



Republic of the Philippines  
Department of Health  
**OFFICE OF THE SECRETARY**

**ADMINISTRATIVE ORDER**

No. ~~2011~~ - 2012-0003  
mva

**FEB 08 2012**

**SUBJECT: Guidelines on Strengthening Laboratory Confirmation of Suspected Measles Cases**

**I. BACKGROUND AND RATIONALE**

In 2005, the countries of the Western Pacific Region of the World Health Organization (WHO) adopted the goal of measles elimination. To achieve this, the WHO recommends that countries strengthen three areas of their Expanded Programme for Immunization. These areas are: 1) universal high population immunity through increased coverage; 2) high case-based quality surveillance; and 3) adequate laboratory support for confirmation of diagnosis.

World Health Organization guidelines in support of the goal of measles elimination state that any person satisfying the suspect case definition of measles shall be immediately reported, investigated and a blood specimen collected to confirm whether the suspected case is indeed a case of measles. Effective surveillance for measles entails establishing case-based surveillance that includes investigation and laboratory testing of specimens from all suspected cases. Such surveillance system will also identify rubella cases through laboratory confirmation. As the country progresses towards measles elimination, reporting of rubella cases will be implemented and incorporated into the measles surveillance system.

The laboratory plays an important role in measles surveillance. In the elimination phase, it is well established that surveillance based on clinical recognition of cases is inaccurate and that laboratory confirmation of all suspected cases is critical for effective surveillance and proper program planning. It is in this light that confirmation of suspected measles cases through laboratory testing needs to be enhanced.

**1. Monitoring and verifying virus transmission:**

- Confirmation of suspect cases/ outbreaks: confirm the clinical diagnosis, especially in the early stages of an outbreak
- Identification of measles and rubella virus strains and genetic characteristics of viral isolates
- Differentiate endemic or imported cases: monitor circulation of wild genotypes to define pathways of transmission/importation

## 2. Monitoring susceptibility profile of the population

- Determination of the age distribution of susceptibility to measles and rubella in order to assess population at risk and appropriate intervention to reduce risk.
- Evaluation of the impact of the immunization campaigns

Quality measles surveillance requires laboratory confirmation of at least 80% of the reported suspected measles cases. However, **during elimination phase, all suspected measles cases require laboratory confirmation.** This shall serve as the evidence of the country's achievement and maintenance of the elimination status.

## II. OBJECTIVES

### A. General Objective:

This administrative issuance sets the guidelines for strengthening laboratories in support of the goal of measles elimination.

### B. Specific Objectives:

- To strengthen measles case-based surveillance
- To strengthen detection of rubella cases through measles surveillance and laboratory confirmation
- To evaluate impact of the measles and rubella routine and supplemental immunization activities in interrupting measles transmission and achieving control of rubella through efficient laboratory confirmation
- To establish the use of dried blood spot (DBS) and nasopharyngeal swab (NPS) as other methods of confirming suspect cases
- To implement standards for the collection, handling, storage and transport of DBS and NPS samples

## III. SCOPE AND COVERAGE

This issuance shall apply to the entire health sector, to include public and private health facilities both at the national and local government units involved in disease surveillance and response activities (*refer to AO. No. 2007-0036: Guidelines on the Philippine Integrated Disease Surveillance and Response*).

## IV. DEFINITION OF TERMS, ABBREVIATIONS & ACRONYMS

Case Investigation Form (CIF)	Refers to reporting form that allows collection of standard information to acquire epidemiological study of disease incidence and disease patterns
Cluster	Defined as two or more persons presenting with manifestations of a suspect measles case that are detected with onset of illness within a period of 7 to 21 days and in the same geographical area and/or are epidemiologically linked

Contacts	Are all persons living in a household or other close quarters with the case during the infectious period (5 days before to 5 days after the onset of rash)
Disease Reporting Unit (DRU)	This includes all health facilities (rural health units, hospitals, laboratories, seaports and airports are considered DRUs)
Epidemiologically-Linked Measles Case	Defined as a suspected measles case who was not discarded and who: <ul style="list-style-type: none"> <li>▪ had contact with a laboratory confirmed case or another epidemiologically-linked case within 7-21 days before rash onset and</li> <li>▪ the other epidemiologically-linked or laboratory confirmed case was infectious at the time of contact (i.e. contact was 5 days before and 5 days after rash onset)</li> </ul>
Immunoglobulin Class M (IgM)	An antibody detected to confirm suspect measles cases
Provincial Epidemiology and Surveillance Unit (PESU)	Refers to the unit established in the Provincial Health Offices that provides services on public health surveillance and epidemiology
Philippine Integrated Disease Surveillance and Response (PIDSR)	Refers to the Philippines process of coordination, prioritizing, and streamlining of core surveillance activities (e.g., data collection, reporting, laboratory and epidemiological confirmation, analysis and feedback), support functions (e.g., training, monitoring, financial and logistics) and response (e.g., epidemic investigation) with the aim of making the system more efficient and effective in providing timely, accurate and relevant information for action
Regional Epidemiology and Surveillance Unit (RESU)	Refers to the unit established in the Centers for Health Development or the DOH regional offices that provide services on public health surveillance and epidemiology
Research Institute For Tropical Medicine (RITM)	It houses the Department of Virology which is the national measles reference laboratory
Rural Health Unit (RHU)	Refers to the unit established in the rural health units that provides services on public health surveillance and epidemiology
Suspected Measles Case	Any individual, regardless of age, with history of fever (38°C or more) or hot to touch, generalized non-vesicular rash of 3 or more days duration; and at least one of the following cough, coryza, or conjunctivitis

## V. DECLARATION OF POLICIES

- A. Global Immunization Vision Strategy (GIVS) proposed a new measles mortality reduction of 90% by 2010 with the following major challenges: (i) measles mortality reduction activities in several large countries with high measles burden, (ii) enhanced efforts are needed to improve immunization systems to ensure that at least 95% of infants are vaccinated with measles before their first birthday, (iii) continue conduct "follow-up" SIAs every 3-4 years until their routine system are capable of providing two opportunities for measles immunization to >90% of every birth cohort, (iv) disease surveillance at district, provincial and national levels need to be strengthened to enable case-based surveillance with testing of clinical specimens from suspected cases in the laboratories. This was endorsed in the World Health Assembly 2005
- B. In 1996, the Regional Office of the Western Pacific (WPRO) established a "Plan of Action (POA) for Accelerated Measles Control". By 2003, the region's vision had moved to elimination with the publication of the "Western Pacific Regional POA for Measles Elimination", that covered the years 2003-2005. The Regional Office published the Field Guidelines for Measles Elimination in 2004 and in 2005, a second Regional Committee (RC) resolution established 2012 as the target date for measles elimination
- C. In light of the proven efficacy and safety of the Regional Advisory (RA) 27/3 bases rubella vaccine, WHO recommends its use in all countries where control or elimination of Congenital Rubella Syndrome (CRS) is considered a public health priority. Current efforts in global measles control shall be used as an opportunity to pursue control of rubella through the use of Measles Rubella (MR) and Measles Mumps Rubella (MMR) vaccines
- D. WHO-UNICEF comprehensive strategy for reducing measles mortality among priority countries with the following goals: (i) achieving and maintaining high coverage (>90%) of the 1<sup>st</sup> dose of measles-containing vaccine (MCV1) among all children by the age of 12 months in every district through routine immunization services, (ii) ensuring that all children receive a second opportunity for measles immunization, (iii) enhancing measles surveillance with integration of epidemiological and laboratory information, and (iv) providing appropriate clinical management for measles cases

## VI. IMPLEMENTING MECHANISM

The primary function of the laboratory in measles surveillance is confirming suspect measles cases, either through serology, molecular detection of the virus or virus isolation. The testing of serum specimen for the presence of anti-measles IgM antibodies remains the gold standard for laboratory confirmation of suspect cases occurring both sporadically, in clusters or during outbreaks. Other means of confirmation includes testing of DBS for presence of anti-measles IgM antibodies and/or culture and isolation of measles virus from suspect or clinically confirmed measles cases.

In countries with measles elimination goal and implementing case-based surveillance, the recommendation is to collect either serum or DBS specimen within the first 28 days of rash onset from all suspect measles cases. While the collection of NPS/ OPS specimen for viral isolation must be within the first 5 days of rash onset. Viral isolation provides evidence of elimination of indigenous measles virus, including outbreak source and transmission pathways.

#### **A. Laboratory Procedures for Case Confirmation**

##### **1. Use of Serum Samples for IgM testing**

A single serum sample obtained at the first contact with the patient at any time within 28 days after rash onset shall be taken from ALL suspected measles cases. Serum sample collection remains the GOLD STANDARD for confirming suspect cases under surveillance. Measles-specific IgM antibodies appear within the first few days of rash onset and decline rapidly after one month. Specimen shall be sent to RITM within 72 hours after collection with the completely filled-up Measles Case Investigation Form (Annex A.).

Provided that the epidemiological linkage among cases has been established within a household, specimen shall be taken from the **index case** (first person that fit the case definition of suspected measles case).

In line with this, it is very important to identify accurate information regarding the **exposure history** of suspect measles case. The CIF shall contain basic epidemiological information on time, date, place and history of contact with a known measles case. This will help identify origin and path of measles virus transmission.

**Patients admitted at hospital facilities and those DRUs (RHU/Health Centers) that have the capacity to collect, store and transport shall have serum sample collected.** Procedures for collection, storage and transport of sample are contained in the Annex B.

##### **2. Use of alternative sample (dried blood spot )**

Dried blood spot samples shall only be used as an alternative means of specimen collection where there is difficulty in extracting blood (e.g. very young infants, no medical technologist or certified phlebotomist), maintaining the specimen at 2-8°C during storage and transport (i.e. selected island barangays, municipalities or RHUs, and provinces, lack of specimen storage facilities and no local courier). Collection shall be done by medical, paramedical and other trained personnel (doctors, medical technologists, nurses, midwives, etc).

##### **3. Use of Oropharyngeal and/or Nasopharyngeal swab for virus isolation**

To monitor transmission pathways of measles virus during outbreaks, it is important to collect sample for viral isolation and characterization. Oropharyngeal and Nasopharyngeal swab (OPS/NPS) are the most appropriate specimens for virus isolation, OPS/NPS shall be collected as soon as possible within five (5) days of rash onset from any cluster of suspected measles cases. The probability that the measles virus can be isolated is highest during the first 3 days of rash onset.

In collecting NPS, the response team shall consider the guide below in collecting samples of cases in a cluster/ outbreak:

- 3 cases - collect at least 1-2 samples
- 5 cases - collect a minimum of 3 samples
- 10 cases – collect a minimum of 5 samples
- $\geq 10$  cases - collect a minimum of 10 samples

Viral isolation is significant to confirm whether the transmissions of indigenous measles strains have been fully eliminated or not. This will certify if the country has achieved the measles elimination goal.

Single or sporadic measles case with history of travel or unknown history of travel shall be collected with samples for both IgM testing (eg. Serum or DBS) and viral isolation (NPS) to determine transmission pathway and differentiate between importation and indigenous transmission.

## **B. Roles and Responsibilities**

### **1. Research Institute for Tropical Medicine**

- a. Shall receive all specimens from the DRUs and other allied health units
- b. Shall inform the RESU if specimen/s arrives at RITM in bad condition and if recollection is needed
- c. Shall process/test the specimens and send timely result to the National Epidemiology Center (NEC), National Center for Disease and Prevention Control (NCDPC) and RESUs
- d. Shall build and develop laboratory capacity and networking of laboratories at the national and local levels if needed
- e. Shall collaborate with WHO to strengthen molecular surveillance and virus identification and share laboratory results (both serology and virus identification) on a monthly basis with WHO country office

### **2. National Epidemiology Center**

- a. Shall oversee the implementation of high quality measles surveillance in all regions, province and cities
- b. Shall monitor and provide technical support to all regions, provinces and cities that are experiencing low surveillance performance
- c. Shall facilitate the provision of logistics for specimen collection to all RESUs
- d. Shall facilitate/ensure transport of specimens from the RESU/PESU and RHU
- e. Shall ensure the adequacy & timeliness of specimen collected
- f. Shall perform the overall coordination and data management

3. Center for Health Development

- a. Shall coordinate and investigate suspect measles cases
- b. Shall monitor and provide technical support to all provinces and cities that are experiencing low surveillance performance
- c. Shall be responsible for regional level surveillance data management
- d. Shall ensure the proper and timely collection, storage and transport of specimens to the National Measles Reference Laboratory (e.g. RITM)
- e. Shall facilitate the provision of logistics to the hospitals and LGUs
- f. Shall send laboratory results to concerned LGUs

4. PESU/PHO/RHU

- a. Shall coordinate the reporting and investigation of suspect measles cases
- b. Shall be responsible for provincial/city level surveillance data management
- c. Shall ensure the proper and timely collection, storage and transport of specimens to the national reference laboratory, RITM and or CHD
- d. Shall facilitate the provision of logistics to the hospitals and LGUs
- e. Shall ensure that CIFs are completely filled up sealed in a separate plastic bag and enclosed in the shipping box
- f. Shall send laboratory results to concerned LGUs

5. All Hospitals

- a. All suspected measles cases in-patient and out-patient wards shall be completely investigated with proper documentation
- b. Shall collect blood specimen for all suspected measles cases at first contact or within 28 days from rash onset following the standard procedure in specimen collection
- c. Shall properly label, store and/transport blood specimens to ESU/RITM
- d. Shall ensure that CIFs are completely filled up sealed in a separate plastic bag and enclosed in the shipping box
- e. Shall keep specimen collection kits properly and check for the expiration date
- f. Shall coordinate with CHD/ESU for the laboratory results

**C. Funding Requirements**

Budgetary requirements such as laboratory expenses, logistic support and other laboratory requirements for testing shall be charged against the Research Institute for Tropical Medicine through the support of Department of Health and World Health Organization. The freight and handling of specimen shall be charged against the different CHDs, subject to the usual accounting and auditing rules and regulations.

## **VII. REPEALING CLAUSE**

The provisions of previous Orders and other related issuances inconsistent or contrary with the provisions of this Administrative Order are hereby revised, modified, repealed or rescinded accordingly. All other provisions of existing issuance which are not affected by this Order shall remain valid and in effect.

## **VIII. EFFECTIVITY**

This order shall take effect immediately.



**ENRIQUE T. ONA, MD, FPCS, FACS**  
Secretary of Health



# ANNEX A – CASE INVESTIGATION FORM FOR MEASLES SURVEILLANCE



Philippine Integrated Disease  
Surveillance and Response

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## Case Investigation Form

### Measles

(ICD 10 Code: B05)

Name of DRU:				Type: <input type="checkbox"/> RHU <input type="checkbox"/> CHO <input type="checkbox"/> Gov't Hospital <input type="checkbox"/> Private Hospital <input type="checkbox"/> Clinic			
Address:				<input type="checkbox"/> Gov't Laboratory <input type="checkbox"/> Private Laboratory <input type="checkbox"/> Airport/Seaport			
<b>I. PATIENT INFORMATION:</b>		Patient Number:		Patient's First Name		Middle Name	
						Last Name	
Complete Address:				Sex: <input type="checkbox"/> Male		Date of Birth: MM DD YY	
District: ILHZ:				<input type="checkbox"/> Female		Age: <input type="checkbox"/> Days <input type="checkbox"/> Months <input type="checkbox"/> Years	
Patient Admitted? <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown				Date Admitted/Seen/Consult: MM DD YY		Date Onset of Illness: MM DD YY	
Date of Report: MM DD YY				Date of Investigation: MM DD YY			
Name of Investigator:				Contact Nos.:			
<b>II. CLINICAL INFORMATION:</b>				<b>III. VITAMIN A AND VACCINATION HISTORY:</b>			
Fever: <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U Date onset: ____/____/____ Rash: <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U Date onset: ____/____/____ Cough: <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U Runny nose/coryza: <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U Red eyes/conjunctivitis: <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U Other symptoms: _____ Are there any complications? <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U If YES, specify: _____				Patient received measles-containing vaccine (MCV)? <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U No. of documented MCV received: _____ Date of last dose of MCV received: ____/____/____ If NO, state the reasons: <input type="checkbox"/> Mother was busy <input type="checkbox"/> Child was sick <input type="checkbox"/> Forgot the schedule <input type="checkbox"/> No vaccine available <input type="checkbox"/> Against belief <input type="checkbox"/> Not eligible for vaccination <input type="checkbox"/> Medical contraindication <input type="checkbox"/> Fear of side effects Other reasons, specify: _____ Patient received vaccination during special campaigns? <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U Was the patient given therapeutic Vitamin A during this illness?: <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U			
<b>IV. EXPOSURE HISTORY:</b>							
Is there a history of travel to an area with known measles transmission 7-18 days prior to the appearance of rash? <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U							
Where did exposure probably occur? <input type="checkbox"/> Day care <input type="checkbox"/> Home/ dormitory <input type="checkbox"/> School <input type="checkbox"/> Health Care Facilities <input type="checkbox"/> Community <input type="checkbox"/> Unknown <input type="checkbox"/> Other, specify _____							
Was there contact with a laboratory confirmed Measles case 7-18 days prior to rash onset? <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U							
Are there other measles cases in the community? <input type="checkbox"/> Y <input type="checkbox"/> N <input type="checkbox"/> U							
<b>V. LABORATORY TESTS:</b>							
Specimen	Collected?	If YES, date taken	Date sent to RITM	Date received RITM	Measles IgM Result	Rubella IgM Result	Date result
Serum	<input type="checkbox"/> Y <input type="checkbox"/> N	____/____/____	____/____/____	____/____/____			____/____/____
Dried blood	<input type="checkbox"/> Y <input type="checkbox"/> N	____/____/____	____/____/____	____/____/____			____/____/____
NP swab	<input type="checkbox"/> Y <input type="checkbox"/> N	____/____/____	____/____/____	____/____/____			____/____/____
Urine	<input type="checkbox"/> Y <input type="checkbox"/> N	____/____/____	____/____/____	____/____/____			____/____/____
NP aspirate	<input type="checkbox"/> Y <input type="checkbox"/> N	____/____/____	____/____/____	____/____/____			____/____/____
Throat swab	<input type="checkbox"/> Y <input type="checkbox"/> N	____/____/____	____/____/____	____/____/____			____/____/____
<b>VI. CLASSIFICATION AND OUTCOME:</b>							
CASE CLASSIFICATION				OUTCOME		FINAL DIAGNOSIS	
<input type="checkbox"/> Laboratory Confirmed <input type="checkbox"/> Epidemiologically-linked <input type="checkbox"/> Clinically-confirmed <input type="checkbox"/> Discarded Case				<input type="checkbox"/> Alive <input type="checkbox"/> Died Date died: ____/____/____ <input type="checkbox"/> Unknown			

*emph*

## Case Investigation Form

**Measles****CASE DEFINITION/CLASSIFICATION:**

- **Suspected case:** Any individual, regardless of age, with the following signs and symptoms:
  - history of fever (38°C or more) or hot to touch; and
  - generalized non-vesicular rash of 3 or more days duration; and
  - at least one of the following: cough, coryza, or conjunctivitis
- **Laboratory-confirmed case:** Suspected case that is laboratory-confirmed.
- **Epidemiologically-linked:** An epidemiologically-linked measles case is defined as a suspected measles case who was not discarded and who:
  - had contact with another epidemiologically-linked case or a laboratory confirmed case 7-21 days before rash onset and
  - the other epidemiologically-linked or laboratory confirmed case was infectious at the time of contact (i.e., contact was 4 days before to 4 days after rash onset in the other epidemiologically-linked or laboratory confirmed case)
- **Clinically-confirmed:** A suspected measles case, that, for any reason, is not completely investigated\* (e.g. death before investigation, no blood sample) or has equivocal laboratory test results.

\*Such cases represent failures of the surveillance system to adequately classify a case
- **Discarded or not measles case:** A suspected measles case with an adequate specimen that is not serologically confirmed or is confirmed positive for other diseases such as rubella or dengue.

**LABORATORY CONFIRMATION:**

- Positive serologic test result for anti-measles IgM antibodies
- Fourfold rise in anti-measles IgG antibodies in acute and convalescent serum
- Isolation of measles virus
- Dot immunobinding assay
- Polymerase chain reaction testing for measles nucleic acid

**Therapeutic Dosage of Vitamin A for Measles cases:**

- 50,000 IU for children <6 months old
- 100,000 IU for children 6 to 11 months old
- 200,000 IU for children 12 to 71 months old

Note: The therapeutic dosage of Vitamin A for measles cases should be given upon diagnosis regardless of when the last dose of vitamin A capsule was given.



## **ANNEX B – GUIDELINES FOR SPECIMEN COLLECTION & STORAGE**

### **B.1. WHOLE BLOOD FOR SEROLOGY**

#### **I. Timing of Collection**

Obtain a single serum sample at the first contact with a suspected case of measles or any time within 28 days after onset of rash

#### **II. Pre-specimen Collection**

1. Prepare all materials needed.
2. Completely and legibly fill up the PIDSR Measles Case Investigation Form (CIF).

#### **III. Collection and Storage**

1. Make the patient comfortable with the arm accessible to the medical technologist/ phlebotomist.
2. Clean the venipuncture site with alcohol then prick with a sterile, single use syringe with gauge 23 needle.
3. Collect at least 5 ml (1ml for infants and younger children) of blood from the patient.
4. Label the red top Vacutainer™ tube with the patients' name, age, sex, date of birth, date of blood extraction. The information on the label must be legible and shall match the information on the CIF. Label must remain attached under all conditions of storage and transport.
5. Keep blood at room temperature, undisturbed until a clot is formed.
6. Allow the clot to retract in the refrigerator (approximately 4 to 8°C) or in a transport box with 4 frozen ice packs. Do not freeze the specimen. Centrifuge at 1000 x G for 10 minutes to separate serum from the clot. If there is no centrifuge, the blood shall be kept in a refrigerator for no longer than 24 hours until there is complete retraction of the clot from the serum. Carefully transfer serum aseptically to a properly labelled vial.

#### **IV. Processing and Handling**

1. Carefully transfer or decant the serum into a cryovial labelled with the patient's name, age/sex and date of collection. Avoid mixing red blood cells with the serum as these hemolyse during storage.
2. Store serum at 4-8°C until shipment takes place, or for a maximum of 7 days. If a delay in shipment is anticipated, serum samples must be frozen at -20°C or lower.

#### **V. Transport**

1. The serum sample shall be placed in a sealable plastic bag or pouches containing absorbent materials such as cotton wool to soak up any leakage that may occur. Insulated containers shall be used to contain the sealed bags of specimen.
2. The case investigation form shall be sealed in a separate plastic bag and enclosed within the shipping box.
3. Place the specimens in the transport box with frozen ice packs no less than 4 pieces fitted around the specimens.

4. Arrange shipment such that arrival of specimens at RITM does not fall on weekends or holidays. Otherwise, make advance notice of such arrival via telephone, fax or e-mail.
5. Address shipment to: **Head, Virology Department**  
Research Institute for Tropical Medicine  
Filinvest Corporate Compound  
Alabang, Muntinlupa City  
Tel. Nos: 02-8097120

**NOTE: SPECIMENS SHALL BE SHIPPED WITHIN 48 HOURS (2 DAYS) AFTER COLLECTION TO ENSURE ARRIVAL AT RITM WITHIN 72 HOURS (3 DAYS)**

#### VI. Rejection Criteria

1. Inadequate sample collection.
2. Samples without CIF.
3. Improperly labelled sample/ unlabelled sample.
4. Samples with visible contamination.
5. Spillage or breakage in transit.
6. Grossly hemolyzed sample
7. Improperly shipped ( no cold chain)

### **B.2. DRIED BLOOD SPOT (DBS) FOR SEROLOGY**

#### I. Timing of Collection

3-28 days from onset of rash.

#### II. Pre-specimen Collection

1. Prepare materials.
2. Completely and legibly fill up the Measles CIF.

#### III. Collection and Storage

1. Label the DBS card with patient's name, age, sex, date of birth, date of collection and the Disease Reporting Unit (DRU). The information on the label must be legible and shall match the information on the CIF. Label must remain attached under all conditions of storage and transport.
2. Clean each individual's finger (either side of the middle or 4<sup>th</sup> finger), or the side of the heel in the case of a very young children (those who are not capable of walking on their own) with alcohol, then prick with a sterile, single use microlancet;
3. Hold the finger or side of the heel face down to the DBS card (i.e. Whatman S & S No. 903) and allow blood to fill completely at least 3 circles. Do not touch the finger or heel to the paper. Blood must soak all the way through the paper.
4. Alternatively, a venous blood sample (about 1 ml) can be collected and immediately dropped unto the circles of the DBS card.
5. Allow the blood spots to dry thoroughly (at least 60 minutes). Do not heat. Place the dried blood spots in a re-sealable bag with desiccant (if available) individually to prevent possible cross contamination and to protect from dust and moisture during storage.
6. Samples can be stored at room temperature for a maximum of two weeks if immediate shipment is not possible.

#### IV. Specimen Handling and Transport

1. Place the individually packed DBS in an envelope big enough to accommodate the number of DBS. Ship the DBS sample via ordinary postage.
2. Although samples do not need to be kept refrigerated or frozen during transport, it is advisable to store in a cool place and transport to the laboratory/RITM, Virology Department as soon as possible to the address mentioned above.

#### V. Rejection Criteria

1. Inadequate sample collection (less than 3 circles filled, circle not completely filled, blood not soaked through).
2. Samples without CIF.
3. Improperly labelled sample.
4. Samples with visible contamination (mouldy).

**NOTE: DBS IS USED AS AN ALTERNATIVE FOR WHOLE BLOOD EXTRACTION ONLY UNDER SPECIFIED CIRCUMSTANCES.**



## DIAGRAM-Collection of Dried Blood Spots (DBS)

### 1. Equipment required



#### Initial requirements

Pencil/pen – Label card with name, DOB, Sex  
Case Investigation form

#### Vinyl/latex gloves for person taking blood

#### For blood collection

Single use lancet  
Alcohol swab

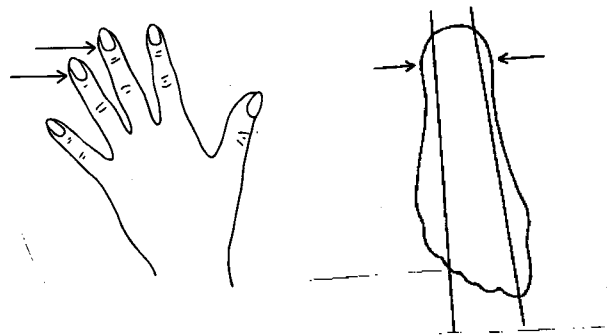
Filter paper collection card

Ziplock bag (small plastic bag) with desiccant

### 2. Pricking the finger or sides of heel

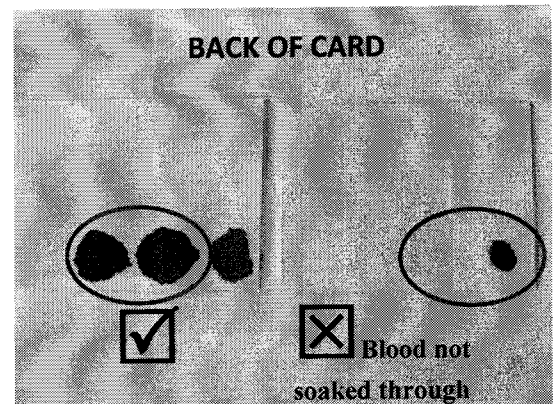
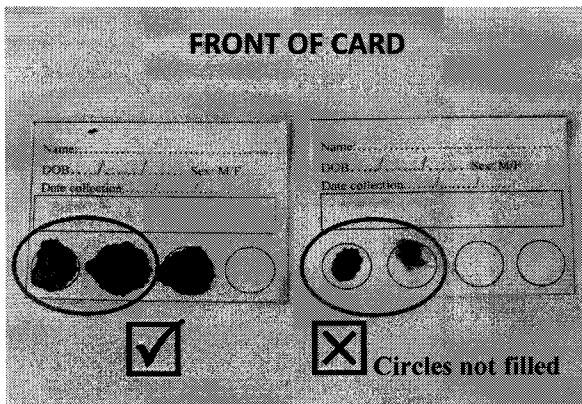


Arrows show the best areas for skin puncture



### 3. Collecting an adequate sample

Fill the circle completely with blood drop. Blood must soak all the way through the paper



The best flow of blood for a dried blood sample is obtained if the skin puncture is directed to either side of the middle or 4th finger, the skin is softest, least sensitive and has many blood vessels.  
For infants and small children the sides of the heel as pictured are the best areas for skin puncture

Hold finger face down to card and allow blood to drop onto card to fill at least 3 circles. Do not touch finger to card. ***Air dry blood spots at room temperature completely for at least 1 hr before packaging with desiccant. DO NOT HEAT.***

A handwritten signature in the bottom right corner.

### **B.3. OROPHARYNGEAL/NASOPHARYNGEAL SWAB (OPS/NPS) FOR VIRUS ISOLATION**

#### **I. Timing of Collection:**

Collect specimen as soon as possible after rash onset. Sample collected 5 days after rash onset have much lower chances for successful isolation of the virus.

#### **II. Pre- specimen collection**

1. Take out only the number of Viral Transport Media (VTM) needed from the freezer (-20 °C)/refrigerator freezer where they are stored.
2. Frozen VTM shall be thawed just before use. If the collection site is far from a refrigerator, have a thermo box with 4-6 frozen ice packs at hand to maintain a refrigerated temperature during collection.
3. Check VTM for turbidity. The medium shall be clear and pinkish. Tap the tube to mix contents.
4. Check also the integrity of the swab and tongue depressor pouch to ensure sterility. Do not use swabs or tongue depressor that has been opened.
5. Completely and legibly fill up the CIF.

#### **III. Specimen Collection and Storage**

Label the VTM tube with the patient's Full Name and date of collection. The information on the label must be legible and shall match the information on the CIF. Label must remain attached under all conditions of storage and transport.

##### **Nasopharyngeal swab (NPS)**

1. Using a flexible Dacron or Rayon tip swab, measure from the base of the nostril towards the auditory pit. Divide the length into half in order to know into what extent will be inserted into the nostril (usually 5–6 cm in adults) to ensure that it reaches the posterior pharynx.
2. With the patient seated, tilt the head slightly backwards. Insert the swab into the nostril parallel to the palate.
3. Rotate swab applying a little force taking large quantities of mucosa.
4. Repeat procedure in the other nostril using the same swab (if possible)
5. Place the nasopharyngeal swab immediately in the VTM tube to avoid drying of the swab.
6. Break/cut with scissors the end of the swab that sticks out of the tube and close the tube.

##### **Oropharyngeal swab (OPS)**

1. With gloved hands, hold down the tongue with a sterile tongue depressor.
2. Have the patient say "aahh" to elevate the uvula.
3. Use a sweeping motion to swab the posterior pharyngeal wall and tonsillar pillars. Apply a little force, taking large quantities of mucosa.
4. Avoid swabbing the soft palate and do not touch the tongue with the swab tip. (N.B.this procedure can induce the gag reflex)
5. Place the oropharyngeal swab immediately in the same VTM tube with the nasopharyngeal swab.
6. Break/cut with scissors the end of the swab that sticks out of the tube and close the tube tightly.

7. Secure the cap with parafilm to prevent leakage during transport.
8. Store inside the refrigerator (2-8°C)/thermobox with ice packs while awaiting transport.

#### IV. Processing and Handling

1. Wrap VTM tubes with specimens in tissue paper or any absorbent material; place upright in a separate 50 ml centrifuge tube or any leak/puncture proof container; place the 50 ml tube or any container in a resealable plastic bag (Ziplock<sup>TM</sup>).
2. Transport to RITM immediately or shall reach RITM within 72 hours after collection.

#### VII. Transport

1. The laboratory request form shall be sealed in a separate plastic bag and enclosed within the shipping box.
2. Place the specimens in the transport box with frozen ice packs no less than 4 pieces fitted around the specimens.
3. Arrange shipment such that arrival of specimens at RITM does not fall on weekends or holidays. Otherwise, make advance notice of such arrival via telephone, fax or e-mail. Transport to the laboratory/RITM, Virology Department as soon as possible to the address mentioned above.

**NOTE: SPECIMENS SHALL BE SHIPPED WITHIN 48 HOURS (2 DAYS) AFTER COLLECTION TO ENSURE ARRIVAL AT RITM WITHIN 72 HOURS (3 DAYS).**

#### VIII. Rejection Criteria

1. Inadequate sample collection.
2. Samples without CIF.
3. Improperly labelled sample.
4. Samples with visible contamination.
5. Spillage or breakage in transit.