Revised National Guidelines for Mpox



Developed by:

Dr. Muhammad Wasif Malik (Principal Scientific Officer CDC-NIH) Mirza Zeeshan Iqbal Baig (Epidemiology & Surveillance Lead UKHSA) Dr. Massab Umair (HOD virology department PHLD NIH) Majid Tahir (Health Education Officer CDC-NIH) Dr. Asif Bettani (Health Advisor UKHSA) Ms. Shazia Adeel (Quality assurance manager) Dr. Hamza Tanveer (FELTP Fellow 14th Cohort) Dr. Sara Saeed (Health Officer BHS) **Reviewed By:** Dr. Muhammad Salman (Chief PHLD-NIH) Dr. Mumtaz Ali Khan (PSO, Deputy Coordinator NCOC) Dr. Safi Malik (Health Advisor M/o NHSR&C) **Approved By:** Dr. Mukhtar Ahmad (PM Coordinator for M/o NHSR&C) Mr. Nadeem Mahbub (Federal Secretary M/o NHSR&C)

Dr. Shabana Saleem (Federal DG M/o NHSR&C)

Source (WHO, UKHSA, CDC)

(15 August, 2024 version 1.1)



Guidelines for Mpox Center for Disease Control National Institutes of Health (NIH) Ministry of National Health Services, Regulations & Coordination Phone: (+92-51) 9255082-9255232



15 August, 2024 (Version: 1.1)

Table of Contents.....

Chapter 1
Guidelines for Mpox Surveillance7
Background7
Global and national burden of disease7
Transmission9
Incubation period and sign and symptoms9
Figure-01 Clinical Presentation
Figure-02 mpox symptoms10
Case Definitions10
Objectives
Outbreak Threshold11
Case Reporting11
Surveillance Mechanism11
Surveillance tool11
Contact tracing
Definition of a contact
Table 1: Types of contacts 12
Contact monitoring
Monitoring of exposed health workers13
Travel-related contact tracing14
Figure-03 Testing algorithm15
Figure-04 Case Investigation Form16
Figure-05 Contact Follow Up Form17
Chapter 2
Prevention and Control of Mpox on Arrival at all International Airports
On Arrival
Wheelchair Handlers (Airlines)- Follow standard and in addition transmission based precautions as per WHO ref in the comment

Baggage handling	
Health staff	
CAA and ASF	19
Porters (Airline)	19
FIA/ Immigration	19
Customs Staff	19
ANF Officials	20
Janitors Cleaning Staff	20
Safe Disposal of Aircraft Waste	20
Lifts, Hand Rails and Baggage Trollies	20
Chapter 3	21
Prevention and Control on Arrival at all International Seaports	21
(KARACHI, PORT BIN QASIM & GAWADAR)	21
Chapter 4	
Prevention and Control on Arrival at all International Land Crossing	22
(THORKUM BORDER, WAGHA BORDER, KARTARPUR CORRIDOR, SO TAFTHAN BORDER & KHOKHARPAR BORDER)	
Chapter 5	23
Mpox Detection	23
Introduction	23
Indications for Testing	23
Sample Collection and Management	
Specimen to be collected	24
Figure-06 Specimen Collection and Storage	25
Sample Transportation	25
Figure-07 Sample collection criteria	26
Laboratory Testing	26
Molecular Methods	27
DNA Extraction	27
Molecular Detection	27
Figure-08 Testing Algorithm	
Interpretation of Results	
Differential Diagnosis	
Sample referral to Public Health Laboratories	
Data Reporting	
	Page 3

Mpox virus (MPVX): Real-time PCR protocol	31
Master Mix	31
Template	31
PCR Amplification Condition for G2R_G assay	32
PCR Amplification Condition for G2R-WA Assay	32
PCR Amplification Condition for C3L Assay	32
Primers and Probes	33
Chapter 6	35
Safe Handling and Waste Management of Suspected Mpox Virus Specimens	35
Principles of Biosafety: Primary and Secondary Protection Barriers	35
MPXV Biosafety during Specimen Processing and NAAT	35
Disinfection	35
Inactivation of Specimens Prior to Analysis	36
Waste Management	36
Chapter 7	37
Risk Communication and Community Engagement	37
Background	37
Modes of Transmission	37
Preventive Measures to be considered for RCCE activities	37
Avoid Contact with Infected Animals	37
Personal Hygiene	38
Avoid contact with infected person	
Vaccination	
Public Awareness	
Communication strategies	38
Communication Process	
Focus of awareness	39
Chapter 8	40
Infection Prevention and Control (IPC)	40
Background	40
GUIDELINES FOR IPC	
Vaccination	40
Case management of Mpox	41
B1- Isolation of people with mpox in non-hospital setting	42

B2-	Precautions for preventing mpox virus transmission in Hospital setting, PPEs42
Du	ration of Isolation Precautions for Patients with Suspected or Confirmed Mpox Infection43
C-	Hand Hygiene43
vi.	Contain and dispose of contaminated waste, such as dressings, bandages, or disposable glove43
D-	Environmental Infection Control (Healthcare settings)43
E-	Domestic settings
F-	Waste45
G-	Visitation45
Chapt	t er 9 46
Guide	line for Waste Management
Bao	
Ma	terial and Equipment46
Col	or Code for segregation of waste material46
MP	X Waste categorization47
No	n-Hazardous Infectious Waste (Packing Materials / Papers waste etc)47
Ha	zardous Infectious Waste
Dis	posal of Hazardous Infectious Waste (Sharps)47
Dis	posal of Hazardous Infectious/ Biological Waste47
Ha	ndling of waste of home isolated patients47
Ha	ndling of waste of healthcare facilities48
Dis	posal of MPX waste in Laboratory49
Saf	e handling of linen49
Wa	ste Container Management
Pre	cautionary Measures
	Figure-09 Coding for each waste category and the type of packaging and the appropriate treatment as summarized in the table below51

Chapter 1 Guidelines for Mpox Surveillance

This document serves to provide interim guidance/ recommendations to carry out mpox surveillance activities mainly case investigation, contact tracing and isolation. For the development of this document WHO, UKHSA and CDC guidelines were referred to and adopted within the country context.

Background

Mpox (previously Monkey pox) is an infectious disease caused by monkey pox virus. Mpox is an enveloped double-stranded DNA virus of the Orthopoxvirus genus in the Poxviridae family, which includes variola, cowpox, vaccinia and other viruses. The two genetic clades of the virus are clade I and II. The mpox virus was discovered in Denmark (1958) in monkeys. The first human case of mpox was reported in 1970 in the Democratic Republic of the Congo (DRC). Since then, cases reported in other central and western African countries including Cameroon, Central African Republic, Cote d'Ivoire, Democratic Republic of the Congo, Gabon, Liberia, Nigeria, Republic of the Congo, and Sierra Leone.

Majority of cases were reported from in Democratic Republic of the Congo. Outside Africa, cases were reported from USA, UK, Germany, Brazil, Spain, Columbia, Singapore, Australia, India and Israel etc which were linked with international travel or imported animals. In May, 2022 the World Health Organization declared mpox as a Public Health Emergency of International Concern. The natural reservoir of the virus is unknown whilst various small mammals such as squirrels, monkeys and rodents are susceptible.

Global and national burden of disease

As of 30^{th} June 2024, a total of 99,176 cases and 208 deaths associated with mpox have been reported globally. The distribution of cases among all region with highest number of cases in Americas (n= 62,904) followed by Europe (n= 27,529), Africa (n= 4,232), Western Pacific (n= 3,491), South East Asia (n= 925) and Eastern Mediterranean (n= 95) whilst 116 countries from all six region have reported mpox cases. From 1 January 2022 to 30 June 2024, the ten countries that reported the highest cumulative number of confirmed cases globally are the United States of America (n = 33 191), Brazil (n = 11,212), Spain (n = 8, 084), France (n = 4,272), Colombia (n = 4,249), Mexico (n = 4,124), the United Kingdom (n = 3,952), Peru (n = 3,875), Germany (n = 3,857), and the Democratic Republic of the Congo (2,999). This marks the first time the Democratic Republic of the Congo has featured among the top ten countries that have reported the highest cumulative number of confirmed cases globally. The updated numbers till the date will be uploaded by WHO soon.

A total of 934 new cases were reported in June 2024, comparable to the number of new cases reported for May 2024, including some cases retrospectively reported for previous months. For the second month in a row, most cases in June 2024 were reported from the African Region (61%), followed by the Region of the Americas (19%), and the European Region (11%). In June 2024, 16 of 26 (62%) reporting countries showed an increase in cases compared to May 2024. The Democratic Republic of the Congo reported the highest relative increase in the African Region (n = 543 vs 459), Spain (n = 54 vs 38) reported the highest increase in the European Region, Colombia (n = 11 vs zero) reported the highest increase in the Western Pacific Region, and no country reported an increase in the South-East Asia Region. No case was reported to WHO by the Eastern Mediterranean Region.

As per recent risk assessment (August, 2024) conducted by WHO, the Eastern Democratic Republic of the Congo and neighboring countries ranked **High**, affecting mostly adults and spreading predominantly through sexual contact (linked to clade-lb), areas of the Democratic Republic of the Congo where mpox is endemic has also been ranked **High**, affecting mostly children and spreading through multiple modes of transmission (linked to clade-la) whilst Nigeria and countries of West, Central and East Africa where mpox is endemic ranked **Moderate**, affecting children and adults and spreading through multiple modes of transmission (linked to both clades I and II), rest of the countries in Africa and around the world also ranked **Moderate**, where outbreaks affect mainly men who have sex with men and spread predominantly through sexual contact (linked to clade-IIb).

In June 2024, a total of 934 new laboratory confirmed cases of mpox and four deaths were reported to WHO from 26 countries, showing continuing transmission of mpox across the world. The most affected WHO regions, by number of laboratory-confirmed cases were, the African Region (n=567), the Region of the Americas (n=175), the European Region (n=100), the Western Pacific Region (n=81) and the South-East Asia Region (n=11). The Eastern Mediterranean region did not report cases in June 2024. As reporting from countries to WHO has been declining, the current reported global data most likely underestimate the actual number of mpox cases. Within the African Region, the Democratic Republic of the Congo reported most (96%) of the confirmed cases during June, 2024. The WHO recent reports also features an update on the geographic expansion of mpox in the WHO African Region from July – August 2024.

Four new countries in Eastern Africa (Burundi, Kenya, Rwanda, and Uganda) reported their first mpox cases. In 2024, Burundi reported 61 cases distributed across several districts till 9th August, Kenya reported one confirmed case of mpox in Taita Taveta County (on Kenya-Tanzania border) on 29th July 2024, Rwanda notified four laboratory-confirmed mpox cases till 7th August and Uganda reported two confirmed cases till 2nd August. Upon genomic sequencing of all cases clade-I has been identified and are linked to the expanding outbreak in East and Central Africa and all cases sequenced to date from these countries are clade-I. Central African Republic (CAR) declared an mpox outbreak on 26 July 2024 and reported 26 confirmed cases. Separately, Côte d'Ivoire reported six cases till 2nd August and experiencing an outbreak of mpox linked to clade-II MPXV and 22 cases have been reported from 8th May to 6th July from South Africa.

Globally, during last six months (1 January 2024 – 30 June 2024) cases fluctuated between 700 to 1000 (averaging 866 cases per month), with most cases reported by the African Region, followed by the Americas and European Regions. Globally, 96.4% of confirmed cases are male patients, with a median age of 34 years (interquartile range: 29 - 41 years). The age and sex distribution of cases remain stable over time, especially outside the African Region. Among cases with age data available, 1.3% were 0 - 17 years while 0.4% of aged 0 - 4 years. In clade-IIb affected countries, children have been least affected. Conversely, in historically affected countries such as the Democratic Republic of the Congo, children under 15 years of age represent most reported mpox cases. Among modes of transmission, sexual contact is the most commonly reported (83.8%), followed by person-to-person non-sexual contact. This pattern has persisted over the last six months, with 95.6% of new cases reporting sexual contact. Detailed information on transmission is not available for most cases in the African Region; available information suggests modes of transmission in this region are more diverse including human-to-human transmission due to different types of physical or close direct or indirect contact and, in some settings, also zoonotic exposure. Among symptoms the most common was any rash (88.5%) followed by fever (57.9%) and systemic rash or genital rash (54.8%). The symptomatology of cases has been very consistent over time in the global outbreak.

The mucosal (including genital) lesions have always been a feature of mpox, among cases exposed through sexual contact in the Democratic Republic of the Congo, some individuals present only with genital lesions, rather than the more typical extensive rash associated with clade-I MPXV. Around 52% of cases are living with HIV. This proportion approximates that reported cases in the last six months are related to a common risk factor of sexual exposure. Information on HIV status is not available for most cases in the African Region, and the above description might not be fully representative of the cases with different demographics in this region.

In 2022, around 100 countries reported mpox cases for the first time, with human-to-human transmission continuing for several months. This is the first time that sustained community transmission has occurred outside of previously known affected areas of west or central Africa. Keeping these factors in consideration IHR committee declared mpox as a Public Health Emergency of International concern (PHEIC) in July 2022 which was later on lifted in 2023. With upsurge of cases (clad 1) from non-endemic countries and considering the factors relating to emergency WHO IHR committee again declared mpox as PHEIC on 14th August, 2024.

The first ever case of mpox in Pakistan was reported to WHO on 20th April 2023. As of 15th August, 2024, a total of 11 confirmed cases and one death associated with mpox have been reported. The evidence of local transmission has not yet been established.

Transmission

Human-to-human transmission of mpox can occur through direct contact with infectious lesions of the skin or mucous membranes or body fluids from those lesions, this includes face-to-face, skin-to-skin, mouth-to-mouth or mouth-to-skin contact and through respiratory droplets (and possibly short-range aerosols requiring prolonged close contact). The mpox virus enters the body through broken skin, mucosal surfaces (e. g. oral, pharyngeal, ocular, genital or anal), or via the respiratory tract.

The infectious period can vary, but generally patients are considered infectious from the time of symptom onset until skin lesions have crusted, the scabs have fallen off and a fresh layer of skin has formed underneath. Transmission can also occur from the environment to humans from contaminated clothing or linens that have infectious skin particles (also described as fomite transmission). If shaken, these particles can disperse into the air and be inhaled, land on broken skin or mucosal membranes and lead to transmission and infection.

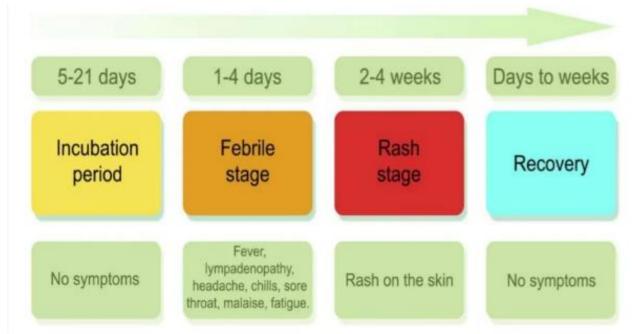
Incubation period and sign and symptoms

The incubation period of mpox ranges from 5 to 21 days. Typically, the prodromal phase of clinical illness lasts 1-5 days during which time patients may experience fever, headache, back pain, muscle aches, and lymphadenopathy. This is followed by a second phase, which typically occurs after the fever subsides, with the appearance of skin and/or mucosal rash, which might include a single or multiple lesions. Typically, the lesions progress through macules, papules, vesicles, and pustules, before crusting over and desquamating over a period of 2 to 4 weeks. In the context of this outbreak, patients are presenting more mucosal lesions than previously described, and often these are localized in the genital or perineal/perianal area as well as in the mouth and on the eyes. Lesions might appear at different stages of progression and it has been observed that the rash can develop prior to typical prodromal or constitutional symptoms (such as fever, fatigue). Ano-rectal pain and bleeding (e.g. due to proctitis) has also been reported more often in this outbreak. In addition, lymphadenopathy (swollen lymph nodes) is a classic feature of mpox. Some people can be infected without developing any symptoms.

Figure-01 Clinical Presentation



Figure-02 mpox symptoms



Case Definitions

Suspected Cases: Any person having skin rash/lesion (may include single or multiple oral, conjunctival, urethral, penile, vaginal, or ano-rectal lesions) with or without fever (>38.3°C), headache, lymphadenopathy, myalgia (muscle pain/body aches), back pain, profound weakness, any respiratory

symptom and fatigue. Contact of probable/confirmed case developing febrile prodromal illness compatible with mpox.

Probable case: A suspected case with an epidemiological link to confirmed cases or probable case during last 21 days.

Confirmed case: A person with laboratory confirmed MPXV infection by detection of unique sequences of viral DNA by real-time polymerase chain reaction (PCR) and/or sequencing.

Objectives

- Identify mpox cases and sources of infection.
- Rapidly identify new cases and prevent further transmission at points of entry
- Perform contact tracing.
- Identify vulnerable population and to implement public health measures.
- Perform the epidemiological analysis of the surveillance data to formulate recommendations for prevention and control.

Outbreak Threshold

One confirmed case of mpox is considered as an outbreak while one suspected case will be considered as an alert.

Case Reporting

All mpox cases detected by surveillance teams and clinicians will be immediately reported to federal and provincial emergency operation centers (EOCs). The bottom-up approach will be used for the flow of information (health facility – district surveillance and response unit – provincial surveillance and response unit - provincial EOCs/PDSRUs/provincial health department – NCOC-NIH/ M/o NHSR&C – WHO country/ regional office). Probable and confirmed cases of mpox should be reported as early as possible, including a minimum dataset of epidemiologically relevant information.

Surveillance Mechanism

The suspected/ probable case will be identified on the basis of case definition by:

- 1. Border health services (BHS) and points of entry (POE)
- 2. Health care facility
- 3. Active case identification by RRT.
- 4. Self-Reporting
- 5. Diagnostic Laboratory

Once the suspected case will be identified, sample will be sent to National/ provincial public health lab for confirmation. RRTs will perform case investigation and contacts will be identified. After the lab confirmation of the suspected case, RRT will perform contact tracing and samples of symptomatic cases will be sent to the designated labs for testing. The referral facility will also be identified for isolation or quarantine and case management. The risk communication will be done simultaneously.

Surveillance tool

A pre-structured questionnaire for both cases and contacts will be adopted to collect the epidemiological information. Initially line list will be updated using MS Excel. Later on surveillance data will be captured on DHIS2 using existing integrated Disease Surveillance and Response System. Frequency of reporting will be done on daily basis.

Contact tracing

Contact tracing is a key public health measure to control the spread of infectious diseases. It allows for the interruption of chains of transmission and can also help people at a higher risk of developing severe disease. Cases should be promptly interviewed as soon as possible to elicit the names and contact information of all potential contacts and identify places visited where contact with other people may have occurred. Contacts of cases should be notified within 24 hours of identification and advised to monitor their health status and seek medical care if they develop symptoms. Depending on their level of exposure, information sheet will be provided to contacts to observe their regular health status and to inform health care authorities in case of development of symptoms. The contacts will be followed up and monitored regularly by RRT team till the completion incubation period or on the availability of lab test.

Definition of a contact

A contact is defined as a person who has been exposed to an infected person during the infectious period i.e. the period beginning with the onset of the index case's first symptoms and ending when their skin lesions have crusted, the scabs have fallen off and a fresh layer of skin has formed underneath. Types of contacts on the basis of exposure are as follows:

Table 1: Types of contacts

Exposure Risk	Description	Public health Measures for contacts
High risk contact	Direct exposure of broken skin or mucous membranes to mpox case, their body fluids or potentially infectious material (including clothing or bedding) without wearing appropriate PPE	 Passive monitoring Provide information sheet. Avoid sexual or intimate contact and other activities involving skin to skin contact for 21 days from last exposure. Avoid contact with immunosuppressed people, pregnant women, and children aged under 5 years where possible for 21 days from last exposure. Consider exclusion from work following a risk assessment for 21 days if work involves skin to skin contact with immunosuppressed people, pregnant women or children aged under 5 years (not limited to healthcare workers)
Medium Risk contact	 Intact skin-only contact with an mpox case, their body fluids or potentially infectious material or contaminated fomite. Passengers seated directly next to mpox case on plane No direct contact but within one meter for at least 15 minutes with an mpox case 	 Passive monitoring Provide information sheet Avoid sexual or intimate contact and other activities involving skin to skin contact for 21 days from last exposure

· · · · · · · · · · · · · · · · · · ·		
	without wearing appropriate	
	PPE	
Low Risk	Contact with mpox case or	Will be observed till the end of incubation period (21
contact	environment contaminated	days).
	with MPXV while wearing	
	appropriate PPE	
	 Healthcare worker (HCW) 	
	involved in care of an mpox	
	case not wearing appropriate	
	PPE without direct contact and	
	maintained a distance	
	between one and 3 meters and	
	no direct contact with	
	contaminated objects	
	Community contact between	
	one and 3 meters of an mpox	
	case	
	 Passengers seated within 3 	
	rows from an mpox case on	
	plane	
	plane	

Contact monitoring

Contacts should be monitored by the RRT or should self-monitor on regular basis for the onset of signs or symptoms for a period of 21 days from the last contact with the probable or confirmed case or their contaminated materials during the infectious period. Signs and symptoms of concern include headache, fever, chills, sore throat, malaise, fatigue, rash, and lymphadenopathy. Contacts should monitor their temperature twice daily.

Any individual with signs and symptoms compatible with MPXV infection; or being considered a suspected, probable, or confirmed case of mpox by jurisdictional health authorities; or who has been identified as a contact of a mpox case and, therefore, is subject to health monitoring, should avoid undertaking any travel, including international, until they are determined as no longer constituting a public health risk. The federal and provincial health departments may have an emergency contact no for any assistance.

Monitoring of exposed health workers

Any health worker who has involved in caring or dealing with probable or confirmed mpox case or worked with a relevant laboratory specimen will observe and inform for development of any symptoms that could suggest mpox, especially within the 21-day period after the last date of exposure. The WHO recommends that health workers with an occupational exposure to MPXV should notify infection control, occupational health, and public health authorities for further guidance regarding IPC practices and management. Moreover, health care staff will comply IPC practices as per recommended guidelines in the SOP and will report in case of any breach.

Health workers who have occupational exposure to patients with mpox or possibly contaminated materials (such as by a needle stick or other percutaneous sharps injury, fomites or contact with a case while not wearing appropriate PPE) should follow national infection control guidance. Such contacts do not need to be excluded from work duty if asymptomatic, but should actively monitor for symptoms,

which includes measurement of temperature twice daily for 21 days following the exposure; conversely, they should not work with vulnerable patients during this period. Prior to reporting for work each day, the health worker should be interviewed regarding evidence of any relevant signs or symptoms as above. Where vaccines are available, post-exposure vaccination within four days of exposure (or up to 14 days in the absence of symptoms) is recommended for health workers, including laboratory personnel, who met a case or potentially infectious material without use of appropriate PPE.

Travel-related contact tracing

Public health officials will work with border health security/points of entry, and other national health authorities to facilitate international contact tracing, when required, during travel or upon return, in order to assess potential risk of exposure and to identify contacts (passengers and others) who may have had exposure to a case while travelling. If a probable or confirmed case is reported in a long-distance travel (e.g., lasting more than 6 hours), travelers seated in the same row, two rows in front and two rows behind the sick traveler as well as the cabin crew who served the case, can be contacted to assess the risk of exposure and monitoring requirements. Any passenger or crew team member who did not report physical contact with a symptomatic case and was wearing PPE such as face mask for COVID-19 should not be considered a mpox contact. More specific evaluations for each scenario need to be assessed on a case-by-case basis by national and local health authorities.

Border Health Services will coordinate with aligned departments to provide detailed information of the cases and contacts for all inbound travel through airport, seaport and land crossings.

Figure-03 Testing algorithm

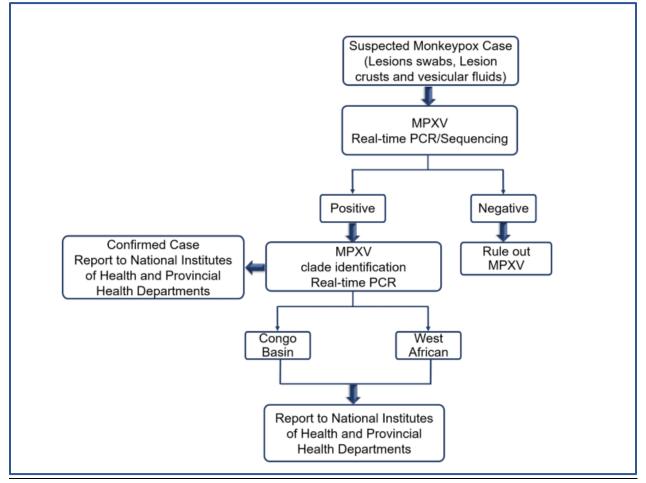


Figure-04 Case Investigation Form

Mpox case investigation form							
Demographic Information							
Unique identifier number/CNIC		Date	/				
Name		Age/ Gender					
Permeant address		Relationship with patient					
Residential Address during last one month		Contact number					
Marital status		Education					
Occupation		Interviewer Name					
Reporting site/Distt		Interviewer Contact					
	Medical History	/ Clinical Presentation					
HIV Status	Pos 🗌 /Neg 🛄	Rash	Yes /No				
Smallpox vaccination	Yes /No	Fever	Yes /No				
Any history if sexually transmitted infection	Yes /No	Fatigue	Yes 🛄 /No 🛄				
Name of infection (Chlamydia, Gonorrhea, Genital Herpes, Syphilis, fungal infection etc.)		Genital lesions	Yes //No				
Warts	Yes /No	Lymphadenopathy	Yes /No				
ТВ	Yes /No	Muscular pain	Yes /No				
Any antiviral treatment	Yes 🗌 /No 📃	Sore throat	Yes /No				
Hospitalization	Yes /No	Headache	Yes /No				
Intensive care	Yes 🗌 /No 📃	Respiratory symptoms	Yes /No				
Home isolation as advised by doctor	Yes /No	Any other Symptoms					
Type of Rash	Pus blister macule papule	Date of Rash	/				
Case classification	Suspected Probable Confirms	Date of Onset of symptoms	/				
	E	xposure					
Contact with case	Yes /No	House/Flat	Yes /No				
Date of contact	/	Work	Yes /No				
Travel History	Yes /No	Travel	Yes /No				
Travel country		Animal contact	Yes /No				
Place of the contact		Setting of contact (eg. Mass gathering, hotel, market, school etc.)					
Mode of transmission							
Animal handling	Yes 🗌 /No 📃	Type of Contact	Physical 🔲 Fomite 🥅 Sexual 🦳				
Health facility	Yes /No	Type of Sexual contact	Marital 🔛 Extramarital 🔛				
Lab	Yes 🗌 /No 📃	Date of last sexual activity	/				
Others							
	Lab	nformation					
Swab	Yes /No	Genital secretion	Yes /No				
Crust	Yes /No	Oropharyngeal	Yes /No				
Date of sample collection	//	Lab result	Yes /No				

Figure-05 Contact Follow Up Form

Contact Follow up Form								
Personal details								
Unique ID/ CNIC		Date						
Name		Current Address						
Age/Gender		Current Contact Number						
	M	onitoring						
Monitoring start date		Monitoring End date						
Day1	Symptomatic Asymptomatic	Day11	Symptomatic Asymptomatic					
Day2	Symptomatic Asymptomatic	Day12	Symptomatic Asymptomatic					
Day3	Symptomatic Asymptomatic	Day13	Symptomatic					
Day4	Symptomatic Asymptomatic	Day14	Symptomatic Asymptomatic					
Day5	Symptomatic Asymptomatic	Day15	Symptomatic Asymptomatic					
Day6	Symptomatic Asymptomatic	Day16	Symptomatic Asymptomatic					
Day7	Symptomatic Asymptomatic	Day17	Symptomatic Asymptomatic					
Day8	Symptomatic Asymptomatic	Day18	Symptomatic Asymptomatic					
Day9	Symptomatic Asymptomatic	Day19	Symptomatic Asymptomatic					
Day10	Symptomatic Asymptomatic	Day20	Symptomatic Asymptomatic					
		Day21	Symptomatic					
Clinical signs and symptoms								
Date of onset of symptoms		Date of Samples						
Type of symptoms		Type of Samples						

Chapter 2

Prevention and Control of Mpox on Arrival at all International Airports

Based on available information at this time, WHO does not recommend that Member States adopt any measures that restrict international traffic for either incoming or outgoing travelers. However, precautionary measures are advised for those planning or those who undertook international travels. Infection prevention and control (IPC) measures are critical at points of entry to prevent the spread of infectious diseases. Here are some guidelines to follow:

On Arrival

- i. Air traffic control tower shall inform BHS-P for safe disembarkation of the disease suspected passenger. The suspected passenger should wear medical mask
- ii. To prevent the exposure from the suspected diseases passenger the aircraft will be allowed by the air traffic control tower to let disembark the said passenger at apron and not through routine channel of bridge area. The passenger will be moved immediately in CAA ambulance to the designated isolation area after immigration.
- iii. Immigration of the Suspect to be done by FIA under proper preventive measures. Handle the documents wearing mask and gloves and keep them in a zip lock bag.
- iv. The practice of protocols shall be stopped at once to prevent any chance of exposure from suspected disease passenger and further outbreak of diseases of international concern. CAA, FIA, ASF, ANF, Custom and Airlines staff shall ensure stoppage of this practice and shall ensure their staff do not stand at and around the health counters to protect them self and to prevent further spread of the diseases.

Wheelchair Handlers (Airlines)- Follow standard and in addition transmission based precautions as per WHO ref in the comment.

- i. Staff carrying wheelchairs must wear masks and gloves.
- ii. Cleaning and disinfection of wheelchair with bleach after transporting the suspected passenger each time.

Baggage handling

- v. All baggage handlers must wear masks and gloves while handling baggage.
- vi. BHS-P fumigators to carry out disinfection of all the baggage as soon as it is disembarked from the aircraft. BHS-P fumigator after such flight having diseases suspected passenger will disinfect the luggage, luggage area, FIA counter Area, Health inspection Area, corridors, high touch surfaces of escalator, toilets and as well as arrival lounge if any transit passengers stay there for next flight for deport where required in this connection the concerned airline Operators is held responsible to carry out all process under required Public Health Preventive measures.

Health staff

- i. Ensure hand hygiene and wear proper PPEs (mask and gloves).
- ii. Do not come in close contact with passengers.
- iii. Be vigilant and observe each and every passenger one by one specially Umra pilgrims. Anyone observed with any skin lesion on exposed parts or who falls on case definition

must be isolated immediately from the rest and inform team lead for further management and transfer for isolation.

- iv. Strict disinfection measures of the premises, including the toilets, to be followed.
- v. Proper disposal of used PPEs according to the guidelines needs to be ensured.

CAA and ASF

- i. CAA and ASF shall implement social distancing measures, such as marking out appropriate distances for queuing and seating arrangements and ensuring compliance, to minimize the risk of transmission. CAA and ASF shall facilitate BHS-P staff at Airports and avoid issuing flight passes for protocol to minimize the chances of spreading the infection.
- ii. Civil Aviation shall ensure provision of a suitable place to BHS-P Airport Health Department at cargo areas of all International airports so that the required screening of cargo goods/parcel/livestock could be done to avoid spread of infection.

Porters (Airline)

CAA Porters handling baggage shall wear masks and gloves while handling baggage. Frequent sanitization of the Trolley handles and hand hygiene (frequent hand washing with soap and water or using hand sanitizer) is recommended.

FIA/ Immigration

- i. To observe public health preventive measures while handling the passengers.
- ii. Wear face masks and gloves when handling passports and other documents of the passengers.
- iii. Hand hygiene (frequent hand washing with soap and water or using hand sanitizer) and sanitization of counters' front and desk area as well as frequently touched surfaces after each flight.

Customs Staff

- i. Customs officers / officials to observe standard public health preventive measures and wear masks, gloves and use hand sanitizer while handling and screening baggage and guiding the passengers at arrival.
- ii. Hand hygiene (frequent hand washing with soap and water or using hand sanitizer)
- iii. The following details need to be captured for any consignment / detail of cargo (Goods or any livestock or Plants) being imported in the country.

S.No	Consignment	Containing	Tracking	Passport	Company	Origin	Nationality	Placement	Transit
	No.		ID	No	Concern	of		detail on ship	Airport
						Country			
						& city			
						-			

Any cargo / consignment / Goods etc. at arrival shall be cleared only after Health clearance at airport and issuance of Health clearance certificate by BHS-P through Airport Health Department.

ANF Officials

- i. ANF officer/officials to observe Public Health Preventive Measure including wearing PPE's, mask, and gloves and using sanitizer while performing their duty for checking of baggage of the diseases suspected passenger.
- ii. Hand hygiene (frequent hand washing with soap and water or using hand sanitizer)
- iii. Sanitization and disinfection of counters.

Janitors Cleaning Staff

- i. Janitors and cleaning staff deputed to clean premises and toilets to wear masks and gloves for self-protection.
- ii. Use 0.1 % bleach solution to clean the toilets
- iii. Thorough washing of the toilets with detergent and bleach.
- iv. Ensure frequent disinfection of urinals.
- v. Proper and safe waste disposal of toilets waste bins.
- vi. Frequent monitoring and supervision of hygienic and sanitary conditions of the Airport and its premises.
- vii. Hand hygiene (frequent hand washing with soap and water or using hand sanitizer)

Safe Disposal of Aircraft Waste

The concerned CAA agency engaged in proper safe disposal of aircraft waste shall strictly comply with the waste disposal guidelines.

Lifts, Hand Rails and Baggage Trollies

As they come in frequent contact with passengers. These should be disinfected after each flight by BHS-P Staff and CAA.

Chapter 3

Prevention and Control on Arrival at all International Seaports

(KARACHI, PORT BIN QASIM & GAWADAR)

- i. No personnel from any agency should be allowed to get on board of the ships/Conveyance before the Port Health Department visit and inspection of the ship and Health Clearance irrespective of the origin of the ship Voyage.
- ii. No Personnel from any Agency/Department should be allowed to get on board without proper PPEs (personal protective equipment) irrespective of non-endemic Mpox reporting countries bound/voyaged ships or other.
- iii. All the Pax/Crew on the board is advised to use PPEs including mask and gloves.
- iv. All the second hand goods/ containers must be Disinfected/ under the supervision of Port Health Department and certified by the Port Health Officer.
- v. The Pakistan Customs is advised to include the Fumigation Certificate of Port Health Department in their system Web Based One Custom (WeBOC) and give access to BHS-P and Port Health Department for submission and verification of Health Documents. It is necessary that WeBOC is required so that we could trace out the origin of contamination of the goods from the infected region. Custom is also directed to submit the manifesto to BHS-P staff of the Port Health in detail as mentioned below;

S.No	Container No.	Loaded with	Company Concern.	Origin of Country	Nationality	Placement detail on ship	Transit Port
------	------------------	----------------	---------------------	----------------------	-------------	-----------------------------	--------------

- i. All health-related data must be shared with the Port Health Authorities on daily/regular basis.
- ii. All Port authorities are hereby directed to take routine preventive health measures like disinfection etc within the territory of the port of berth area under the supervision of Port Health Officers.
- iii. In case of a suspected crew on board the master of the ship is advised to stay on the outer anchorage of the port and immediately inform the BHS-P about the case.
- iv. In case of a suspected case on board the ship Master/Ship Surgeon immediately isolate the suspected crew in his room or a specified area for this purpose. The Ship Master without any delay must inform the Port Health Authority about the case for further necessary Health measures.
- v. In case of a confirmed Mpox case on board, all the Crew must be quarantined for 21 days and should not be allowed any short leave.
- vi. In case of a confirmed case on board the ship should undergo proper disinfection/Fumigation procedures under the supervision of Port Health Department.
- vii. Strict personal hygiene including frequent hand washing practices should be encouraged.
- viii. The Animal Quarantine Department is directed to take necessary preventive measures while handling the animals as Mpox is a zoonotic disease and can spread from animal to human being.

Chapter 4

Prevention and Control on Arrival at all International Land Crossing

(THORKUM BORDER, WAGHA BORDER, KARTARPUR CORRIDOR, SOST BORDER, CHAMAN BORDER, TAFTHAN BORDER & KHOKHARPAR BORDER)

- i. All functionaries shall adopt Public Health preventive measures while performing their duties and if found anyone suspected to have MPOX should report to BHS-P staff posted at that area.
- ii. Ensure monitoring/ screening of passenger and disinfection of baggage cargo arriving from affected areas in the containers (I trucks) or carried in hand by person without vehicle so that they are maintained in such a condition that they are free of contamination.
- iii. Customs department shall note down and submit the detail of the containers (loaded with goods / livestock and coming from MPOX outbreak, endemic or non-affected countries
- iv. BHS-P Staff will screen the passenger coming in the vehicle or pedestrian through thermal scanner and by observation being properly dressed with PPE.
- v. If a passenger is suspected to have communicable disease and coming from the infected region of Mpox then he/she will be quarantined in isolation center for 21 days before joining the community
- vi. Proper Screening of container truck drivers and assistants and obtaining health declaration form.
- vii. Disinfection of containers when required under the supervision of BHS-P staff.

Chapter 5 Mpox Detection

This guidelines are based on the World Health Organization and PAN American Health Organization interim guidance on "Laboratory testing for the mpox virus" and is intended to provide guidance to provincial public health laboratories and any other lab having requisite capacity for lab testing of mpox virus.

Introduction

Mpox is an infectious viral disease caused by the mpox virus (MPXV), a member of the *orthopoxvirus* genus within the *Poxviridae* family. MPXV is a double-stranded DNA virus around 190 kb in size having two phylogenetically distinct clades: Clade I (Congo Basin) and the Clade II (West African). Clade II encompass two phylogenetically distinct subclades, IIa and IIb. All sequences in the ongoing 2022-24 mpox outbreak, as of August, 2024, are from Clade II, or more specifically, Clade IIb. Among these, the vast majority have been associated with the B.1 lineage of Clade IIb. However, there has been recent upsurge of clade-I MPXV in Eastern and Central African countries.

The incubation period of Mpox ranges from 5 to 21 days. Typically, the prodromal phase of clinical illness lasts 1-5 days during which time patients may experience fever, headache, back pain, muscle aches, and lymphadenopathy. This is followed by a second phase which typically occurs after the fever subsides, with the appearance of skin and/or mucosal rash, which might include a single or multiple lesions. Typically, the lesions progress through macules, papules, vesicles, and pustules, before crusting over and desquamating over a period of 2 to 4 weeks. Rashes with swollen lymph nodes is the distinctive feature of Mpox.

Because of the range of conditions that cause skin rashes and because clinical presentation it can be challenging to differentiate mpox solely based on the clinical presentation. Therefore, the decision to test should be based on both clinical and epidemiological factors, linked to an assessment of the likelihood of infection.

Given the current multiple detection of MXPV world-wide, any individual meeting the definition for a suspected case should be offered testing. In this sense, the National Institutes of Health (NIH) recommends to provincial health departments to ensure the timely identification of suspect cases, the timely collection of samples and the implementation of molecular detection protocols at the provincial public health reference laboratories and relevant labs according to the existing capacity. Where necessary, shipping of samples to NIH may be considered.

Address: Department of Virology, Public Health Laboratories Division, National Institutes of Health, Islamabad. +92 (51) 9255082; +92 (51) 9255238.

Indications for Testing

Any individual meeting the definition for a suspected case should be offered testing. The decision to test should be based on both clinical and epidemiological factors, linked to an assessment of the likelihood of infection. Due to the range of conditions that cause skin rashes and sign/symptoms resembling to other diseases (chickenpox, measles) it can be challenging to differentiate mpox solely based on the clinical presentation. It is therefore important to consider other potential causes of discrete skin lesions or a disseminated rash; examples of other etiologies for similar-appearing skin lesions at the different stages of development include herpes simplex virus, varicella zoster virus, measles etc.

Sample Collection and Management

Safety Procedures

Use of standard operating procedures (SOPs) must be ensured, and laboratory personnel must be trained for appropriate use of personal protective equipment (PPE) including disposable anti fluid gown, latex gloves, goggles or full-face cover, face mask, head cover, shoe covers, and for the disposal of used PPE. Additionally, staff should be appropriately trained for specimen collection, storage, packaging, and transport.

Biological Risk Management

Measures should be taken to minimize the risk of laboratory transmission based on a risk assessment at institutional level when testing routine clinical specimens from confirmed or suspected mpox patients. These may include limiting the number of staff testing specimens only to those with proven competency, wearing appropriate PPE, using rigorously applied standard precautions, using effective disinfectants (which include quaternary ammonium compounds and 0.5% (or 200ppm) bleach (0.5%), and avoiding any procedures that could generate aerosols. Rigorous adherence to infection prevention and control guidelines must be ensured during specimen collection and handling.

It is recommended that all manipulations of specimens originating from suspected, probable, or confirmed cases of mpox in the laboratory be conducted according to a risk-based approach. Each laboratory should conduct an Institutional risk assessment and when manipulating biological specimens, core biosafety requirements (biosafety level 2) must be met and heightened control measures should be applied based on risk assessment.

MPXV may be contracted during the specimen processing stage from contaminated material or faulty processes. Therefore, heightened biosafety measures are recommended in addition to the core requirements, including the following for the purpose of clinical testing without virus propagation:

- Specimens from patients with suspected MPXV infection must be handled in a functioning and certified Class II biosafety cabinet, prior to sample inactivation. Properly inactivated specimens do not require a biosafety cabinet.
- Laboratory personnel should wear appropriate PPE, especially for handling specimens before inactivation.

• Where use of a centrifuge is required for a procedure, safety cups or sealed rotors should be used. Additional control measures should be considered for specific procedures, including aerosol-forming procedures, according to the local risk assessment. For more information on core biosafety requirements and heightened control measures, please see the fourth edition of the WHO Biosafety Manual [6].

Specimen to be collected

The recommended specimen type for laboratory confirmation of mpox virus is **skin lesion material**, including:

- Swabs of lesion surface and/or exudate,
- Roofs from more than one lesion, or
- Lesion crusts
- Lesions swabs, crusts and vesicular fluids should not be mixed in the same tube.

Swab the lesion vigorously using Dacron or polyester flocked swabs, to ensure adequate viral DNA is collected. Both dry swabs and swabs placed in viral transport media (VTM) can be used. Two lesions of the same type should be collected in one single tube, preferably from different locations on the body and which differ in appearance. In addition to a lesion specimen, the collection of an oropharyngeal swab is encouraged. However, data on the accuracy of this specimen type for diagnosis is limited for mpox virus, therefore a negative throat swab specimen should be interpreted with caution.

Figure-06 Specimen Collection and Storage

Specimen Type	Collection Materials	Storage Temperature	Collection purpose
Skin lesion material, including: • Swabs of lesion exudate • Lesion roofs • Lesion crusts	Dacron or polyester flocked swabs with VTM or dry swab	Refrigerate (2-8 °C) or freeze (- 20°C or lower) within 1 hour of collection; -20°C or lower after 7 days *	Recommended for diagnosis
Oropharyngeal swab	Dacron or polyester flocked swabs with VTM or dry swab	Refrigerate (2-8 °C) or freeze (- 20°C or lower) within 1 hour of collection; -20°C or lower after 7 days *	Recommended for diagnosis if feasible, in addition to skin lesion material
Serum	Serum-separating tubes	Refrigerate (2–8 °C) or freeze (-20 °C or lower) within 1 hour of collection; -20°C or lower after 7 days	To be considered for serology for diagnosis/differential diagnosis (following ethics guidelines)

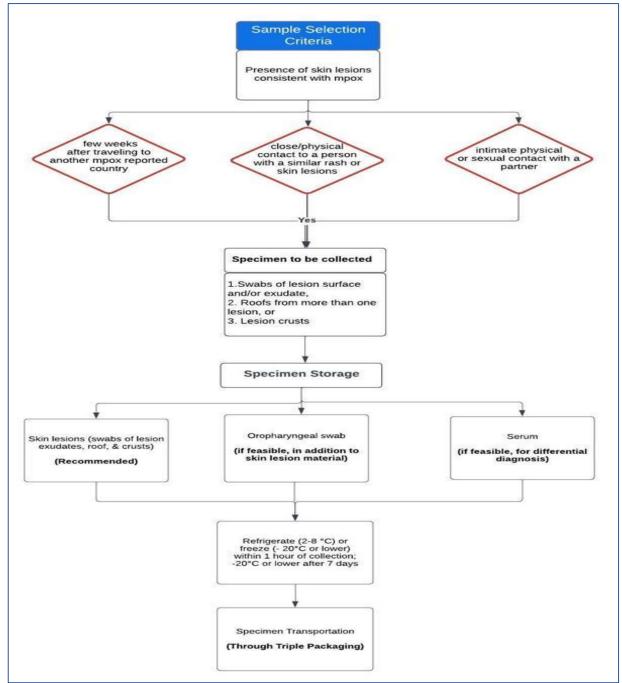
*Long term specimen storage (>60 days from collection) is recommended at -70°C. Repeated freeze-thaw cycles should be avoided because they can reduce the quality of specimens.

Sample Transportation

Specimens should be stored, refrigerated or frozen within an hour of collection and transported to the laboratory as soon as possible after collection. All specimens being transported should have;

- Appropriate triple packaging and proper labeling
- Proper labelling
- Patient history form (Annex-I)
- Coolers and cold packs or dry ice





Laboratory Testing

Testing for the presence of MPXV should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. Measures should be taken to minimize the risk of laboratory transmission based on risk assessment when testing routine clinical specimens from confirmed or suspected mpox patients. Laboratories with no molecular diagnostic protocol implemented for MPXV detection should send suspected clinical samples (strictly fitting case definition) to the National Institutes of Health, Islamabad.

Molecular Methods

Confirmation of MPXV infection is based on nucleic acid amplification testing (NAAT), using realtime or conventional polymerase chain reaction (PCR), for detection of unique sequences of viral DNA. PCR can be used alone, or in combination with sequencing. Few groups have developed validated PCR protocols for the detection of MPXV, some of which include distinction of Clade I and II. Real-time PCR kits that can detect MPXV are also commercially available, and they can be selected through the online FIND database for diagnostic kits [7]. To ensure the quality and reliability of the selected kit, it is recommended to procure CE-IVD kits which preferably targets two genes/targets of MPXV.

DNA Extraction

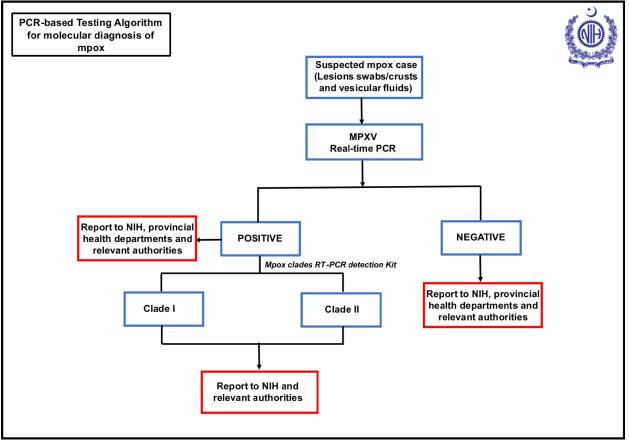
DNA can be extracted from samples mentioned above using any standard extraction protocols or kits. In general, the sample lysis step in DNA extraction inactivates any live virus. Thus, it is recommended that the sample lysis step is performed under a Class II biosafety cabinet. For crust samples, DNA extraction kit for tissue samples should be used to insure appropriate sample lysis.

Molecular Detection

Based on the protocol suggested for the detection of MPXV by Li et al., [8], a working protocol has been designed and mentioned as Annex-II. Further, the commercial Real-time PCR kits (CE-IVD) for mpox virus detection can also be utilized. These protocols are based on the initial detection of MPXV through a real-time PCR that detects all MPXV strains. If positive, the sample may be subjected to detection of specific clades I and II using real-time PCR.

It is important that each NAAT run must include external, internal, positive and negative controls to validate the results. Controls provide the information about (1) sample quality, (2) nucleic acid quality, and (3) process quality. In order to avoid contamination, negative controls on every run should be utilized to ensure contamination has not occurred. Sample integrity controls (e.g., RNase P), extraction, positive and inhibition controls should also be added to distinguish a false negative from a true negative. Controls should be utilized following laboratory SOPs. If any of the assay controls fail, testing should be repeated.

Figure-08 Testing Algorithm



All positive cases on PCR will not be processed for genomic sequencing, only representative sample during the surge will be processed for sequencing.

Interpretation of Results

Confirmation of MPXV infection should consider clinical and epidemiological information. Positive detection by confirmation of MPXV via PCR in suspected cases confirm MPXV infection. When the clinical presentation and epidemiology suggest an infection with MPXV despite negative PCR results, serological testing may be useful to further investigate prior infection for epidemiological purposes. A number of factors could contribute to false-negative results, such as poor quality of specimen, wrong handling or shipping, or technical reasons inherent to the test, DNA extraction failure.

Moreover, the genetic sequencing data, generated either by sanger or next-generation sequencing (NGS) methods also provide additional information regarding virus characteristics, its origin/clade/lineage and epidemiology. It also provides additional information regarding introductions or community transmission.

Differential Diagnosis

It is important to consider other potential causes of discrete skin lesions or a disseminated rash and other etiologies for similar-appearing skin lesions at the different stages of development including herpes simplex virus, varicella zoster virus, enterovirus, measles, chikungunya, dengue, Treponema pallidum (syphilis), bacterial skin infections, parapoxviruses and among others.

S.No	Laboratories/Institute	City/Province	Focal Person	Contact #
1	National Institute of Health (NIH)	Islamabad	Dr. Massab Umair	051-9255082; 0345-5176169
2	Punjab AIDS Control Program (PACP)	Lahore, Punjab	Dr. Hasnain Javed	0333-4591434
3	Institute of Public Health (IPH)	Lahore, Punjab		
4	Armed Forces Institute of Pathology (AFIP)	Rawalpindi, Punjab	Maj. Gen. Eijaz Ghani	0300-9543557
5	Khyber Medical University (KMU)	Peshawar, Khyber Pakhtunkhuwa	Dr. Yasar Yousafzai	0321-9054010
6	Fatima Jinnah General & Chest Hospital	Quetta, Balochistan	Mr. Adnan Mughal	0322-8120020
7	Dow University of Health Sciences (DUHS)	Karachi, Sindh	Dr. Saeed Khan	0333-2276556
8	Aga Khan University Hospital (AKUH)	Karachi, Sindh	Dr. Zahra Hasan	0300-8247815

Sample referral to Public Health Laboratories

Data Reporting

Because of the public health risks associated with a single case of mpox, clinicians should report suspected cases immediately to provincial and federal health authorities, regardless of whether they are also exploring other potential diagnosis. Cases should be reported immediately, according to the case definitions. All the confirmed cases should be notified immediately to National Institutes of Health and Provincial Health Departments and relevant authorities. Laboratories that perform sequencing (sanger or next-generation sequencing) are encouraged to submit MPXV genome to GISAID or NCBI.



Monkeypox Testing Request Form

Department of Virology National Institute of Health Islamabad, Pakistan Phone +92 (051) 9255082 (8am - 4pm) Website: www.nih.org.pk

LAB DETAILS	
Lab name and address	Phone/Cell #
PATIENT DETAILS	
Full Name	Gender Male Female
	Date of Birth D D M M Y Y Y Y Age
SAMPLE DETAILS	
Sample type Lesion swabs/dry crusts in VTM [Mandatory] EDTA whole blood 	LAB ID
□ Plasma □ Serum	Date of collection D D M M Y Y Time

□ Other (please specify)

CLINICAL/EPIDEMIOLOGICAL DETAILS

Foreign Travel within previous 21days?

YES/NO						
Date of travel	D	D	М	M	Y	Y
Date returned	D	D	М	м	Y	Y
Onset date	D	D	М	М	Y	Y
Countries visited						

Symptoms

- □ Fever/Chills □ Headache
- Muscle/Body Ache
 Rash
- Ulcer
- □ Swollen Lymph nodes □ Cough

Travel Vaccination History

Other clinical details/ Exposure history

s this c	ase gay,	bisex	cual or a i	man	who has sex with men?
	Yes		No		Prefer not to say.
Recent	sexual pa	artnei	rs with		
	Men				Prefer not to say.
	Women				No recent casual partner
ny uni	usual activ	vities	?		
					npox cases?

Suspected Diagnosis?

REFERRED BY

Signature

Y

Annex-II

Mpox virus (MPVX): Real-time PCR protocol

Assays for the generic detection of MPXV (species: *Mpox virus*, genus: *Orthopoxvirus*) and the detection of its two clades: 1&2

- Assay with G2R_G primers and probe: detects all MPXV strains
- Assay with G2R_WA primers and probe: detects Western African clade viruses
- Assay with C3L primers and probe: detects Congo Basin clade viruses
- Primers and probes sequences at the end of the document.
- All probes are hydrolysis ("TaqMan") probes labelled with the FAM dye and the BHQ-1 quencher.

Master Mix

	Volume per reaction	Volume per reaction
Reagent	EXPRESS qPCR Supermix	TaqMan [®] Universal PCR
	Universal ²	Master Mix ³
Reaction buffer (2x)	10 µl	10 µl
Forward primer (10 μ M)	0.8 µl	0.8 μΙ
Reverse primer (10 µM)	0.8 μΙ	0.8 μl
Probe (10 μM)	0.4 μl	0.4 µl
Nuclease Free Water	3.0 μl	3.0 μl
Total Volume	15 µl	1

Template

Pipette **5 μl** of sample DNA in 15 μl master mix (total reaction volume: 20 μl). Finally add **5 μl** negative and positive control to assess the validity of the run.

EXPRESS qPCR Supermix	Supermix Universal TaqMan® Universal PCR N		Master Mix	
	Condition		Condition	
UNG Incubation	50°C – 2min	UNG Incubation	50°C – 2min	
Polymerase Activation	95°C - 06min	Polymerase Activation	95°C - 10min	
PCR Amplification (45 Cycle)	95°C - 15sec	PCR Amplification (45 Cycle)	95°C - 15sec	
Fluorescence Acquisition (FAM)	60°C – 30sec	Fluorescence Acquisition (FAM)	60°C – 30sec	

PCR Amplification Condition for G2R_G assay

PCR Amplification Condition for G2R-WA Assay

EXPRESS qPCR Supermix I	Jniversal	TaqMan [®] Universal PCR Master Mix			
	Condition		Condition		
UNG Incubation	50°C – 2min	UNG Incubation	50°C – 2min		
Polymerase Activation	95°C - 06min	Polymerase Activation	95°C - 10min		
PCR Amplification (45 Cycle)	95°C - 15sec	PCR Amplification (45 Cycle)	95°C - 15sec		
Fluorescence Acquisition (FAM)	62°C – 30sec	Fluorescence Acquisition (FAM)	62°C – 30sec		

PCR Amplification Condition for C3L Assay

EXPRESS qPCR Supermix Universal		TaqMan [®] Universal PCR Master Mix		
	Condition		Condition	
UNG Incubation	50°C – 2min	UNG Incubation	50°C – 2min	
Polymerase Activation	95°C - 06min	Polymerase Activation	95°C - 10min	

PCR Amplification (45 Cycle)	95°C - 15sec	PCR Amplification (45 Cycle)	95°C - 15sec
Fluorescence Acquisition (FAM)	60°C – 30sec	Fluorescence Acquisition (FAM)	60°C – 30sec

Primers and Probes

Assay for Generic Detection of Mpox Virus (G2R_G assay) ¹				
Primers/Probe	Sequence (5' > 3')	Length		
G2R_G Forward Primer	GGAAAATGTAAAGACAACGAATACAG	26		
G2R_G Reverse Primer	GCTATCACATAATCTGGAAGCGTA	24		
G2R_G Probe	FAM-AAGCCGTAATCTATGTTGTCTATCGTGTCC-BHQ1	30		
Assa	ay for Detection of Western African Clade Virus (G2R-WA Assay)	1		
G2R_WA Forward Primer	CACACCGTCTCTTCCACAGA	20		
G2R_WA Reverse Primer	GATACAGGTTAATTTCCACATCG	23		
G2R_WA Probe FAM-AACCCGTCGTAACCAGCAATACATTT-BHQ1		26		
	Assay for Detection of Congo Basin Clade Viruses (C3L Assay) ¹			
C3L Forward Primer	TGTCTACCTGGATACAGAAAGCAA	26		
C3L Reverse Primer	GGCATCTCCGTTTAATACATTGAT	24		
C3L Probe	FAM-CCCATATATGCTAAATGTACCGGTACCGGA-BHQ1	30		

- 1. Li et al., Journal of Virological Methods 169, 223–7 (2010).
- 2. Invitrogen, cat. no.: 11785-200, 11785-01K, 11795-200 or 11795-01K.
- 3. Applied Biosystems, cat. no.: 4304437, 4364338, 4364340, 4305719, 4318157 or 4326708.

Disclaimer: The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the National Institute of Health in preference to others of a similar nature that are not mentioned.

Chapter 6

Safe Handling and Waste Management of Suspected Mpox Virus Specimens

Manipulation of specimens with the potential of containing MPXV should be carefully assessed by laboratories. A biohazard risk assessment should be performed by each laboratory, incorporating points listed below. It is recommended that clinical laboratories other than microbiology laboratories are advised to perform MPXV risk assessments.

Principles of Biosafety: Primary and Secondary Protection Barriers

- Biosafety aims to protect laboratory personnel from biologic agents through primary and secondary barriers, facility practices, and PPE.
- Primary barriers, such as BSCs, enclosed containers, centrifuge safety cups, and sealed containers, provide direct protection to individuals from hazards.
- Facility design can serve as a secondary barrier to hazards through effective ventilation, anterooms, and airlocks.
- PPE is considered a secondary barrier and varies depending on the laboratory task and specimens involved. Common PPE includes protective laboratory coats or gowns, eye and face protection (e.g., safety glasses, visors, goggles, face shield), and gloves.
- Biosafety guidelines prioritize the use of primary equipment or facility barriers over PPE for shielding workers from hazards [1].

MPXV Biosafety during Specimen Processing and NAAT

- The CDC USA recommends using a Class II biological safety cabinet (BSC) or other containment device as the primary barrier when handling specimens suspected of containing MPXV. Lesion specimens should be processed in BSL-2 facilities.
- If a BSC is not available, a containment device such as a glove box should be used with personal protective equipment (PPE).
- The CDC USA recommends viral inactivation when testing lesions from suspected MPXV patients; guidelines are available in the "Inactivation of Specimens Prior to Analysis" section.
- To minimize risk, BSCs should be used for primary specimen manipulation, and laboratory aerosolization procedures should be avoided whenever feasible, regardless of staff vaccination status [2].

Disinfection

- Proper disinfection of laboratory areas and equipment used to handle specimens from suspected or confirmed MPXV cases is crucial to minimize the risk of infection.
- MPXV is classified by the Environmental Protection Agency (EPA) as a tier 1 enveloped virus, and approved disinfectants that target the virus's lipid envelope can effectively neutralize it.
- A list of disinfectants approved for use in hospitals can be found on the EPA website [3].

Inactivation of Specimens Prior to Analysis

Clinical laboratories that conduct molecular testing on specimens from patients suspected of having MPXV infection may consider performing an inactivation step before performing clinical testing. The UK Health Security Agency released a report on August 22, 2022 [4], which summarized assessments of several inactivation reagents, these include

- Buffer AVL by QIAGEN
- 70% ethanol
- InhibiSURE Viral Inactivation Medium by Thermo Scientific
- L6 Buffer by Severn Biotech
- Nuclisens Lysis Buffer by bioMérieux
- MagBead Viral RNA Lysis Buffer by Neuromics
- Panther Fusion Specimen Lysis Tubes by HOLOGIC
- Molecular Sample Solution (MMS) by E&O Laboratories.
- i. Heat treatment for **15-30 minutes at 65°C and 15 minutes at 95°C** will inactivate MPXV in specimens.
- ii. If a laboratory determines that an inactivation step is necessary for specimens that may contain MPXV, the entire testing process should be evaluated for any downstream effect the inactivation process may have on results. The inactivation process should be a component of the initial test validation, when possible.
- iii. For a recommended protocol, please refer to the guidelines regarding MPXV inactivation for nucleic acid testing.

Waste Management

- i. The following guidelines should be followed to ensure safe disposal of waste generated during processing of mpox specimens:
- ii. Waste generated during the processing of mpox specimens should be segregated from other laboratory waste and placed in a biohazard bag for safe disposal.
- Surfaces and equipment that come into contact with the sample must be decontaminated before and after work using effective disinfectants such as quaternary ammonium compounds and 0.5% (or 200ppm) bleach [6].
- iv. All biohazardous waste must be autoclaved under appropriate conditions before disposal to ensure its safe disposal.
- v. After autoclaving, the biohazard bags should be disposed of following local regulations, which may involve incineration, chemical treatment, or other approved methods.
- vi. All waste disposal activities should be documented, including the type and quantity of waste generated, the method of decontamination, the date and time of disposal, and the method of disposal, to maintain proper records and compliance.

Chapter 7

Risk Communication and Community Engagement

Background

A contagious viral disease, mpox is primarily found in Central and West African countries. The virus is similar to smallpox, but is less severe in nature. As a zoonotic disease, it can be transmitted from animals to human. The mode of transmission is through physical contact with someone who is infectious with contaminated material or with infected animal. As of 26th April 2023, two cases have been reported in Pakistan and both have travel history to other country. No evidence of local transmission has not been found yet. The preventive measures, such as vaccination, avoiding contact with infected people, practicing good personal hygiene, isolating infected individuals, and promoting public awareness can help to control the spread of the disease.

Modes of Transmission

The transmission of mpox virus occurs through various routes:

- Animal to human: Virus can be transmitted through direct contact with the infected animal. The virus can also be transmitted through bites, rashes, scabs, crusts or fluids from sores, saliva, or infected bodily fluids, including respiratory secretions.
- **Human-to-Human:** Virus can also be transmitted through human-to-human contact. This can occur through close contact with someone who has rashes. The close contact can be defined as face-to-face such as talking, breathing or singing close to one another which can generate droplets or short-range aerosols, skin-to-skin transmission such as touching or vaginal/anal sex, mouth-to-mouth such as kissing or mouth-to-skin contact such as oral sex or kissing the skin.
- Environmental transmission: Environment can become contaminated with the mpox virus, when an infectious person touches clothing, bedding, towels, objects, electronics and surfaces. Someone else who touches these items may become infected if they have any cuts or abrasions or they accidentally touch their eyes, nose mouth or other mucous membranes. This is known as fomite transmission. Airborne transmission has not been established and studies are underway to learn more.
- Ingestion of Contaminated Food: The virus can also be transmitted through the consumption of contaminated bush meat (meat of wild animals such as rodents or primates) which is not cooked properly.

Preventive Measures to be considered for RCCE activities

Following measures can be taken to control the spread of disease:

Avoid Contact with Infected Animals

- i. Avoid contact with infected animals, particularly rodents and primates. Avoid handling or consuming raw/not properly cooked bush meat.
- ii. Individuals who handle infected animals/animal samples/animal by-products either in field or in laboratories should follow appropriate biosafety measures to avoid/minimize the exposure.
- iii. Strict compliance to standard operating procedures for use of appropriate personal protective equipment, sanitization, environmental cleaning and proper waste management and disposal are essential to minimize the chance of acquiring infection.

Personal Hygiene

- i. Practicing good personal hygiene, such as regular hand washing/sanitization is essential in preventing the spread of the virus.
- ii. It is also important to avoid sharing personal items of infected/suspected individual such as towels, clothing, razors etc.

Avoid contact with infected person

- i. Infected individuals presented with signs / symptoms like rash, skin lesions etc. should be isolated for 21 days.
- ii. Contacts who do not have such symptoms are advised to observe themselves for 21 days after exposure. In case, someone develops said signs and symptoms, should immediately report to the healthcare provider.
- iii. Healthcare staff involved in surveillance and case management should use proper personal protective equipment (PPE) which includes masks, gloves, gowns and head caps.

Vaccination

Vaccination is the most effective way to prevent mpox infection. The smallpox vaccine provides immunity against Mpox as well. People who have not received the smallpox vaccine are more susceptible to the virus. If vaccination is to be administered, it must be given during first four days of exposure before the onset of symptoms.

Public Awareness

Being a re-emerging disease and its prevention, treatment and control measures are well known, so it is important to spread its awareness among communities in routine health education activities. Educating the public about the risks of mpox transmission and preventive measures can help to prevent the spread of the virus. This includes promoting safe food handling practices, compliance of personal hygiene practices, and encouraging individuals to seek medical attention if they experience symptoms.

Communication strategies

- i. Presently the risk of disease spread in Pakistan is very low, so the communication activities should be focused on major international airports and the cities with high number of inbound travelers.
- ii. Targeted activities of community awareness will be launched to avoid panic and spread of rumors which includes public service messages, IEC material, and community engagement.
- iii. Guidelines and public health social measure, shared by NIH will be used across the country for mass awareness, and community engagement.
- iv. To manage the infodemic, all types of media will be engaged to handle the situation carefully and only verified and reliable information will be shared or disseminated by a designated media focal person.

Communication Process

- i. Coordinator NCOC-NIH will be a focal person / spokesperson, will support the national, provincial and regional public information focal persons with updated information and guidelines.
- ii. All national, provincial and regional health departments will notify the focal person / media spokesperson to share the information regarding the disease. All focal person may coordinate with NCOC for media related issues.
- iii. No one except the focal persons should be allowed to share any of the information regarding the issue.

Focus of awareness

- i. Isolation of infected persons with signs and symptoms in a separate room or dedicated space of healthcare facility.
- ii. Patients will be evaluated clinically by the physician, and home isolation may be advised by the physician accordingly.
- iii. If any house has an active patient of mpox,
 - they should use a separate bathroom, or cleaning after each use
 - they should clean and disinfect frequently touched surfaces with soap, water and alcohol based or sodium hypochlorite solution etc. as mentioned in IPC guidelines
 - they should use separate utensils, objects, electronics, or clean well with soap and water/disinfectant before sharing
- iv. Not to share towels, bedding, razors, combs or clothes of infected and active case of Mpox
- v. If any house has an active patient of Mpox, they should do their own laundry (lift bedding, clothes and towels carefully without shaking them, put materials in a plastic bag before carrying it to the washing machine and wash them with hot water > 60°C degree. It is recommended to use disposable utensils and crockery and properly dispose after use.
- vi. Encouraging everyone in the house to clean their hands regularly with soap and water or an alcohol-based hand sanitizer.

Chapter 8

Infection Prevention and Control (IPC)

Background

A rare viral disease, caused by infection with mpox virus. Spread may occur when a person comes into close contact with an animal, human, or materials contaminated with the virus. The virus enters the body through broken skin (even if not visible), the respiratory tract, or the mucous membranes (eyes, nose, or mouth). Person-to-person spread is uncommon, but may occur through:

- contact with clothing or linens (such as bedding or towels) used by an infected person
- direct contact with mpox skin lesions or scabs
- large droplet respiratory spread from prolonged close contact with an individual with a mpox rash

There is no specific treatment for the disease and, therefore, infection prevention and control measures are essential in managing the spread of the disease.

GUIDELINES FOR IPC

Vaccination

Three vaccines against mpox are currently available globally, but the supplies are very limited. It is recommended that after vaccination, continue to take care to avoid catching and spreading Mpox because it takes several weeks to develop immunity after being vaccinated. Secondly, it is not known to what extent the vaccines protect and stop infecting others as efficacy data in the current multi-country outbreak setting is limited.

Some countries are recommending vaccination for persons at risk. However vaccines for an eradicated disease called smallpox, may also be useful for Mpox. Two of these vaccines are MVA-BN and LC16. Another vaccine available is "JYNNEOS" is a 2-dose vaccine developed to protect against mpox and smallpox infections.

It is recommended that mass vaccination campaigns should not be rolled out, rather we recommend that you get vaccine if:

- i. You had known contact with positive case of Mpox
- ii. You had a sex partner in the past 2 weeks who was diagnosed with mpox
- iii. You are a gay, bisexual, or other man who has sex with men or a transgender, nonbinary, or gender-diverse person who in the past 6 months has had any of the following:
 - A new diagnosis of one or more sexually transmitted diseases (e.g., chlamydia, gonorrhea, or syphilis)
 - More than one sex partner
- iv. You have had any of the following in the past 6 months:
 - Sex at a commercial sex venue (like a sex club or bathhouse)
 - Sex related to a large commercial event or in a geographic area (city or county for example) where mpox virus transmission is occurring
- i. You have a sex partner with any of the above risks
- ii. You anticipate experiencing any of the above scenarios
- iii. You have HIV or other causes of immune suppression and have had recent or anticipate future risk of mpox exposure from any of the above scenarios
- iv. You work in settings where you may be exposed to mpox:

- You work with orthopoxviruses in a laboratory
- You are part of an orthopoxvirus and health care worker response team

You should NOT get the vaccine if:

- You had a severe allergic reaction (such as anaphylaxis) after getting your first dose of the JYNNEOS vaccine.
- It is also recommended that vaccine should be administered at least 3-4 days prior to the onset of symptoms in case of any exposure.
- There is no specific treatment for the disease and, therefore, infection prevention and control measures are essential in managing the spread of the disease

Case management of Mpox

Case management of a confirmed mpox patient involves several steps to ensure proper treatment and prevent the spread of the virus. Here are some guidelines for case management:

- 1. **Isolation:** The patient should be isolated in a single room with a private bathroom and provided with appropriate personal protective equipment (PPE) to prevent transmission of the virus to healthcare workers and other patients.
- 2. **Symptomatic treatment**: Treatment for mpox is primarily supportive and symptomatic. Patients should be given antipyretics for fever, analgesics for pain relief, and fluids to maintain hydration.
- 3. Antiviral treatment: There is no specific antiviral treatment for mpox, but some antiviral medications, such as cidofovir as advised by the physician, have shown efficacy in treating severe cases.
- 4. **Infection prevention and control**: Strict infection prevention and control measures should be followed, including hand hygiene, environmental cleaning, and disinfection. Healthcare workers should wear appropriate PPE at all times when caring for the patient.
- 5. **Contact tracing**: Close contacts of the patient should be identified, monitored for symptoms, and isolated if necessary.
- 6. **Public health reporting**: Confirmed cases of mpox must be reported to local district and provincial health departments, who will provide guidance on additional measures to prevent the spread of the virus.

It is important to note that mpox is a rare disease, and most people who contract the virus have a mild illness that resolves on its own without treatment. However, in severe cases, the virus can cause significant morbidity and mortality, and prompt and appropriate case management is essential to prevent transmission and improve outcomes. In order to prevent the spread of mpox to others, persons with mpox should **isolate at home, or in hospital if needed, for the duration of the infectious period** (from onset of symptoms until lesions have healed and scabs fall off). Covering lesions and wearing a medical mask when in the presence of others may help prevent spread.

- A- Identification of Cases: The first step in infection prevention and control of Mpox is to identify the cases. Case definitions should be widely circulated.
- B- Isolation Precautions: People with mpox shall remain isolated for the duration of illness to help prevent transmission. (Note; The following considerations may change with the evolving situation of the outbreak

B1- Isolation of people with mpox in non-hospital setting

People with mpox should follow these recommendations until infection has resolved:

- Friends, family or others should not visit
- Avoid close contact with others
- Avoid close contact with pets in the home and other animals
- Do not engage in sexual activity
- Do not share potentially contaminated items, such as bed linens, clothing, towels, wash cloths, drinking glasses or eating utensils
- Routinely clean and disinfect commonly touched surfaces and items, such as counters or light switches
- Wear well-fitting medical mask when in close contact with others at home
- Avoid use of contact lenses to prevent inadvertent infection of the eye
- Avoid shaving rash-covered areas of the body as this can lead to spread of the virus

Bathroom usage

- i. If possible, use a separate bathroom if there are others who live in the same household.
- ii. If there is not a separate bathroom in the home, the patient should clean and disinfect surfaces such as counters, toilet seats, faucets
- Limit exposure to others
 - iii. Avoid contact with unaffected individuals until the rash has resolved, the scabs have fallen off, and a fresh layer of intact skin has formed
 - iv. Isolate in a room or area separate from other household members and pets when possible
 - v. Limit use of spaces, items, and food that are shared with other household members.
 - vi. Do not share dishes and other eating utensils

Limit contamination within household

- vii. Try to avoid contaminating upholstered furniture and other porous materials that cannot be laundered by placing coversheets, waterproof mattress covers, blankets, or tarps over these surfaces
- viii. Additional precautions such as steam cleaning can be considered if there is concern about contamination

Considerations for isolating with animals in the home

- ix. People with mpox should avoid contact with animals (specifically mammals), including pets
- x. If you notice an animal that had contact with an infected person appears sick (such as lethargy, lack of appetite, coughing, bloating, nasal or eye secretions or crust, fever, rash) inform the health officials and contact veterinarian

B2- Precautions for preventing mpox virus transmission in Hospital setting, PPEs

In addition to Standard Precautions, if a patient seeking care is suspected to have mpox infection, control team should be notified immediately and additional infection control precautions (as described below) should be implemented. Activities that could resuspend dried material from lesions (e.g., use of portable fans, dry dusting, sweeping, vacuuming) should be avoided.

Patient Placement & Transportation

A patient with suspected or confirmed mpox infection should preferably be placed in a singleperson isolation room. The door should be kept closed. The patient should have a dedicated bathroom. Transport and movement of the patient outside of the room should be limited. If the patient is transported outside of their room, they should use well-fitting medical mask and have any exposed skin lesions covered with a sheet or gown. Intubation, extubation and any procedures likely to spread oral secretions should be performed in an airborne infection isolation room.

Personal Protective Equipment (PPE)

PPE used by healthcare personnel who enter the patient's room should include:

- Gloves
- Gown
- Eye protection (i.e., goggles or a face shield that covers the front and sides of the face)
- Respirator equipped with N95 filters or higher

Duration of Isolation Precautions for Patients with Suspected or Confirmed Mpox Infection

For patients with suspected or confirmed mpox infection in a healthcare setting:

- Those with **suspected mpox** infection should have recommended isolation precautions, maintained until infection is ruled out
- Those with **confirmed mpox** infection should have recommended isolation precautions, maintained until all lesions have crusted, those crusts have separated, and a fresh layer of healthy skin has formed underneath.

Decisions regarding discontinuation of isolation precautions in a healthcare facility may need to be made in consultation with the local health department.

C- Hand Hygiene

- i. Use of an alcohol-based hand rub or hand washing with soap and water should be performed by people with mpox and household contacts after touching rash material, clothing, linens, or environmental surfaces that may have had contact with rash material.
- ii. Cover all skin rashes to the extent possible by wearing long sleeves or full length clothes. Gloves can be considered for covering rashes on the hands when not in isolation such as when receiving medical care.
- iii. People with mpox should use well-fitting medical mask, if close contact with others cannot be avoided, such as when receiving medical care.
- iv. Other household members should wear a respirator or a well-fitting mask when in close contact (e.g., within 6 feet) with the person with mpox for more than a brief encounter.
- v. When possible, the person with mpox should change their own bandages and handle contaminated linens while wearing disposable gloves, followed by immediate handwashing after removing gloves.
 - As a last resort, if assistance is needed with these activities, a household member should avoid extensive contact and wear, at a minimum, disposable medical gloves and a well-fitting mask or respirator. Any clothing that contacts the rash during dressing changes should be immediately laundered. Gloves should be disposed of after use, followed by handwashing.
- vi. Contain and dispose of contaminated waste, such as dressings, bandages, or disposable glove

D- Environmental Infection Control (Healthcare settings)

Decontamination of a room, laundry and waste

Individuals cleaning or decontaminating rooms that a patient with Mpox has spent significant time in should:

- i. Wear appropriate **PPE** to avoid direct contact with contaminated material during the process including gloves, gown, eye protection i.e. goggles and Respirator equipped with N95 filters or higher
- ii. Contaminated **clothing and linens should be collected** and bagged before the room is cleaned. These clothing or linen items should not be shaken or handled in a manner that may disperse infectious particles. Items of potentially infected clothing or linen should be placed in a water soluble (alginate) bag, sealed or tied and placed inside an impermeable bag for transport to the laundry facility.
- iii. After contaminated clothing and linens have been removed, the rooms can be cleaned and disinfected as per standard terminal cleaning of an isolation room. The mpox virus will be destroyed through the use of hospital detergents followed by disinfection with 1000ppm available chlorine (sodium hypochlorite). As an alternative, 5000ppm available chlorine may be used on its own. Pay particular attention to frequently touched surfaces such as tables, door handles, toilet flush handles and taps.
- iv. The protective cover over mattresses can be cleaned by wiping with detergent solution, removing excess fluid and then wiping with 1000ppm available chlorine and allowed to air dry. As an alternative, 5000ppm available chlorine may be used on its own. A damaged protective cover or soiled cover should be replaced and the old cover should be double wrapped and sealed, and sent for incineration.
- v. Carpets and soft furnishings should be steam cleaned. PPE worn when removing clothing and linens should be disposed of in a manner consistent with the Waste guidance below.

Cleaning of common areas

- i. For those rooms where the case may have spent limited time, appropriate PPE for cleaning includes a surgical face mask, visor or goggles, disposable gloves and apron to protect the individual from potential splashes from the detergent and sodium hypochlorite used.
- ii. Cleaning should include the use of hospital detergents followed by disinfection with 1000ppm available chlorine. As an alternative, 5000ppm available chlorine may be used on its own. Carpets and soft furnishings should be steam cleaned, where possible.
- iii. All PPE and disposable materials should be disposed of in a manner consistent with the Waste guidance below.

Laundry

After removing clothing and linens from the rooms as described above, they must be washed in a standard washing machine with hot water (over 60° C) and detergent; bleach may also be added but is not necessary.

Waste (Clinics)

Waste from someone suspected or confirmed as having mpox can be treated as healthcare (clinical) category B waste. It can be disposed of in an orange bag for alternative treatment and does not have to be sent for incineration.

Diagnostic samples and Culture samples

Diagnostic samples from all suspected or confirmed mpox cases should be transported as Category B samples whereas Laboratory cultures are assigned to Category A.

E- Domestic settings

If cleaning is required in a domestic setting, such as a home or a car, individuals should be made aware that they should not attempt to clean or decontaminate the area themselves. The local health official should be contacted and they will arrange for specialist decontamination of the affected areas and disposal of any waste.

Cleaning of domestic settings should be carried out in the following order:

- i. Contaminated clothing and linens should be collected first before the room is cleaned. These clothing or linen items should not be shaken or handled in a manner that may disperse infectious particles.
- ii. Items that have been in direct contact with the skin of an infected person, for example duvets, pillows, blankets, sheets, towels or underwear can be sealed, bagged and destroyed as Category A waste.
- iii. All other clothing and linen items can be washed in a standard washing machine with hot water (over 60° C) and detergent, using an extended washing cycle. Washed items should not be placed into areas where they may be re-contaminated during the cleaning process.
- iv. Domestic settings, including car interiors, should be cleaned using a HEPA filtered vacuum cleaner, and the vacuum cleaner contents should be disposed of as Category A waste.
- v. Hard surfaces should be cleaned using detergents similar to those used in a hospital setting, followed by disinfection with 1000ppm available chlorine and allowed to air dry. As an alternative, 5000ppm available chlorine may be used on its own.
- vi. Soft furnishings, such as carpets, sofas, curtains, mattresses and car interiors, should then be professionally steam cleaned by individuals wearing full PPE as described above. Duvets and pillows that have not been in direct contact with the skin of an infected person and cannot be washed in a home washing machine may be steam cleaned.

F- Waste

- i. Waste generated by someone with mpox who is self-isolating at home can be placed in the usual domestic waste stream.
- ii. Waste created by a healthcare worker in a domestic setting should be disposed of as a category B clinical waste.

G- Visitation

Visitors to patients with mpox infection should be limited to those essential for the patient's care and wellbeing (e.g., parents of a child, spouse). Decisions about who might visit, including whether the visitor stays or sleeps in the room with the patient, typically take into consideration the patient's age, the patient's ability to advocate for themselves, ability of the visitor to adhere to infection prevention and control recommendations, whether the visitor already had higher risk exposure to the patient, and other aspects. In general, visitors with contagious diseases should not be visiting patients in healthcare settings to minimize the risk of transmission to others.

Chapter 9 Guideline for Waste Management

Background:

The purpose of this document is to summarize existing guidance for the disposal of contaminated waste, such as gauze, wound dressings, culture material, patient samples, glassware and other contaminated items, from Mpox (MPX) suspected or confirmed cases that visit NIH or samples sent to NIH for testing/confirmation from different healthcare facilities of the country.

This procedure applies to all divisions, departments & laboratories of the institute which are dealing with monkey pox viral testing or patient testing etc. This document can be used by public health organizations and local/regional health units to support the development of Mpox waste disposal guidance.

- i. The Chiefs and officer In-charge of all divisions and departments are responsible for the compliance of whole process.
- ii. All section in-charges are responsible for source segregation of the waste, then proper disposal from the department, maintenance of logbook / data form for the same along with the implementation and execution of departmental SOP.
- iii. Arrangement of incineration / shredder and maintenance of logbook for incinerated waste is the responsibility of designated Incinerator operator.

Material and Equipment

Waste required to be disposed off

- Autoclave
- Incinerator
- Shredder
- Color coded containers for waste collection
- Personal protective equipment like rubber gloves, rubber long shoes, face mask, goggles or face shield etc.
- First Aid Box
- Color coded waste Disposal bags
- Sharp disposal box
- Waste transportation trolleys

Careful segregation of waste material into different categories helps to minimize quantities of hazardous waste.

Color Code for segregation of waste material

- Yellow Puncture proof container for Glassware/sharps
- Red Infectious/biological/ Hazardous material
- Black Non-infectious waste (paper, primary packaging material, etc.)

MPX Waste categorization

United Nations Regulation 2814 states that the waste associated with the Mpox virus is considered a Category **A** waste, as it is identified as such by. However, waste from patients infected with the West African Clade of Mpox may be determined exempt from Category A Infectious Substance Regulations, however, local public health authorities must be contacted for further guidance

Non-Hazardous Infectious Waste (Packing Materials / Papers waste etc)

- i. Collect the material in specified **Black** colored container.
- ii. Transport and store the material in the designated area for scrap in room near incinerator.
- iii. Material is incinerated / burned, and record is maintained in the relevant form / logbook accordingly.

Hazardous Infectious Waste

All the infectious waste is collected in **RED colored** leak proof container with **red bin liner**. All expired reference / standard material, chemical / reagents / solvents, microbial cultures, cultures and stocks of MPV agents from laboratory work, waste from MPV patients (e.g. swabs, bandages and disposable medical devices), pathological waste, urine and excreta from patients, sharps (glass implements, needles, syringes, scalpels, blades etc), used filters, used membrane filters, used PPE (disposable gloves, masks, caps, shoes covers, overalls), used cotton plugs etc.

Disposal of Hazardous Infectious Waste (Sharps)

- i. Collect the material in a **yellow**-colored rigid, impact resistant, puncture proof and sealable container of appropriate size
- ii. The design of container must protect handlers from being injured during collection and transport.
- iii. Sharps containers must be single used which is disposed of with the waste inside in incinerator.
- iv. Reusable sharps are autoclave as infectious waste in appropriate puncture resistant container.
- v. Maintain records in the relevant logbook accordingly.

Disposal of Hazardous Infectious/ Biological Waste

- i. All cultures, stocks, residual specimens, and mpox virus waste should be decontaminated before on-site disposal using an approved method, such as autoclaving.
- ii. Materials decontaminated outside the immediate laboratory should be placed in a durable, leakproof RED colored container and closed for transport from the laboratory.

Handling of waste of home isolated patients

- i. Individuals with Mpox isolating at home should use a dedicated, lined garbage can in the room where they are isolating.
- ii. All waste that has been in direct contact with skin should be disposed of in a sealed double plastic bag, then placed into the dedicated garbage.
- iii. The waste that is generated from caring for a patient with MPX, such as bandages and PPE, should be placed in strong bags and securely tied before disposal and eventual collection by municipal waste services. All disposable garbage bags should be placed into a second disposable bag, tied securely, before being disposed of as usual with your domestic waste. If such services are not available, as an interim measure and according to local policies, safely burying or controlled burning may be done until more sustainable and environmentally friendly measures can be put in place.

- iv. An individual should not put any waste into recycling bins until an individual has ended their selfisolation. Waste should all be double bagged and disposed of as described above
- v. Vacuum cleaner waste, including disposable filters if your vacuum cleaner has one, should be carefully emptied into a disposable garbage bag.
- vi. Patients in quarantine or receiving care at home should ensure appropriate management of all waste (such as bandages) and potentially contaminated materials to prevent the disease from being transmitted from infected humans to susceptible animals at home (including pets), or to peri-domestic animals, especially rodents.
- vii. Always clean surfaces first with detergent and water followed by disinfection with an approved disinfectant with virucidal activities: Disinfect using 70% alcohol or hypochlorite solution (concentration of 1000ppm, usually 2 sachets to 4.5L of water) To prevent cross-contamination, cleaning must always be carried out from the cleanest area first and finish in the dirtiest area last, and always clean from top to bottom. Particular attention should be paid to toilets and frequently touched surfaces.
- viii. Use damp mopping, avoid dry sweeping to prevent dispersion of particles Carpeting and household furnishing should be steam cleaned where possible Avoid vacuuming
- ix. <u>Risks from domestic waste to humans within the household</u> arising from directly handling contaminated waste, such as gauze and wound dressings, generated within the home setting can be reduced through practices such as performing regular hand hygiene, wearing gloves, discarding contaminated items directly, not touching the outside of the waste container or other surfaces with contaminated gloves.
- x. <u>Risks from domestic waste to humans collecting the garbage</u> can be reduced by using strong bags, ensuring bags are securely tied, double bagging waste and reinforcing routine practices for management of waste (i.e., good hand hygiene, gloves if bags are handled).

Handling of waste of healthcare facilities

- i. Standard Precautions should be applied for all patient care, including for patients with suspected Mpox.
- ii. Use a pre-identified route from patient treatment areas (or, in laboratories and other facilities, areas where MPX waste is generated) to a secure storage location within the facility that serves as a waste holding area, either prior to inactivation on-site or for storage prior to transport for off-site inactivation.
- iii. Transport MPX waste from the point of origin within the generating facility to a secure holding area with the use of covered push carts, bins, or other leak-proof containers to ensure that there is no release or spillage of the waste.
- iv. Decontaminate the outside surfaces of all waste containers before moving them.
- v. Use designated elevators, such as freight elevators, if possible. Cleaning up spills.
- vi. Develop spill clean-up protocols, assemble spill clean-up kits, and train staff on how to respond to and clean up spills consisting of blood, body fluids, and other potentially infectious or contaminated materials within the facility. Spill clean-up kits typically contain absorbent materials (such as clay cat litter or other absorbent granules), an appropriate disinfectant, and tools for clean-up (including of bulk materials). Spill clean-up kits may also include personal protective equipment (PPE) or other supplies workers may need to safely manage spills.
- vii. Packaging waste for off-site treatment (if applicable). Store the waste at designated area (under lock and key) prior to waste vendor transport. Storage facilities should comply with requirements

for storage time, temperature controls, and capacity. Emergency permits may be required for extended storage periods or to manage increased volumes of waste.

- viii. It's better to have measures for separating the areas for Category A waste storage from other waste, locating the Category A wastes on impermeable/non-porous surfaces (i.e., floors without carpet, cracks, or gaps) and providing protection and security against spillage, weather, putrescence (i.e., rotting), pest infestation, trespassers, and theft. The waste holding area should adequately accommodate the volume of packaged waste that may develop between waste transport vendor pickups (e.g., 24-hour, 48-hour, or 72-hour intervals) and should be secure at all times with access limited to authorized employees only
- ix. Cleaning and disinfection of surfaces PPE (gloves [heavy duty], gown, respirator [e.g.N95, FFP2] and eye protection) should be worn by health workers while cleaning and disinfecting patient care equipment and patient care areas or isolation rooms where patients were suspected or confirmed to have Mpox.
- x. Use dedicated footwear that can be decontaminated. Disposable shoe covers are not recommended. Wet cleaning methods are preferred. Use dedicated cleaning material.
- xi. Always clean surfaces first with detergent and water followed by disinfection with an approved disinfectant with virucidal activities: Disinfect using 70% alcohol or hypochlorite solution (concentration of 1000ppm, usually 2 sachets to 4.5L of water) To prevent cross-contamination, cleaning must always be carried out from the cleanest area first and finish in the dirtiest area last, and always clean from top to bottom. Particular attention should be paid to toilets and frequently touched surfaces.
- xii. Use disposable or dedicated patient care equipment and clean and disinfect equipment before use on other patients.
- xiii. Dishes can be washed with detergent in automated dishwasher or manually cleaned in hot water (>55°C) while wearing domestic gloves.
- xiv. Mpox waste generated in an ambulance transporting a patient to a hospital may need to be left at the hospital to be packaged or treated.

Disposal of MPX waste in Laboratory

- i. Place all Biological waste materials in the appropriate **Red** colored container having a biohazard symbol.
- ii. Incinerate the material directly (Ensure transportation in covered trolley with designated trained handler wearing all required PPE (rubber boots, rubber gloves, masks, overall)
- iii. To be on safe side it's better to decontaminate the material in an autoclave for an appropriate period according to the type of material and container
- iv. Drain decontaminated liquid material in the diluted form in sewerage tanks
- v. Maintain records in the relevant logbook accordingly

Safe handling of linen

- i. Carefully lift and roll linens. Do not shake linen or laundry as this may disperse infectious particles.
- ii. These items should be carefully placed into designated container or bag for transport to laundry services. Linens, towels, and clothing from the patient with MPX should be laundered separately from other household laundry

- iii. Linens can be machine washed with hot water at > 60°C with laundry detergent and dried according to routine procedures, preferably at high heat.
- iv. If machine washing is not possible and hot water is not available, linens can be soaked in a large drum using a stick to stir with care taken to avoid splashing. The linens should be soaked in chlorine, rinsed with clean water and allowed to fully dry.
- v. Workers in laundry area should follow standard and transmission-based precautions including: minimize handling, in particular avoid shaking of linen and laundry; - wear gloves, apron or gown, a respirator (e.g.N95, FFP2) and eye protection.
- vi. Clothing and linens of the person with MPX can be reused after washing with soap and preferably hot water (> 60°C) or soaked in chlorine* if hot water is not available (25,26,38)
- vii. Workers in laundry area should follow standard and transmission-based precautions including: minimize handling, in particular avoid shaking of linen and laundry; wear gloves, apron or gown, a respirator (e g N95, FFP2) and eye protection

Waste Container Management

- i. Avoid storing waste containers where they have the potential to freeze or are exposed to high heat.
- ii. Waste containers should be in good condition, not rusted or dented.
- iii. Train laboratory personnel on safe procedures to transfer chemicals to waste containers.
- iv. Make sure waste containers are compatible with the waste type they are expected to contain.
- v. Do not fill the waste bag to capacity. When they are three-quarters full, replace with new bin liner and send this one with goose neck sealing for disposal as per requirement
- vi. Do not roll waste containers on their side or edge.

Precautionary Measures

- i. There is no substitute for applying **GMPP (good microbiological procedures and practices) and using "common sense"** during handling of the waste material including potentially harmful waste.
- ii. Consult material safety data sheet provided with the chemicals; take maximum care with the highly combustible or explosive materials to avoid direct contact
- iii. Do not mix chemical wastes as unexpected reactions can occur
- iv. In order to protect, prevent or minimize the damages through the potential routes of exposures i.e. inhalation and dermal contacts wear use respirators.
- v. Engineering control devices i.e. exhaust fan ventilation; fume hoods etc must be in working condition before handling the chemical
- vi. Segregate the waste at source to avoid mixing

Figure-09 Coding for each waste category and the type of packaging and the appropriate treatment as summarized in the table below

Category	Type of waste	Packaging	Color code	Treatment
Sharps	 Waste sharps including Metals: Needles, syringes with fixed needles, needles from needle tip cutter or burner, scalpels, blades, or any other contaminated sharp object that may cause puncture and cuts. This includes both used, discarded and contaminated metal sharps Glassware: Broken or discarded and contaminated glass including medicine vials and ampoules except those contaminated with cytotoxic wastes. 	Purpose – made puncture proof rigid container	yellow	 Incineration Disinfection (by soaking the washed glass waste after cleaning with detergent and Sodium Hypochlorite treatment) or through autoclaving or microwaving
Infectious & / or contaminated non- sharps	 Soiled Waste: Items contaminated with blood, body fluids like dressings, plaster casts, cotton swabs and bags containing residual or discarded blood and blood components Animal Anatomical Waste : Experimental animal carcasses, body parts, organs, tissues, including the waste generated from animals used in experiments or testing 	Sealable, strong / heavy duty leak proof plastic bag or container	Red	Incineration
Contaminated Waste (Recyclable)	 Wastes generated from disposable items such as tubing, bottles, intravenous tubes and sets, catheters, urine bags, syringes (without needles and fixed needle syringes) and vacationers with their needles cut) and gloves 	Red colored non chlorinated plastic bags or containers	Red	Autoclaving or microwaving/ followed by shredding or combination of sterilization and shredding

non-infectious or healthcare general waste	Card boards, paper etc	Sealable, plastic lined, puncture proof rigid plastic container	Black	Designated area for non-hazardous waste
Non-infectious wet waste	Kitchen &/or compost waste	Sealable, plastic lined, puncture proof rigid plastic container	Green	Designated land fill