INTERIM BIOSAFETY GUIDANCE FOR SPECIMEN COLLECTION, HANDLING, AND TESTING OF MONKEYPOX VIRUS

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May 26, 2022

1. Background

Monkeypox is a rare disease that is caused by infection with monkeypox virus. Monkeypox virus belongs to the *Orthopoxvirus* genus in the family *Poxviridae* and it is an enveloped double-stranded DNA virusn. The *Orthopoxvirus* genus also includes variola virus (which causes smallpox), vaccinia virus (used in the smallpox vaccine), and cowpox virus. Human-to-human transmission of monkeypox virus occurs by direct contact with lesion material or from exposure to respiratory secretions, albeit less common, means of person-to-person spread. African rodents and primates may harbor the virus and infect humans, but the reservoir host is unknown.

In humans, the symptoms of monkeypox are similar to but milder than the symptoms of smallpox. The incubation period is usually 7–14 days but can range from 5–21 days. The illness begins with a fever, headache, muscle aches, backache, swollen lymph nodes, chills, and exhaustion. And then within 3 days, sometimes longer after the appearance of fever, the patient develops a rash, often beginning on the face then spreading to other parts of the body. Lesions progress through the following stages before falling off: macules, papules, vesicles, pustules, and scabs.

In order to prevent the transmission of monkey viruses, individuals that meet the suspected case definition for monkeypox should be offered testing. Thus, specimens should be collected and handled in a safe manner, and measures should also be taken to minimize the risk of transmission based on risk assessment when testing routine clinical specimens from confirmed or suspected monkeypox patients.

This document provides interim biosafety guidance for laboratory personnel and other health professionals working on specimen collection, handling, and testing for monkeypox virus in Ethiopia.

2. Biosafety Practices

It is recommended that all manipulations of specimens originating from suspected, probable or confirmed cases of monkeypox in the laboratory be conducted according to a risk-based approach. Each laboratory should conduct a local (that is, institutional) risk assessment.

- When manipulating biological specimens, core biosafety requirements, similar to those previously referred to as biosafety level 2, must be met and heightened control measures should be applied based on local 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:
- Performing hand hygiene frequently
- Performing cleaning and disinfection procedures
- Specimens from patients with suspected MPXV infection must be handled in a functioning 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.

3. Safety during specimen collection, shipment and storage

If monkeypox is suspected, laboratory or other health workers should collect an appropriate sample and have it transported safely to a laboratory with appropriate capability. Hence, specimens should be collected, packaged, and shipped in accordance with safety.

Laboratory personnel must be trained for appropriate donning and doffing of personal protective equipment (PPE), specimen collection, storage, packaging and transport. All specimens collected for laboratory testing should be regarded as potentially infectious and handled with caution. Measures should be taken to minimize the risk of laboratory transmission based on risk assessment when testing routine clinical specimens from confirmed or suspected monkeypox patients. The measures may include:

- limiting the number of staff testing specimens only to staff with proven competency,
- wearing appropriate PPE,
- using rigorously applied standard precautions, and
- avoiding any procedures that could generate infectious aerosols.
- Where appropriate and available, consideration of vaccination among staff is encouraged.

- Strict adherence to biosafety guidelines or procedures must be ensured during specimen collection and handling

3.1 Safety during Specimen Collection

The recommended specimen type for laboratory confirmation of monkeypox is skin lesion material, including swabs of lesion surface and/or exudate, roofs from more than one lesion, or lesion crusts. In addition to a lesion specimen, the collection of an oropharyngeal swab and blood are encouraged.

- Individuals collecting specimens from suspect or patient with monkeypox should wear appropriate PPE (see annex 1 for donning).
- During skin lesion, swabs and blood collection use gown, and gloves However, if there is a risk of droplets use medical mask and google/ face shield
- During oropharyngeal swabs collection use gown, gloves, mask (if available N95) and google/ face shield. Goggles or a face shield should covers the front and sides of the face)
- Strict adherence to biosafety guidelines or procedures during specimen collection
- Doff PPE properly (see annex 2 how to doff)
- Practice hand hygiene before and after finishing work.
- After specimen collection, contaminated PPE and other materials should be decontaminated and placed in a durable, leak proof container and sealed

3.2 Safety during Packaging and shipment of specimens.

- Transport of specimens should comply with any applicable national and/or international regulations, including the UN Model Regulations and any other applicable regulations depending on the mode of transport being used.
- All specimens being transported should have appropriate triple packaging, labelling and documentation.
- For international transport, specimens from suspected probable or confirmed cases of MPXV, including clinical samples, viral isolates and cultures should be transported as Category A, UN2814 "infectious substance, affecting humans.
- Shipping requires a dangerous goods certified shipper. For information on infectious substances shipping requirements, please see the WHO Guidance on regulations for the transport of infectious substances 2021-2022 (3).

3.3 Safety during Specimen storage

Applied biosecurity addresses laboratory risks and threats that fall outside the realm of accidental exposure or release. Hence, access to areas where monkeypox virus specimens or materials are stored must be limited to those with proper clearance.

- The employees who have access to and work with monkeypox virus materials must be responsible for basic inventory tasks because they are familiar with the type and amount of biological materials present and their location and state.
- Rules and procedures for inventory control must be developed by laboratory managers or principal investigators, together with management and the safety officer.
- All specimens containing monkeypox virus should be stored at lockable freezers at -70°C or below.
- In addition, specimens collected for monkeypox virus testing should be stored in safe manner and access control from unauthorized personnel.

4. Safety during testing specimens for monkeypox virus

Testing for the presence of monkeypox virus 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 when testing routine clinical specimens from confirmed or suspected monkeypox patients. These may include:

- limiting the number of staff testing specimens,
- wearing appropriate personal protective equipment when performing monkeypox virus testing and handling ,
- using rigorously applied standard precautions, and
- avoiding any procedures that could generate infectious aerosols
- Strict adherence to biosafety guidelines or procedures during specimen testing
- After specimen testing, contaminated PPE and other materials should be decontaminated and placed in a durable, leak proof container and closed

4.1 Manipulating Specimens Suspected to Contain Monkeypox Virus

When persons perform laboratory work that involves handling specimens that may contain monkeypox virus, they should pay attention to biosafety practices and good laboratory practices/ good microbiological practices.

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- Routine specimen processing may be handled in BSL-2 facilities, but with more stringent BSL-3 work practices.
- Laboratory workers must wear protective equipment, including disposable gloves, gowns with cuffed sleeves. when performing aerosol generating procedure, wear respirators (e.g. if available N95 Mask) and face protection/goggles (snugly fitting goggles are preferred; if a face shield is used, it should have crown and chin protection plus wrap around the face to the point of the ear) to provide a barrier to mucosal surface exposure.
- Centrifugation must be performed using safety cups or sealed rotors. Rotors or safety cups should be opened in a BSC after centrifugation involving monkeypox specimens.
- If procedures that generate fine-particle aerosols cannot be contained within a BSC, acceptable methods of respiratory protection include particulate respirators; these respirators provide the minimum level of respiratory protection.
- Facilities may consider the use of higher levels of respiratory protection, particularly if vaccination status of staff is not confirmed or if personnel cannot be correctly fitted to disposable models. These higher levels may include the use of powered air purifying respirators.
- Decontamination of work surfaces after the completion of work or at the end of the day is essential. Any hospital detergent-disinfectant currently used by health-care facilities for environmental sanitation may be used. Manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling should be followed.
- If the appropriate safety equipment and/or protocols are not available, consideration should be made to refer specimens to a suitably equipped reference laboratory.

4.2 Routine Chemistry, Hematology, and Urinalysis Laboratories

During handling and processing routine clinical specimens from monkeypox patients (e.g., urine for urinalysis, blood for CBC, chemistries, microbiology), measures should be taken to minimize the risk of laboratory transmission when testing routine clinical specimens from confirmed or suspected monkeypox patients. These may include:

- Limiting the number of staff testing specimens,
- Wearing appropriate personal protective equipment, using rigorously applied standard precautions, and

- Avoiding any procedures that could generate infectious aerosols.

In addition BSL-2 facilities with standard BSL-2 work practices may be used for Molecular Testing, and Analysis of Bacterial or Mycotic Cultures

5. Decontamination

Decontaminate work surfaces with disinfection with 0.5% sodium hypochlorite (200ppm available chlorine) bleach, freshly prepared, quaternary ammonium compounds, or appropriate disinfectants. When you use a disinfectant, you should follow the manufacturer's recommendations for use, such as dilution, contact time, and safe handling of disinfectants.

6. Spills

Allow aerosols to settle and, wearing protective clothing, gently cover the spill with paper towels and apply a suitable disinfectant, starting at the perimeter and working towards the center. Allow sufficient contact time before cleanup.

7. Waste Disposal

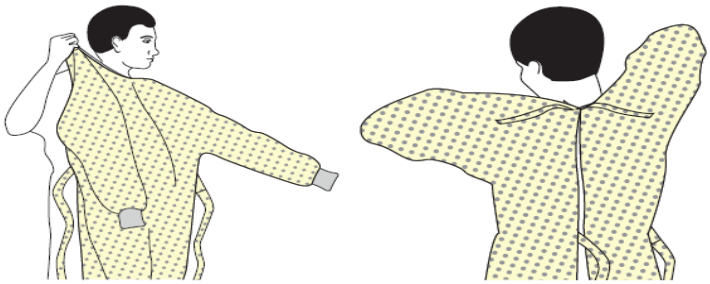
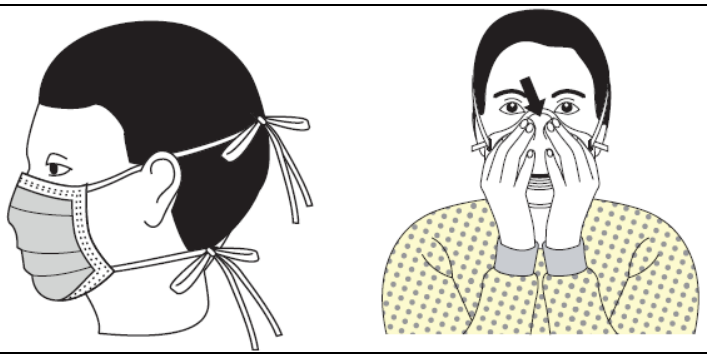
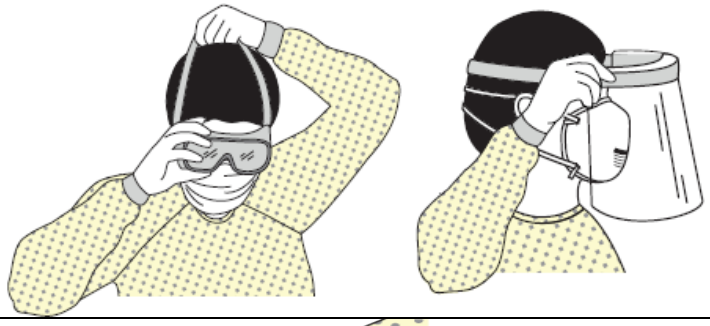
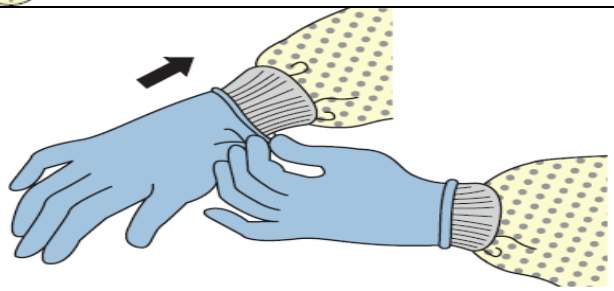
All stocks, and other waste containing monkeypox virus should be decontaminated before disposal onsite by using an approved method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory should be placed in a durable, leak proof container and closed for transport from the laboratory.

Reference

1. CDC. Monkeypox virus. Laboratory Procedures: https://www.cdc.gov/poxvirus/monkeypox/lab-personnel/lab-procedures.html. Accessed May 25, 2022
2. Monkeypox: Guidance for environmental cleaning and decontamination Public Health England v5 11 September 2018
3. World Health Organization. Laboratory testing for the monkeypox virus Interim guidance. 23 May 2022. WHO.
4. Canada Pathogen Safety Data Sheets: Infectious Substances – Monkeypox virus: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/monkeypox-virus.html. Accessed May 26, 2022

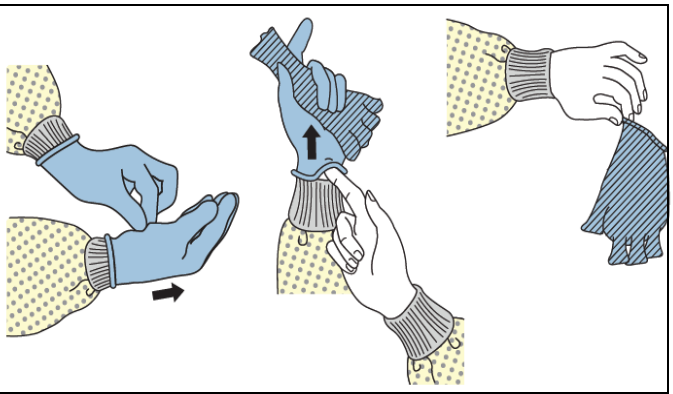
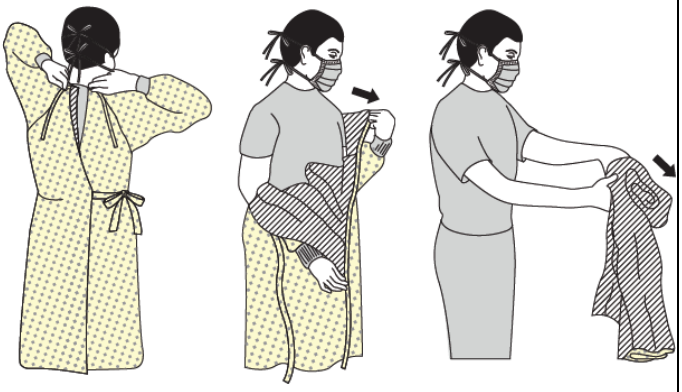
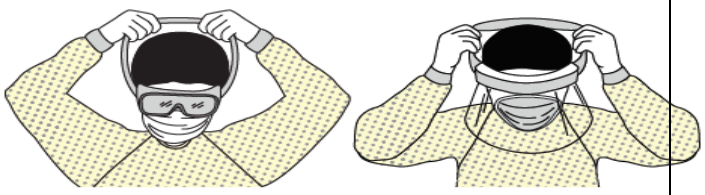
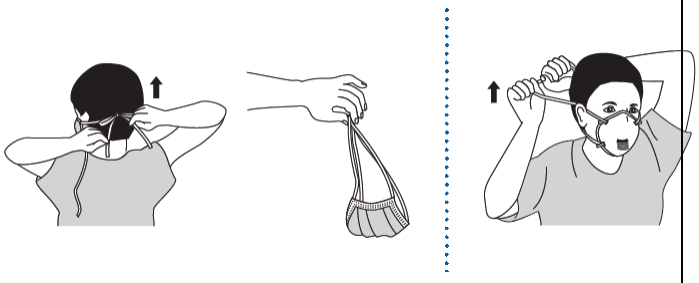
A. Annex 1: PPE Donning Procedures

Table 1: Sequencing for Donning Personnel Protective Equipment (PPE)

GOWN • Fully cover torso from neck to knees, arms to end of wrists, and wrap around the back • Fasten in back of neck and waist • If the gown is with front opening, make sure to close the gown with appropriate button/zipper	
MASK OR RESPIRATOR Secure ties or elastic bands at middle of head and neck • Fit flexible band to nose bridge • Fit snug to face and below chin • Fit-check respirator ➢ Inhale – respirator should collapse ➢ Exhale – check for leakage around face	
GOGGLES OR FACE SHIELD • Position goggles over eyes and secure to the head using the ear pieces or headband • Position face shield over face and secure on brow with headband • Adjust to fit comfortably	
GLOVES • Do gloves last of all PPE • Select correct type and size • Check for any damage • Insert hands into gloves • Extend gloves over gown cuffs	
USE SAFE WORK PRACTICES TO PROTECT YOURSELF AND LIMIT THE SPREAD OF CONTAMINATION	

B. Annex 2: PPE Doffing Procedures

Table 2: Sequencing for Doffing/Removing Personnel Protective Equipment (PPE)

GLOVES • Outside of gloves are contaminated! • If your hands get contaminated during glove removal, immediately wash your hands or use an alcohol-based hand sanitizer • Using a gloved hand, grasp the palm area of the other gloved hand and peel off first glove • Hold removed glove in gloved hand • Slide fingers of ungloved hand under remaining glove at wrist and peel off second glove over first glove • Discard gloves in a waste container	
GOWN • Gown front and sleeves are contaminated! • If your hands get contaminated during gown removal, immediately wash your hands or use an alcohol-based hand sanitizer • Unfasten gown ties, taking care that sleeves don't contact your body when reaching for ties • Pull gown away from neck and shoulders, touching inside of gown only • Turn gown inside out • Fold or roll into a bundle and discard in a waste container	
GOGGLES OR FACE SHIELD • Outside of goggles or face shield are contaminated! • If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer • Remove goggles or face shield from the back by lifting head band or ear pieces If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container	
MASK OR RESPIRATOR • Front of mask/respirator is contaminated — DO NOT TOUCH! • If your hands get contaminated during mask/respirator removal, immediately wash your hands or use an alcohol-based hand sanitizer • Grasp bottom ties or elastics of the mask/respirator, then the ones at the top, and remove without touching the front • Discard in a waste container	
WASH HANDS OR USE AN ALCOHOL-BASED HAND SANITIZER IMMEDIATELY AFTER REMOVING ALL PPE	