Mpox

Biosafety Laboratory Guidance for Handling and Processing Mpox Specimens

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> Partners at the U.S. Department of Transportation, Labor, and Defense departments, the U.S. Environmental Protection Agency, CDC, and the Assistant Secretary for Preparedness and Response have updated released a Safety Advisory Notice regarding the classification of *Monkeypox virus* (MPXV) diagnostic specimens. This notice clarifies that samples from both clade I and II MPXV are designated as Category B infectious substances. Only samples or clinical waste from cultures of clade I MPXV should be designated as Category A infectious substances. Category B (UN3373) categorization includes infectious substances transported for diagnostic or investigational purposes. Submitters should follow all appropriate Category B regulations for packaging and transporting specimens from suspect mpox patients for diagnostic testing.

All clinical specimens may contain potentially infectious agents or organisms. Take precautions when handling specimens suspected or confirmed positive for *Monkeypox virus*. Timely communication between clinical and laboratory staff is essential to minimize the risk of laboratory transmission when handling and testing specimens from patients with possible mpox. Label specimens accordingly and alert the receiving laboratory to ensure that specimens are appropriately handled. Correct handling and storage of specimens during transportation are essential for accurate diagnostic testing.

General Guidance

Monkeypox virus is a member of the *Orthopoxvirus* genus within the *Poxviridae* family. Some federal regulations and guidelines apply to work conducted with the *Monkeypox virus*. See Select Agent Regulations.

According to Advisory Committee on Immunization Practices (ACIP) recommendations, employers should offer pre-exposure orthopoxvirus vaccination to workers at risk of occupational exposure. Two vaccines may be used to prevent mpox disease, JYNNEOS and ACAM2000. Individuals are considered fully vaccinated 14 days after the second dose of the JYNNEOS vaccine or four (4) weeks after the ACAM2000 vaccination. The Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th edition recommends vaccination for laboratorians who work directly with viral cultures or animals contaminated or infected with replication-competent orthopoxvirus (e.g., *Monkeypox virus*). The BMBL and the ACIP recommend booster doses of JYNNEOS every 2 years and ACAM2000 every 3 years for people at occupational risk for virulent replicating orthopoxviruses (e.g., *Monkeypox virus*). They also recommend booster doses at least every 10 years for those at occupational risk for less virulent orthopoxviruses (e.g., cowpox virus and vaccinia virus).

As with all procedures, laboratories should perform a site-specific and activity-specific risk assessment to identify and mitigate risks. Risk assessments and mitigation measures depend on the following:

- The procedures performed
- The hazards involved in the processes and procedures
- The competency level of the personnel who perform the procedures
- The laboratory equipment and facility
- The resources available
- The vaccination status of the personnel who perform the procedures

Follow Bloodborne Pathogens – Worker protections against occupational exposure to infectious diseases | Occupational Safety and Health Administration (OSHA) when handling clinical specimens, all of which may contain infectious agents or organisms. These recommendations include hand hygiene and specific personal protective equipment (PPE) determined by the potential for exposure to blood, body fluids, and infectious material. PPE, such as laboratory coats or gowns, gloves, eye protection, respiratory protection, and face shield, can help protect the skin and mucous membranes of the eyes, nose, and mouth. Avoid procedures that could generate infectious aerosols.

For more information, see:

- Biological Risk Assessment: General Considerations for Laboratories
- Core Infection Prevention and Control Practices for Safe Healthcare Delivery in All Settings
- Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard
- Occupational Safety and Health Administration (OSHA) Personal Protective Equipment Standard
- Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition, Section II – Biological Risk Assessment, pages 9-20 and Section IV -Laboratory Biosafety Level Criteria, pages 32-69
- Mpox: Experts Give Virus Variants New Names

Select Agent Regulations

Specimens identified as clade I ***Monkeypox virus*** are regulated as a select agent (SA). Entities that possess, use, or transfer this material must comply with the HHS Select Agent and Toxin Regulations [42 CFR § 73]. Specimens identified as clade II *Monkeypox virus* are excluded from SA regulations. However, if a generic mpox test that does not identify the clade was used, the material is regulated as a select agent unless another exemption or exclusion applies.

Specimens identified as orthopoxvirus or non-variola orthopoxvirus are not select agents and, thus, are not regulated material. See SA Grams – 2022 | Resources | Federal Select Agent Program (selectagents.gov) for more information on the 2022 U.S. Mpox Outbreak & FSAP Regulations.

Anyone that possesses a Select Agent or toxin that is contained in a **diagnostic or verification specimen** or sample is exempt from the requirements of the Select Agent regulations for that specific select agent or toxin if you do the following (more details found here: FAQ: Report of Identification of Select Agent or Toxin | Compliance | Federal Select Agent Program (selectagents.gov) :

- Unless directed otherwise by the Federal Select Agent Program (FSAP), within seven (7) calendar days after identification of the select agent or toxin, either transfer the Select Agent or toxin in accordance with section 16 of the select agent regulations (requires prior approval by FSAP) or destroy.
- Before transfer or destruction, secure the select agent or toxin to prevent theft, loss, or release.
- Report the identification of the select agent or toxin based on the reporting criteria listed in the regulations (within seven (7) calendar days after the identification of mpox clade undetermined using a generic mpox assay or mpox clade I).
- Maintain copies of all APHIS/CDC Form 4A reports for a period of (3) three years.

Select Agent Destruction and Inactivation Options

Destruction (such as by disinfection or sterilization) of an identified select agent (mpox clade undetermined using a generic mpox assay or mpox clade I) must be done on-site or transferred in accordance with section 16 of the select agent regulations (requires prior approval by FSAP) within 7 calendar days. Sterilization procedures can be used to inactivate virus if using an autoclave operating within permitted parameters as outlined by the manufacturer and validated by the operator.

For information on inactivation for non-registered laboratories that identify a select agent, including clade I MPXV, refer to: https:/selectagents.gov/compliance/faq/inactivation.htm . Some lysis buffers (depending on the buffer ingredients) may also be effective at rendering the select agent non-viable and meet the exclusion criteria (refer to relevant publications or email poxviruslab@cdc.gov for additional details). Additional information on inactivation of a select agent, including clade I MPXV, identified in a clinical sample, available at https:/selectagents.gov/compliance/faq/inactivation.htm.

Chemical disinfection procedures are available using an EPA-registered hospital-grade disinfectant with an emerging viral pathogen claim (see decontamination section below).

Biosafety Considerations for Diagnostic Testing

Facilities that process and test mpox lesion materials including swabs of lesion surface and exudate, and lesion crusts, should have the necessary equipment, engineering controls, personal protective equipment, appropriate diagnostic assays, and properly trained personnel. If the appropriate safety equipment or protocols are unavailable, consider referring specimens to an equipped reference laboratory that meets the recommendation above.

- Perform routine diagnostic specimen processing in Biosafety Level 2 (BSL-2) laboratory facilities following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 according to site-specific and activity-specific biosafety risk assessments. Additional precautions to reduce exposure risk may include, but are not limited to:
  - Solid-front gowns with cuffed sleeves
  - Double gloves
  - Eye protection (safety glasses, snugly fitting goggles) or face protection (face-shield)
  - NIOSH-approved particulate respirator equipped with N95 filters or higher
  - Limiting the number of laboratory personnel who work during specimen manipulation
  - Laboratory with directional airflow
- Manipulate diagnostic specimens in a certified Class II Biosafety Cabinet (BSC) or other containment devices, especially if there is a potential to generate aerosols (e.g., vortexing or sonication of specimens in an open tube). Do not work with open vessels on the bench top unless it is safe to do so based on site and activity-specific risk assessments (i.e., the specimen has been fully inactivated utilizing an approved inactivation method).
- If you cannot perform a procedure within a BSC, use a combination of PPE and other containment devices (e.g., glove box, centrifuge safety cups, or sealed rotor) designed to create a barrier between the specimen and the laboratory personnel. Perform site-specific and activity-specific biosafety risk assessments to determine if your situation warrants additional biosafety precautions.

For further details, see:

- Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, Section IV – Laboratory Biosafety Levels, pages 37-43 and Appendix N – Clinical Laboratories, pages 529-541

Routine Diagnostic Testing

If a patient is suspected or confirmed for *Monkeypox virus* infection, testing to evaluate other illnesses on the clinical differential should continue while awaiting orthopoxvirus test results. Implement specific biosafety precautions depending on the specimen tested.

- For routine clinical procedures and testing of non-lesion specimens such as urine for urinalysis, blood for analysis [e.g., complete blood count (CBC), chemistries, microbiology] from suspected or confirmed mpox patients:
  - Perform in Biosafety Level 2 (BSL-2) laboratory facilities following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 according to site-specific and activity-specific biosafety risk assessments. For additional routine diagnostic testing information, see BMBL Appendix N – Clinical Laboratories .
  - The quantity of orthopoxvirus in clinical specimens, such as blood and body fluids, is likely low. Take standard universal precautions to protect against potential infectious agents in any specimen. Consistently adhering to Standard Precautions | Section IV and biosafety protocols for protecting laboratory workers will prevent possible exposure to the *Monkeypox virus* in clinical specimens. Limit the number of staff who test specimens and avoid any procedures that have the potential to generate infectious aerosols. See precaution guidance below to prevent exposures for Procedures with a High Likelihood of Generating Aerosols.

- For lesion specimens (including swabs of lesion surface and exudate, and lesion crusts) from patients who are suspected of having mpox and who are being concurrently tested for orthopoxvirus and other differentials [e.g., herpes simplex virus (HSV) or varicella-zoster virus (VZV), which are known to have the highest quantity of *Monkeypox virus*]:
    - Perform in Biosafety Level 2 (BSL-2) laboratory facilities, following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 according to site-specific and activity-specific biosafety risk assessments.
    - Additional PPE, mitigation, and practices should be assessed during the risk assessment process to reduce exposure risk. See Biosafety Considerations for Testing.
- For viral culture of lesion specimens from patients suspected to have mpox for diagnostic purposes other than *Monkeypox virus* (e.g., HSV or VZV):
    - Perform in BSL-2 laboratory facilities, using additional precautions based on the laboratory's site-specific and activity-specific risk assessment to identify and mitigate risks. See Biosafety Considerations for Testing.
    - As stated above, lesions are known to have the highest quantity of *Monkeypox virus*. Once laboratory personnel extract the viral DNA using a validated extraction protocol, the viral DNA is non-infectious. Laboratory personnel can work in a BSL-2 laboratory facility following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 with this material. Instead of culturing lesion specimens, laboratory personnel should consider using diagnostic techniques that extract DNA or RNA, if possible. Refer to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition , Section IV -Laboratory Biosafety Level Criteria, and Section VIII-E Viral Agents.

Culturing Specimens for *Monkeypox Virus*

Culture-based testing for *Monkeypox virus* should not be performed as a routine diagnostic procedure in clinical or diagnostic laboratories. Refer to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition , Section IV - Laboratory Biosafety Level Criteria BSL-3, and Section VIII-E Viral Agents. If a laboratory inadvertently cultures *Monkeypox virus*, sterilization procedures can be used to inactivate virus if using an autoclave operating within permitted parameters as outlined by the manufacturer and validated by the operator.

Molecular Testing and Analysis of Bacterial or Mycotic Cultures

Perform the following procedures in a BSL-2 laboratory facility following standard and special practices, safety equipment, and facility specifications recommended for BSL-2[1] :

- Molecular analysis of extracted nucleic acid preparations
- Routine examination of bacterial and mycotic cultures for diagnostic purposes

[1]BSL-2 procedures apply, unless the viral cultures are being done with lesion specimens awaiting orthopoxvirus test confirmation. Refer to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition , Section IV - Laboratory Biosafety Level Criteria BSL-3, and Section VIII-E Viral Agents, when performing culturing of lesion specimens for diagnostic purposes other than *Monkeypox virus* from an individual suspected of having mpox.

Clinical and Anatomic Pathology

The practice of pathology plays a critical role in determining accurate disease diagnoses by studying organ tissues and fluids. This includes microscopic evaluation and testing of cytology, surgical biopsy, and autopsy specimens.

Risks associated with surgical pathology and some cytology procedures occur when manipulating fresh tissue and body fluids from patients who may have an unknown or known infectious disease or virus, such as the *Monkeypox virus*. Risks are increased in the surgical grossing room during manual specimen handling, tissue dissection, and the preparation of frozen tissue sections using a cryostat. These procedures can result in percutaneous exposures from punctures or cuts, droplet or aerosol exposures from blood and body fluid splashes, and surfaces contaminated with the virus. Clinical laboratory and support staff must be aware of these risks and provide effective mitigation procedures.

The following pathology specimen types are considered inactivated and can be handled in accordance with BSL-2 guidelines:

- Fixed fluid or tissue smears for routine diagnostic staining and microscopic analysis
- Formalin-fixed biopsy or autopsy tissues
- Glutaraldehyde-fixed grids for electron microscopic study

Sufficient incubation time in fixative should be utilized, dependent on tissue/biopsy size, to allow adequate fixative penetration. Orthopoxviruses (such as vaccinia virus and *Monkeypox virus*) may require additional incubation time in the fixative. For larger tissue samples, additional incubation time should be utilized to ensure complete inactivation of the virus.

For information, see:

- Evaluation of Virus Inactivation by Formaldehyde to Enhance Biosafety of Diagnostic Electron Microscopy
- Reassessment of the rate of fixative diffusion
- Autopsy and Handling of Human Remains

Anatomic pathology uses different procedures and workflows than clinical pathology, so the risks and mitigation control needed to protect personnel may differ. At a minimum, all personnel practicing anatomic or clinical pathology should follow Standard Precautions | Section IV when handling clinical specimens, including hand hygiene and using PPE, such as laboratory coats or gowns, gloves, eye protection, or a disposable mask and face shield, to help protect the skin and mucous membranes of the eyes, nose, and mouth. See precaution guidance below to prevent exposures for Procedures with a High Likelihood of Generating Aerosols.

Site- and activity-specific biosafety risk assessments should be performed to determine if additional biosafety precautions are warranted.

Environmental Testing

At this time, the National Wastewater Surveillance System team recommends that untreated wastewater samples be pasteurized (60°C for 1 hour) before processing if they are suspected of containing *Monkeypox virus*. This is due to the potential exposure of laboratory personnel during untreated wastewater processing.

Procedures with a High Likelihood of Generating Aerosols

Laboratory exposures to poxviruses occur primarily through needle-stick injuries, direct contact with the specimen, or aerosols that laboratory procedures may generate. Conduct procedures with a high likelihood of generating aerosols (e.g., vortexing or sonication) in a certified Class II BSC. Use additional precautions to create a barrier between the specimen and personnel. These additional precautions can include centrifuge safety cups, sealed centrifuge rotors, and additional PPE to reduce the risk of exposure to laboratory personnel. Perform site-specific and activity-specific biosafety risk assessments to identify and mitigate risks and to determine if your situation warrants additional biosafety precautions. Situations that may warrant additional biosafety precautions include high testing volumes, use of pneumatic tube systems, and automated testing platforms (e.g., laboratory robotic platforms, etc.). If testing a lesion specimen from a suspected mpox patient, CDC recommends that laboratory personnel perform complete viral inactivation before putting the specimen on any automated platform or placing the platform within a Class II BSC, if available, to perform the work.

If laboratory personnel cannot perform procedures that may generate aerosols in a BSC, acceptable methods of respiratory protection include NIOSH-approved respirators with N95 filters or higher. N95 filtering facepiece respirators provide the minimum level of respiratory protection. Facilities may consider using higher levels of respiratory protection, particularly if personnel cannot be correctly fitted to tight-fitting respirator models. These higher levels may include using loose-fitting NIOSH-approved powered air-purifying respirators equipped with particulate filters.

Decontamination

Perform routine cleaning and disinfection procedures using an EPA-registered, hospital-grade disinfectant with emerging viral pathogens claim. Products with Emerging Viral Pathogens claims may be found on EPA's List Q . Follow the manufacturer's directions for concentration, contact time, and care and handling.

Reevaluate current protocols for cleaning, use of PPE, patient placement, and hand hygiene; see Standard Precautions | Section IV. For example, high-touch surfaces such as patient waiting rooms and equipment present a higher probability of contamination in the work area and should be disinfected frequently. Increase the number of available cleaning supplies, distribute them throughout the laboratory and waiting areas, and encourage staff to clean surfaces and equipment frequently. Reusable PPE should be cleaned and disinfected according to manufacturer instructions because not all disinfectants are compatible, and some may degrade the PPE.

Laboratory Waste Management

Dispose of sharps in appropriate puncture-resistant containers to autoclave as infectious waste. All cultures, stocks, residual specimens, and *Monkeypox virus* waste should be decontaminated before on-site disposal using an approved method, such as autoclaving. Materials decontaminated outside the immediate laboratory should be placed in a durable, leak-proof container and closed for transport from the laboratory. Follow local, regional, state, national, and international regulations for waste disposal. State and local waste disposal regulations vary; for more information, see:

- Environmental Protection Agency Regulations
- State Universal Waste Programs in the United States
- U.S. Department of Transportation's: Managing Solid Waste | F-2 pages 94–97
- Notice of Enforcement Discretion Regarding Mpox Medical Waste
- Safety Advisory Notice – Classification of Mpox Diagnostic Samples and Waste

Resources for Monitoring Healthcare Workers Exposed to *Monkeypox Virus*

Infection Control: Healthcare Settings

Guidelines for monitoring healthcare workers who have unprotected exposures to patients with mpox or laboratory specimens from these patients

Mpox and Smallpox Vaccine Guidance

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