Department of Health
RESEARCH INSTITUTE FOR TROPICAL MEDICINE
Virology Department

GUIDELINES FOR LABORATORY CONFIRMATION OF SUSPECTED HFMD CASES

BACKGROUND

Laboratory confirmation of Hand, Foot, and Mouth Disease (HFMD) is essential to determine the causative agent as it may assist in the clinical management of the disease by preventing further complications. Likewise, identification of the specific agent during outbreaks of HFMD is vital in the prediction of its severity and may provide support in initiating appropriate response.

The genus Enterovirus belongs to the family *Picornaviridae,* which were previously composed of 4 species: Polioviruses, Coxsackie A viruses (CA), Coxsackie B viruses (CB), and Echoviruses. However, recent developments created a new classification of Enteroviruses species into Human Enterovirus group A, B,C, and D. The Human Enterovirus group A specie, is the most common cause of HFMD. The serotype Enterovirus 71 (EV-71) has been known to cause severe complications of HFMD particularly neurological manifestations and deaths.

TYPES OF SAMPLES FOR TESTING

It is to be emphasized that an effective and accurate virological diagnosis is highly dependent on the timing of specimen collection, appropriate clinical sample, and the condition during transport to the laboratory. There are FOUR (4) types of samples that are appropriate for detecting Enteroviruses in cases suspected of HFMD:

1. Oropharyngeal/Throat Swab
2. Vesicular Fluid/Vesicle Swab
3. Rectal Swab
4. Stool

Oropharyngeal or throat swabs collected within 14 days after onset of symptoms (fever, papulovesicular rash on palms and soles, with or without vesicles/ulcers in the mouth – refer to case definition of HFMD for more details), are appropriate for detecting or isolating Enterovirus.

Likewise, **vesicular fluid or vesicle swabs** collected from fresh lesions may also yield enteroviruses and are indicative of current systemic infection. However, in instances that patients consulted a heath facility when respiratory symptoms and vesicles have already disappeared, **stool sample** may be collected as enteroviruses are shed in stool for several weeks (up to 6 weeks).

To increase the possibility of enterovirus detection, collecting throat swab samples for all patients PLUS swabs from at least two (2) vesicles OR from the rectum for patients with no vesicles is recommended.

METHODS OF TESTING

Virus isolation using tissue culture technique is the gold standard in the diagnosis viral infection, however, it is labour extensive, time consuming, and limited only to specialized laboratories. With the development of a more rapid and sensitive molecular techniques, detection of nucleic acid may confirm presence of the specific virus.

The use of a universal primer targeting all human enterovirus groups (A to D) is used as a screening test to detect enterovirus RNA. All samples yielding a positive result will then be subjected to testing, using specific primers targeting EV-71 and Cox A16, the most common cause of HFMD, to detect presence of these viruses. However, in instances that a non-EV71 and Cox A16 enterovirus is detected, further characterization using nucleic acid sequencing will be done to detect enterovirus serotypes.

GUIDELINES FOR SPECIMEN COLLECTION, STORAGE AND TRANSPORT

OROPHARYNGEAL OR THROAT SWAB (OPS) AND VESICULAR SWAB (VS) FOR RNA DETECTION AND VIRUS ISOLATION

I. Timing of Collection:

Collect specimen as soon as possible (within 14 days after the onset of symptoms). Sample collected greater than 14 days after 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 ºC)/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.

<u>Oropharyngeal swab (OPS)</u>

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 tonsilar 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 VTM tube.
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 (2-8ºC)/thermobox with ice packs while awaiting transport.

<u>Vesicular Fluid (VF)</u>

1. Clean the surface of the vesicle with sterile normal saline if available or with tap water to remove any contaminating materials such as body fluids, excreta or drainage.
2. Using a hypodermic needle gauge 26 or 27 attached to a tuberculin syringe, aspirate the fluid contained in the lesion.
3. Transfer the fluid into a separate VTM immediately.

<u>Vesicular Swab (VS)</u>

1. For vesicles that are intact, unroof using the bevelled tip of the sterile hypodermic needle
2. Vigorously rub a sterile swab over the base and margins of the lesion to ensure that epithelial cells containing the viruses will be collected.
3. Immediately place the swab into the same VTM used for the VF if VF was collected.
4. Break/cut with scissors the end of the swab that sticks out of the tube and close the tube tightly.
5. Secure the cap with parafilm to prevent leakage during transport.
6. Store inside the refrigerator (2-8ºC)/thermobox with ice packs while awaiting transport.

GUIDELINES FOR SPECIMEN COLLECTION, STORAGE AND TRANSPORT

OROPHARYNGEAL OR THROAT SWAB (OPS) AND VESICULAR SWAB (VS) FOR RNA DETECTION AND VIRUS ISOLATION cont.

IV. Transport

1. In transporting the specimen, 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™).
2. Put the VTM tubes with specimens in a shipment/carrier box with at least 4 frozen ice packs inside to maintain prescribed temperature: put frozen ice packs in first, at the bottom and at the sides of the carrier box; then place specimens at the middle so that they are surrounded by the ice packs. Cover the carrier box.
3. Place the completely filled-up CIF in a separate zip-locked plastic bag and put on top of the box and secure with tape.
4. Send to the Research Institute for Tropical Medicine (RITM) within 3 days of specimen collection:

Head of Virology Department
Research Institute for Tropical Medicine
Filinvest Corporate Compound
Alabang, Muntinlupa City, 1781
Telefax Number: (02)809-7120

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

V. 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.

GUIDELINES FOR SPECIMEN COLLECTION, STORAGE AND TRANSPORT

RECTAL SWAB (RS) FOR RNA DETECTION AND VIRUS ISOLATION

I. Timing of Collection:

Collect specimen as soon as possible (within 14 days after the onset of symptoms). Sample collected greater than 14 days after onset have much lower chances for successful isolation of the virus. Rectal swabs should be taken from suspected cases with no vesicular lesions.

II. Pre- specimen collection

1. Take out only the number of Viral Transport Media (VTM) needed from the freezer (-20 ºC)/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 to ensure sterility. Do not use swabs that have 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.

Rectal (RS)

1. With gloved hands, gently insert rectal swab 4-6 cm into rectum, roll swab against rectal mucosa, avoid excessive stool sampling.
2. Place the rectal swab immediately in the VTM tube.
3. Break/cut with scissors the end of the swab that sticks out of the tube and close the tube tightly.
4. Secure the cap with parafilm to prevent leakage during transport.
5. Store inside the refrigerator (2-8ºC)/thermobox with ice packs while awaiting transport.

IV. Transport

1. In transporting the specimen, 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™).
2. Put the VTM tubes with specimens in a shipment/carrier box with at least 4 frozen ice packs inside to maintain prescribed temperature: put frozen ice packs in first, at the bottom and at the sides of the carrier box; then place specimens at the middle so that they are surrounded by the ice packs. Cover the carrier box.
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V. 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.

GUIDELINES FOR SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

STOOL FOR RNA DETECTION AND VIRUS ISOLATION

1. Collect 1 stool specimen per case.
2. Stool specimen volume should be at least 8 grams: as big as the size of an adult's thumb; if the stool is watery, fill up ¾ of the specimen collection container.
3. Place the stool sample in a dry, clean and leak proof container; make sure the container is tightly sealed.
4. Properly label the container with stool specimen of the name of the patient and date of collection.
5. Immediately after collection, store specimen in the refrigerator at a temperature of 4 - 8°C while awaiting transport; if refrigerator is unavailable, place specimen in a specimen carrier box with at least 4 frozen ice packs, changing ice packs every 24 hours and just before specimens are shipped to RITM; this is called the "reverse cold chain";
   - DO NOT allow stool specimens to remain at the bedside at room temperature after collection
   - DO NOT allow specimens to remain in specimen carrier without frozen ice packs
6. In transporting the specimen, wrap the container with stool sample with cotton or any absorbent material and put in a zip-locked plastic bag.
7. Put the stool specimen in a shipment/carrier box with at least 4 frozen ice packs inside to maintain prescribed temperature: put frozen ice packs in first, at the bottom and at the sides of the carrier box; then place specimens at the middle so that they are surrounded by the ice packs. Cover the carrier box.
8. Place the completely filled-up CIF in a separate zip-locked plastic bag and put on top of the box and secure with tape.
9. Send to the Research Institute for Tropical Medicine (RITM) within 3 days of specimen collection:

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