to distilled water.

8. Viral transport medium

Hanks' Basal Salt Solution pH 7.4 with HEPES buffer
(commercially available 10x)

Bovine albumin..... 2.0 g
Penicillin/Streptomycin solution (No. 9 below) 1.0 ml
Phenol Red, 0.4% 0.2 ml

Dissolve 2.0 g bovine albumin in 100 ml distilled water. Add 10 ml Hanks' BSS to 80 ml distilled water then add 10ml 2% bovine albumin solution (above) and 0.2 ml phenol red solution. Sterilize by filtration. Add 1 ml penicillin/streptomycin solution. Dispense into sterile vials and store at 4° C.

9. Penicillin / streptomycin solution

Crystalline penicillin G
Streptomycin sulphate

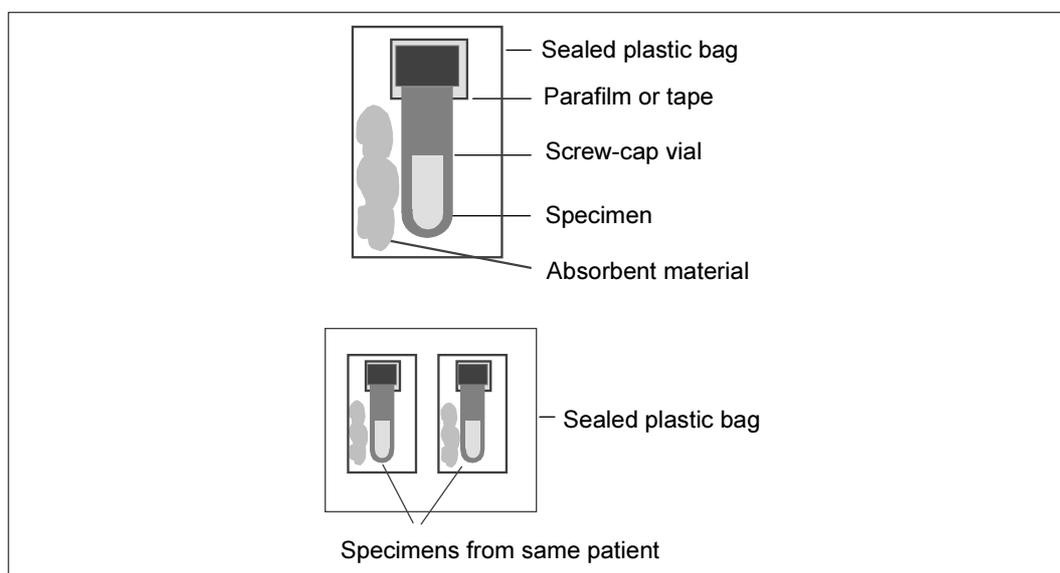
Dissolve 1 x 10⁶ units of penicillin and 1 g streptomycin sulphate in 100 ml sterile PBS. Store 5ml aliquots at -20°C. One ml of this solution in 100 ml medium gives a final concentration of 100 units of penicillin and 100mg of streptomycin per ml.

Annex 3:

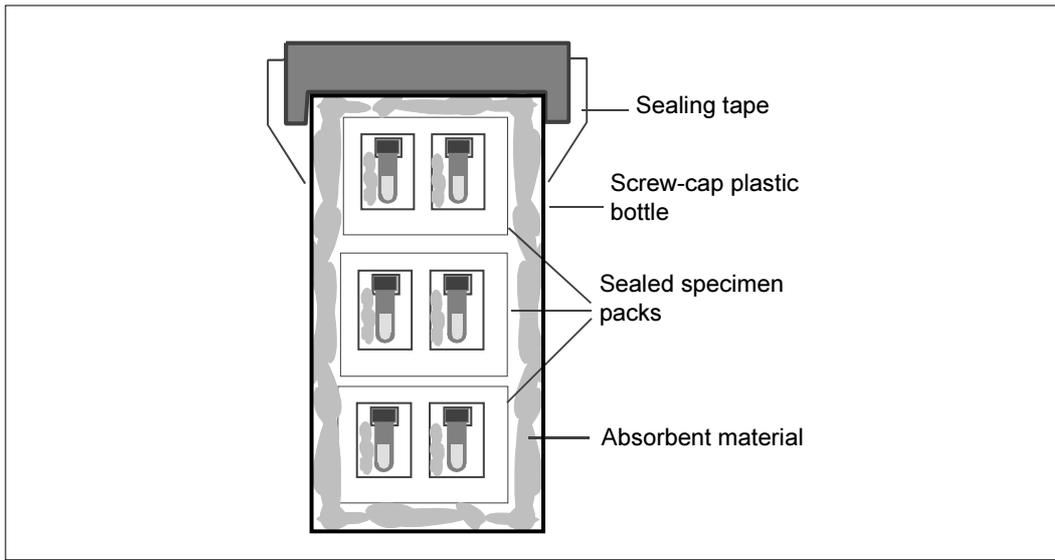
Packaging and shipping requirements for laboratory specimens

1. Correct packaging of diagnostic specimens for transport to laboratories

- Diagnostic specimens for transport to laboratories must be packaged in screw-cap containers of suitable size, for example 2-5 ml specimen containers for serum samples.
- After tightening the cap, sealing tape, for example parafilm or waterproof plastic tape, must be applied over the cap and top of the specimen container.
- The sealed specimen container must be placed in a suitably sized plastic bag together with a small amount of absorbent material, for example cotton wool. The bag must be sealed either using a heated bag sealer or waterproof adhesive tape or alternatively use ziplock plastic bags.
- All specimens should be “double-bagged” in sealed plastic bags. Two or more sealed specimens from the same patient may be placed in a larger plastic bag and sealed. Specimens from different patients should never be sealed in the same bag.



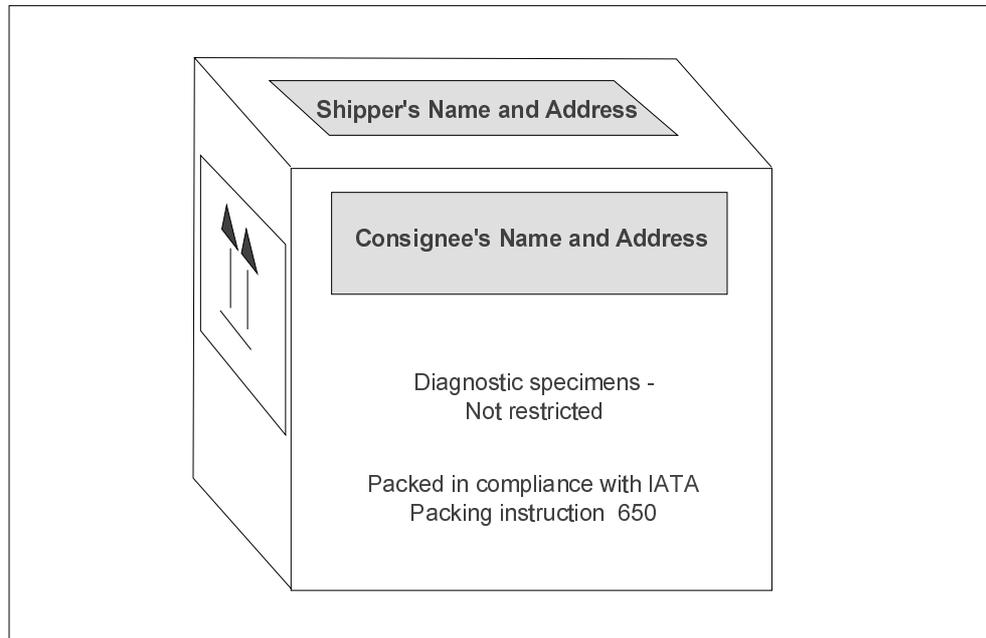
- Sealed bags containing the specimens should be placed inside *secondary* plastic containers with screw-cap lids. Provided the specimens have been double-bagged properly in sealed plastic bags, specimens from several patients may be packed inside the same secondary plastic container. Additional absorbent material should be placed inside the container to absorb any leakage that may occur. The total number of specimens that can be packed inside a single container will depend on the size of the primary containers holding the specimen and the amount of additional packaging material (plastic bag and absorbent material) but could be between 2 to 6 individual specimens.



- Written details of the specimens, any letters or additional information concerning the specimens, and details identifying the shipper and the intended recipient, should be sealed in a plastic bag and taped to the outside of the plastic container.
- Sealed plastic containers should be fitted into insulated containers (polystyrene) with a fibreboard outer packaging or specialized specimen container (similar to vaccine carriers). The insulated container and outer packaging must conform to *IATA Dangerous Goods Regulations Packaging Instruction 650*. The package should contain frozen ice packs, or additional plastic containers containing ice, but should *not* contain dry ice.
- The maximum volume that can be legally packed in a single package is *500 ml*. Since each serum specimen is usually approximately 1 to 2 ml, the 500 ml limit does not represent a problem.
- The inside of the insulated container should be packed with additional materials to prevent the plastic container from moving around during transport.
- Specimens packaged in this way do *not* require a *Declaration of Dangerous Goods*, but if transported by air the airway bill must include the words:

Diagnostic specimens packed in compliance with IATA packing instruction 650.

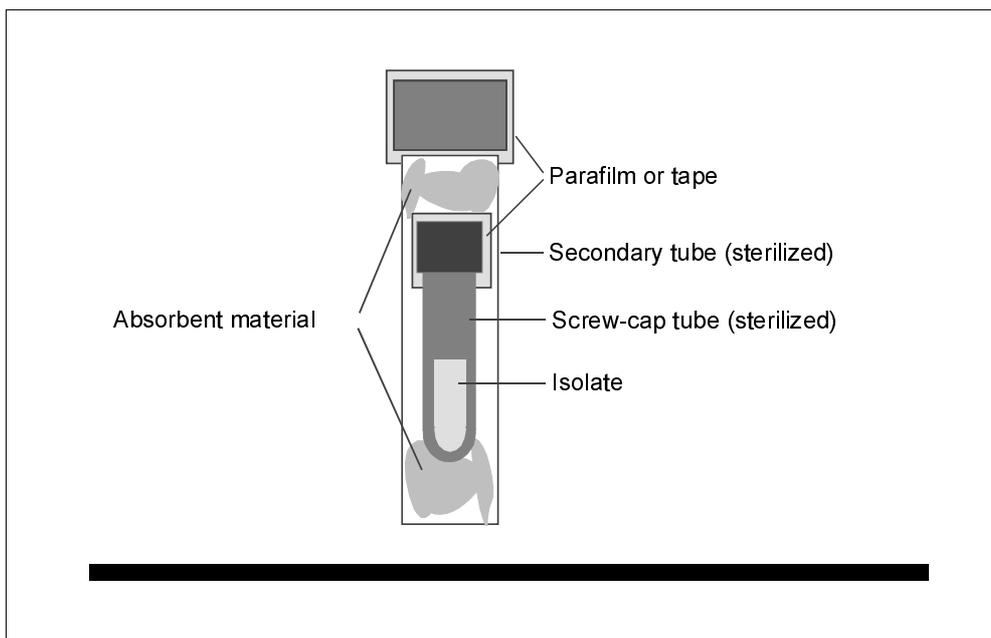
- The outside of the package should be marked as follows:



- It may be of benefit to include an additional label requesting: "Refrigerate package where possible".
- The box should be sealed using wide sealing tape, taking care not to obscure the labels with the tape.
- Suitable reusable secondary containers are available from VWR, catalogue number: VWR 11217-170

2. Correct packaging of viral isolates for transport to reference laboratories

- Viral isolates for transport to reference laboratories must be packaged in sterile screw-cap tubes, such as 1.8 ml cryovials. The tube caps should be sealed with Parafilm or waterproof plastic tape.
- Each sealed tube should be placed inside a secondary sterilized container, which also contains absorbent material, such as cotton wool, to absorb any leakage. Tubes of isolates from the same source and believed to be the same may be packaged in the same secondary container. Tubes containing isolates from different sources, or believed to be different, should be packed in separate secondary containers.
- The completed "tube-set" should be placed within insulated containers (polystyrene) with a fibreboard outer packaging. The insulated container and outer packaging must conform to *IATA Dangerous Goods Regulations Packaging Instruction 602* and must be part of a matching set. Do not mix components from different manufacturers. The "tube-set" should be placed within the polystyrene support cage of the insulated packaging. For best results the insulated packaging should be preconditioned by storing in a freezer, or filling with dry ice, for at least 6 hours before putting the tube-set in place.



- The maximum volume that can be legally packed in a single package is *50 ml*. Since each virus isolate is usually approximately 1 ml, the 50 ml limit does not represent a problem.
- The spaces around the tube-sets should be filled with dry ice, and the lid of the insulated container placed on top. To allow venting of the dry ice, *the top must not be sealed in any way*.
- A list of all viral isolates contained in the package should be included in an envelope taped to the top of the insulated lid, placed under the external fibreboard packaging.
- The outer packaging must be labelled with the following information:
 - The shipper's name, address and contact telephone/fax numbers
 - The UN Classification numbers and proper shipping names:
UN 2814 Infection substances affecting humans (measles virus)
UN 1845 dry ice
 - *The weight of dry ice included in the package when shipment started must also be recorded on the outside packaging*
 - The consignee's name, address and contact telephone/fax numbers
 - ***Infectious substances*** label showing class *6* or *6.2*
 - ***Miscellaneous label*** showing class *9*
- It may be of benefit to include an additional label requesting: "Refrigerate package where possible".
- The box should be sealed using wide sealing tape, taking care not to obscure the labels with the tape and leaving a gap for venting of the dry ice.
- All infectious substances must be accompanied by a *shipper's declaration for dangerous goods*.

Shipper's name, address and contact phone / fax numbers

**UN 2814
INFECTIOUS SUBSTANCES AFFECTING HUMANS
[MEASLES VIRUS]**

UN 1845 DRY ICE Net Quantity: _____ Kgs.

Consignee's name, address and contact phone / fax numbers





 **CLASS 6.2 /95/AUS/3378/HAZPACK**