LABORATORY TESTING

Diagnosis of mpox can be confirmed with laboratory testing using real-time PCR method. Serology and antigen detection are not recommended for diagnosis purpose, as they cross react with other Orthopoxviruses and not offered in Malaysia.

To facilitate accurate diagnosis, correct specimens should be collected from suspected cases, and transported to the National Reference Laboratory [Institute for Medical Research (IMR)/ designated Public Health Laboratory (PHLs)]/ designated laboratories (**Annex 2**) for diagnosis as soon as possible.

1. Specimen Collection, Handling, and Transportation

1.1 Collection of Specimen

i. The type of specimen to be collected depends on the disease phase and clinical signs.

ii. Health care personnel involved in specimen collection for Monkeypox virus (**MPXV)** must wear recommended personal protective equipment (PPE) as per infection control guidelines, i.e., disposable gown, double gloves, respirator N95 and eye protector, such as goggles *(please refer to Chapter 5: Infection Prevention and Control for further details).*

iii. Optimal diagnostic specimens are from skin lesions – vesicular swab with viral transport media (preferred) and lesion, exudate, crusts in sterile container and kept in cold chain (2°C to 8°C).

Table 1: Guidance on types of specimens to be collected for MPXV

Case Category	Disease Phase	Signs / Symptoms	Specimens to Collect
Suspected or probable case	Rash	Vesicles or Pustules	Lesion fluid
		Scabs or Crusts	Lesion scab or crust
Contact	Prodrome	Early stage of fever	Tonsillar swab
			Nasopharyngeal swab

Table 2: Types of samples and collection methods
(Please send **TWO samples** from each lesion)

No	Type of sample		
1.	**Lesion fluid swab**		
	Materials needed	**Procedure**	**Test Method**
	1. Sterile, synthetic or dacron swabs. 2 sets in single tube. 2. Viral transport media (preferred) or sterile container. 3. **(Do not use cotton swabs)**	1. Do not clean the lesion with ethanol or any other disinfectant prior to swabbing. Hold the swab with a firm grasp. Avoid touching the swab shaft at least an inch before the tip if collecting a dry swab and the length of the swab shaft that will be submerged in liquid if using a swab in viral transport media. 2. Apply firm pressure (generally firm enough so that the swab shaft, if plastic, may bend slightly). This may result in discomfort or slight pain, but it is necessary to obtain adequate DNA. a. If a lesion ruptures while swabbing, ensure that swab collects lesion fluid. b. If possible, avoid using swabs that bend too easily which may make applying firm pressure difficult. 3. Swipe the swab back and forth on the lesion surface at least 2 to 3 times then rotate and repeat on the other side of the swab at least 2 to 3 times. a. If material is visible on the swab surface (such as skin material or from lesion fluid that is leaking from the lesion), this is indicative of an adequate collection. Although please note that material may not always be visible on swabs. 4. Place the entire swab in viral transport media (Preferred)	Real-time PCR

2.	**Scab or crust**		
	Materials needed	**Procedure**	**Test Method**
	1. Forceps or other blunt-tipped sterile instrument. 2. Sterile container.	1. Do not clean the lesion with ethanol or any other disinfectant prior to procedure. Use forceps or other blunt-tipped sterile instrument to remove all or a piece of the crust at least 4mm x 4mm. 2. Separate each crust into a dry, sterile container. 3. Cover lesion with band aid.	Real-time PCR
3.	**Tonsillar swab**		
	Materials needed	**Procedure**	**Test Method**
	1. Sterile screw capped container (1.5 to 2 mL) with viral transport media. 2. Sterile dry polyester or Dacron swabs. ***Do not use cotton swab**	1. Swab or brush posterior tonsillar tissue with a sterile dry polyester or Dacron swab. 2. Break off end of applicator into a 1.5-mL or 2-mL screw-capped sterile container or place entire swab in a sterile container with viral transport media.	Real-time PCR
4.	**Nasopharyngeal swab**		
	Materials needed	**Procedure**	**Test Method**
	1. Sterile dry polyester or Dacron swabs – with viral transport media ***Do not use cotton swab**	1. Swab the nasopharynx with a sterile dry polyester or Dacron swab. 2. Break off end of applicator into a 1.5- or 2-mL screw-capped sterile container or place entire swab in a sterile container with viral transport media	Real-time PCR

Refer workflow of laboratory approach in mpox investigations.

1.2 Specimen Transportation

i. All specimens must be maintained at cold temperature (2°C to 8°C) during transportation.
ii. The Laboratory Request Form must be sent together with the specimen/s, and must be attached at the outside of the triple packaging system. Label the outside sample box with 'mpox'.
iii. All specimens from community (case or contacts) must be sent as soon as possible to PHLs. Specimens that are collected from the hospitals are sent designated hospital laboratories or IMR (Annex 2).
iv. Each specimen should be labeled with the patient's name, identification number, collection date, type of specimen, and body location for lesion specimens.
v. Place specimens from a single patient into a biohazard bag.
vi. All specimens should be transported on ice packs at 2°C to 8°C.
vii. Specimens may be stored at 2°C to 8°C up to 48 hours before processing. If specimen cannot be processed within 48 hours, it should be stored at -70°C.
viii. Specimens should be packaged and transported in accordance with IATA rules and regulations for diagnostic specimens (UN 3373).
ix. All MPXV specimens transported to PHLs/ IMR should be packaged by following the Triple Packaging System (Picture 1) which consists of a primary receptacle in a seal able specimen bag wrapped with absorbent material, secondary receptacle (watertight, leak-proof), and an outer box.

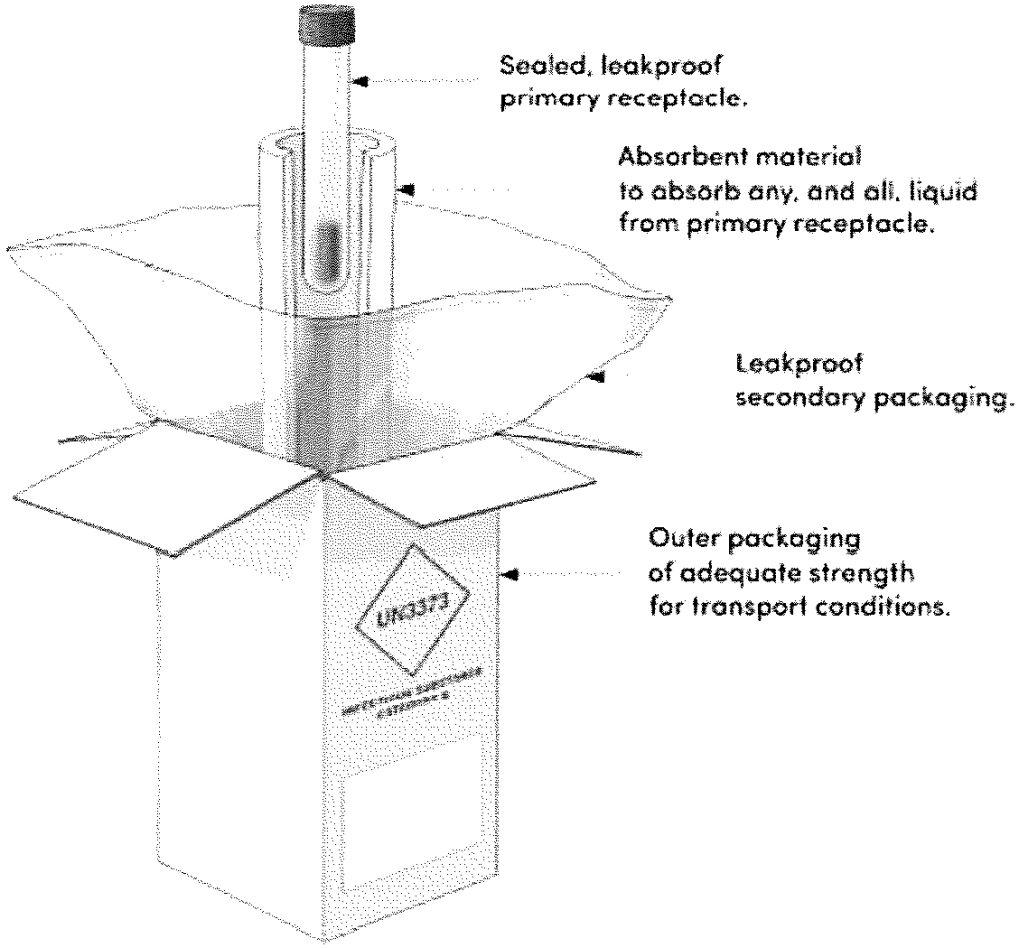


Figure 1: Example of triple packaging materials that may be used to comply with P650 for Category B infectious substances

Source: Illustration created for the 4th edition of the WHO Laboratory Biosafety Manual

1.3 Request Form

In order to interpret test results, it is critical that patient information is provided with the specimens, including:

i. date of onset of fever
ii. date of onset of rash
iii. other clinical signs
iv. date of specimen collection
v. current status of the individual (stage of rash)
vi. nationality/country
vii. travel history to mpox affected country

viii. contact history with mpox patient
ix. specimen type
x. date specimen sent to laboratory
xi. requestor details i.e., name, contact number, email address

Use Specific Laboratory request form to be used for designated laboratories

i. MKAK - *Borang Permohonan Ujian Makmal (Spesimen Klinikal)* with coding MKAK- BPU-U01/Rev2018 – Download from NPHL website https://mkak.moh.gov.my/index.php/muat-turun/borang-dokumen/bahagian-penyakit/13-borang-permohonan-ujian

ii. IMR – *Borang permohonan ujian Virology test request form* Download from IMR website *https://imr.nih.gov.my/en/services-menu/menu-specific-request-form* *or PER-PAT 301.*

iii. Hospital – *Borang PER-PAT 301*

Please call officer on duty (Annex 3) for any queries.

1.4 Laboratory Biosafety Guidelines for Handling and Processing Specimens

Laboratory biorisk assessment must be conducted prior to commencement of any laboratory work to gather the information, evaluate it and use it to inform and justify the implementation of processes, procedures and technologies to control the risks present.

Use of a certified Class II Biological Safety Cabinet (BSC) is recommended for manipulations of mpox specimens - if a BSC cannot be used, the risk of exposure to an inadvertent sample release should be reduced by the appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors).

Use sealed centrifuge rotors or sample cups for centrifugation. Ideally, these rotors or cups should be unloaded in a BSC.

Routine specimen processing may be handled in BSL-2 facilities, but with more stringent BSL-3 work practices. Measures should be taken to minimize the risk of laboratory transmission when testing routine clinical specimens from confirmed or suspected Mpox patients. These may include practicing Good Microbiological Practice and Procedure (GMPP): limiting the number of staffs testing specimens, wearing appropriate personal protective equipment, using rigorously applied standard precautions, and avoiding any procedures that could generate infectious aerosols

Decontamination of work surfaces after the completion of work is essential. Only the approved chemical lists proposed by the companies presented and approved by Hospital Support Services (HSS) Technical Committees should be used. Manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling should be followed.

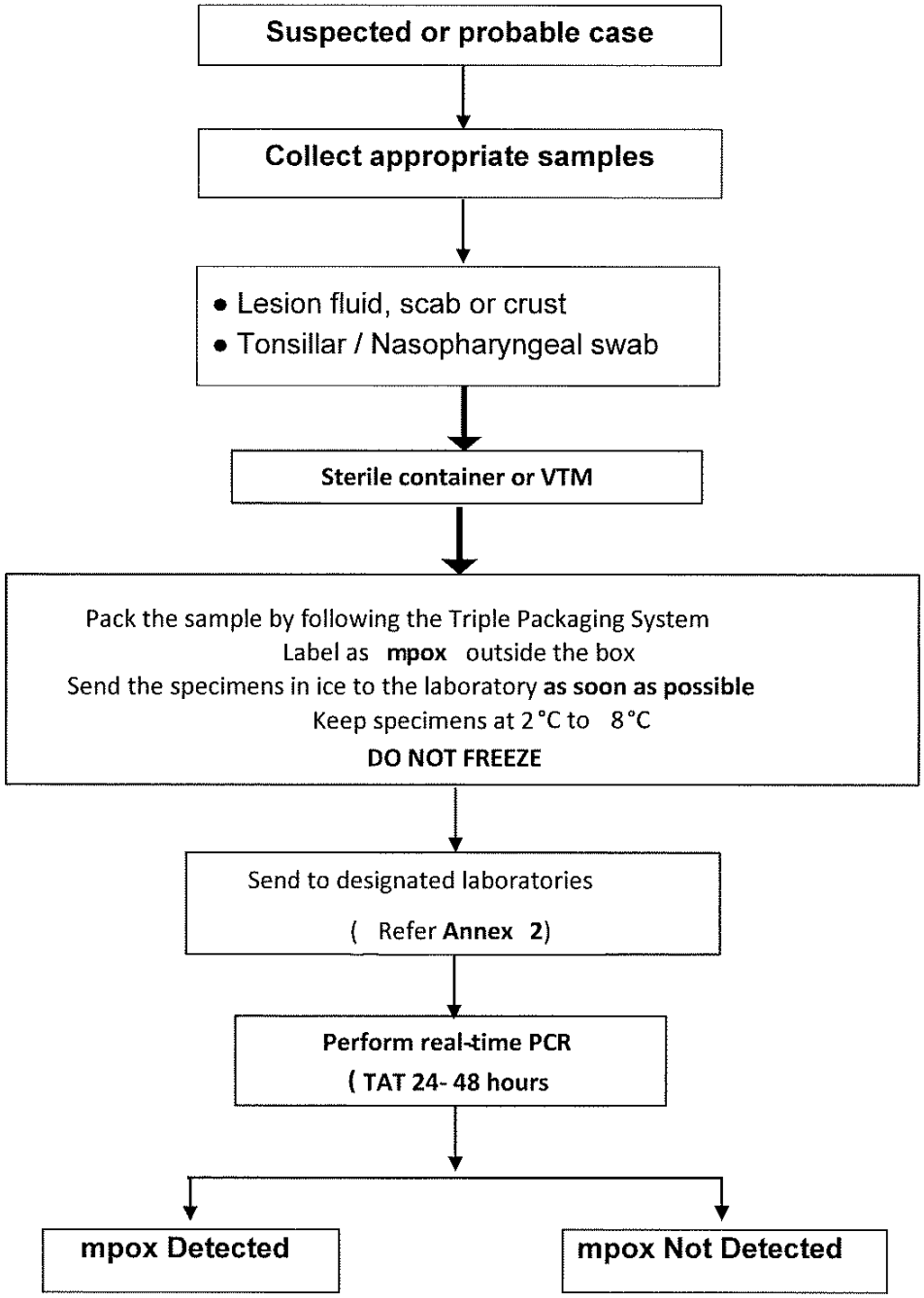
If the appropriate safety equipment and/or protocols are not available, consideration should be made to refer specimens to a suitably equipped reference laboratory.

1.5 Laboratory Waste Disposal Management

All contaminated disposable equipment and PPE (e.g., gown, gloves, mask/respirator N95) used during the manipulation and handling of specimen including specimen collection and processing should be placed in a biohazard bag for disposal with other medical waste. Needles and other sharp instruments should be placed in a sharp container.

Reusable equipment (e.g., goggles, face shield, Powered Air Purifying Respirator) should be disinfected and set aside for reprocessing.

Contaminated waste generated during specimen processing should be handled in accordance with existing facility procedures and local or state regulations for regulated medical waste.



Notes:

1. Reference laboratories: Public Health Laboratory (MKAK) and IMR
2. For other designated laboratories,
    i. 1st case detected, samples will be retested and verified by reference labs
        - PHL (MKA) and private lab: MKAK
        - Hospital laboratory (Hospital Sultanah Maliha): IMR
    ii. Subsequent positive result, verification is not required
    iii. All positive laboratory results must be notified to CPRC MOH
3. All positive samples will be sent to reference labs for sequencing

REFERENCES:

1. Privatisation of Hospital Support Services Cleansing Services: Approved Chemical List (ACL) Revision No. 8.

2. Policies & Procedure on infection Prevention and Control 2019 KKM.

Annex 2

SENARAI MAKMAL YANG BOLEH MENJALANKAN UJIAN MPOX PCR		
1	Makmal Kesihatan Awam Kebangsaan (MKAK)	Klinik Kesihatan di Zon Tengah (Negeri Sembilan, Melaka, Selangor, Pahang, WP Kuala Lumpur dan Putrajaya)
2	Makmal Kesihatan Awam Kebangsaan Ipoh (MKAI)	Klinik Kesihatan di Zon Utara (Perlis, Kedah, Pulau Pinang, dan Perak)
3	Makmal Kesihatan Awam Kebangsaan Kota Kinabalu (MKAKK)	Klinik Kesihatan dan hospital di Sabah dan WP Labuan
4	Makmal Kesihatan Awam Kebangsaan Kota Bharu (MKAKB)	Klinik Kesihatan dan hospital di Kelantan dan Terengganu
5	Makmal Kesihatan Awam Kebangsaan Johor Bharu (MKAJB)	Klinik Kesihatan dan hospital di Johor
6	Hospital Sultanah Maliha, Langkawi	Klinik Kesihatan dan hospital di Langkawi
7	Hospital Umum Sarawak (HUS)	Klinik Kesihatan dan hospital di Sarawak
8	Institut Peyelidikan Perubatan (IMR)	Semua hospital di Zon Tengah dan Zon Utara (selain Langkawi)
9	Neogenix Laboratories Sdn. Bhd.	Hospital atau klinik swasta
10	Innoquest Pathology Sdn. Bhd.	
11	Dunia Wellness Laboratories Sdn. Bhd.	
12	BP Healthcare	