AMERICAN SOCIETY FOR MICROBIOLOGY

Monkeypox Virus (MPXV) Biosafety and Testing Recommendations White Paper

Sept. 30, 2022

Stephanie Mitchell, PhD, D(ABMM), Allen Bateman, PhD, MPH, D(ABMM), Ryan Relich, PhD, D(ABMM), Vincent Munster, PhD, Stella Antonara, PhD, D(ABMM), Romney Humphries, PhD, D(ABMM), Neil Anderson, MD, D(ABMM), Rosemary She, MD, Carrie Anglewicz, MS, Laura Filkins, PhD, D(ABMM)*, Audrey Schuetz, MD, MPH, D(ABMM)*
*Coordinating co-authors

On behalf of the American Society for Microbiology Clinical and Public Health Microbiology Committee, Laboratory Practices Subcommittee

Highlights

- **A multi-national epidemic of clade II monkeypox virus (MPXV) (formerly known as West African clade MPXV) is ongoing.**
- **Lesion materials, including swabs of lesions, are the specimens of choice for detecting MPXV DNA.**
- **It is recommended that laboratories perform a biohazard risk assessment for MPXV.**
- **The Centers for Disease Control and Prevention (CDC) recommends that laboratory personnel perform MPXV inactivation prior to nucleic acid amplification test (NAAT) analysis before placing the specimen on an automated platform outside of the biosafety cabinet (BSC). Inactivation may be achieved by chemical or thermal means to minimize the risk of laboratory-associated infections. The decision of whether to include an inactivation step should be based on the laboratory's risk assessment and test method(s).**
- **After the inactivation step, if performed, specimens collected from patients suspected of MPXV can be handled using standard laboratory precautions at biosafety level-2. Specimens that are not inactivated should be handled according to the measures indicated by the laboratory's risk assessment and following CDC guidance.**

I. Introduction for Use of This Guidance

Monkeypox virus (MPXV) was first described in 1958 among cynomolgus monkeys (*Macaca fascicularis*) imported from Singapore to a Danish animal facility (1). Affected animals developed a non-fatal disease similar in appearance to smallpox and other known poxvirus diseases, but the cause was proven to be distinct. Consequently, the disease was called monkeypox and the orthopoxvirus causing it was named *monkeypox virus* (MPXV; 2). MPXV is an enveloped, double-stranded DNA virus and is one of 12 species currently assigned to the genus *Orthopoxvirus* (subfamily *Chordopoxvirinae*, family *Poxviridae*). Since its first description, additional outbreaks have been reported in monkeys housed in facilities in several countries, including the United States (U.S.). In 1970, the first human cases were identified among people living in parts of West and Central Africa.

Monkeypox Virus (MPXV) Biosafety and Testing Recommendations White Paper

Monkeypox is a zoonotic disease with natural animal hosts, ranging from rodents to non-human primates. Person-to-person transmission can occur when there is close contact with respiratory secretions via droplet transmission, through direct skin contact with lesions of an infected person, or by fomite transmission (3). Cases were originally limited to areas near tropical rainforests but spread into urbanized areas has increased (3). Soon after the first human case, two distinct clades of MPXV were recognized. Originally, these clades were named after the geographic regions of endemicity: West Africa and the Congo Basin. On August 12, 2022, the World Health Organization (WHO) released new nomenclature for these clades: clade I (formerly Congo Basin clade) and clade II (formerly West African clade). Infection with MPXV clade I has traditionally caused more severe disease and it is thought to be more transmissible (3).

The Health and Human Services (HHS) Federal Select Agent Program (FSAP) regulates materials that contain, or is identified as, a select agent. Select agent classification of MXPV depends on the clade. Currently, Clade II is not a select agent, while other MPXV clades are (4). Specimens identified as MXPV clade II by the laboratory are exempt from the HHS FSAP designation. Additionally, if a specimen has been identified by the laboratory as *Orthopoxvirus* but has not yet been confirmed to be MPXV (with or without a clade result), the specimen does not fall under FSAP regulations as it is considered to be a presumptive identification of the MPXV. However, specimens identified as MPXV either without a clade identification or is identified as Clade I, falls under the HHS FSAP designation and requires the appropriate actions for select agents as per 42 CFR 73.

Prior to 2022, most MPXV infections occurred in endemic regions within central and western African countries (5). Most cases identified outside of Africa were associated with individuals with recent travel to those countries. Disease due to MPXV typically presents as a maculopapular rash that can occur on any part of the body and often goes through multiple phases as the disease progresses. The rash can appear vesicular, similar to herpes simplex virus (HSV) and varicella zoster virus (VZV), often scabbing over prior to disease resolution (5). Individuals may also experience other symptoms, such as fever or flu-like symptoms that may occur prior to rash development, swollen lymph nodes, and respiratory symptoms such as nasal secretions/congestion and cough (5). The incubation period for MPXV is approximately 3 weeks. Those who experience flu-like symptoms will develop a rash 1-4 days later (5). Currently, there is no treatment for MPXV specifically; however, given the similarity between MPXV and smallpox, there are efforts to better understand if antivirals against smallpox may be used. Tecovirimat, an inhibitor of a major envelope protein required for the production of extracellular virus, is recommended for those at high risk for severe disease, such as immunocompromised individuals (6). At the time of this guidance, there are two vaccines available for the prevention of MPXV infection: JYNNEOS and ACAM2000. The vaccine is recommended to be administered to those at high risk of contracting the disease, those who are at risk of severe infection, and healthcare professionals including laboratory workers who perform MPXV testing on specimens (7). For specific recommendations and for availability of vaccine, laboratories should consult their local health departments.

In Spring 2022, a rising number of MPXV cases was confirmed in persons from non-endemic countries, many of whom were without a travel history, raising concern for community transmission. Most cases in the current outbreak have been in men who have sex with men (MSM). The number of infected individuals is rising across the U.S. and worldwide; current numbers of laboratory-confirmed cases can be found on the Centers for Disease Control and Prevention (CDC) website (8). The current outbreak is caused by MPXV clade II, which causes less severe disease than clade I. A number of deaths have been reported in the current global epidemic (8).

Currently, laboratory testing for MPXV is offered through the Laboratory Response Network (LRN) and select commercial reference laboratories and clinical laboratories (9). Testing can be performed using either an *Orthopoxvirus* PCR or MPXV PCR. The CDC test protocols and testing guidance are available to clinical laboratories, which are interested in validating these as laboratory developed tests (LDTs) for in-house clinical testing (9) to shorten turn-around-times, but concerns have been raised regarding exposure of laboratory personnel to MPXV during specimen processing and handling.

Prior to implementing MPXV testing, laboratories should perform thorough safety risk assessments for all applicable testing sites and activities. Refer to appropriate resources, including the CDC's Monkeypox Laboratory Procedures and Biosafety Guidelines website (10) and the CDC/National Institutes of Health (NIH) Biosafety in Microbiological and Biomedical Laboratories, 6th Edition (11). Laboratories may consider the outlined testing procedures and MPXV testing-specific biosafety considerations detailed in this white paper as items to include in their risk assessments.

The goal of this white paper is to provide laboratories with an overview of current issues related to MPXV. Discussion points include preferred specimens for MPXV diagnostic testing, biosafety considerations for specimen handling of suspect lesions, biohazard risk assessment suggestions, and guidance for implementation of inactivation methods for nucleic acid amplification testing (NAAT) of lesion specimens.

Specimens for Diagnostic Testing

Lesions (e.g., lesion fluid or exudate, tissue biopsy from lesion, lesion crust) and swabs of lesions are the specimens of choice for detecting MPXV DNA. Swabs may be collected from skin lesions or from lesions on oral, anal, or genital mucous membranes. CDC recommends collection of two swabs per lesion. High numbers of viral particles titers are present in these specimens. CDC protocols for detection of MPXV by NAAT include lesion materials as the only acceptable specimens (12, 13). The U.S. Food and Drug Administration (FDA) states that alternative specimen types such as saliva or blood may be associated with false negative test results (14). In an international collaborative study that included 528 infections among predominantly gay or bisexual men, skin lesions were present in 95% of cases (15). Of those, the majority were in the anogenital area (73%) followed by trunk, arms, or legs (55%). Additionally, mucosal lesions were present in 41% of the cases including anorectal and oropharyngeal mucosa.

In addition to molecular testing for MPXV, lesion biopsies may be examined by histopathology, but immunohistochemistry for MPXV is not widely available. Serology is not currently a recommended method of diagnosis.

Viral culture for MPXV should not be performed as a routine diagnostic procedure (10). If viral culture is performed by laboratories, culture of specimen types that may contain MPXV (such as skin lesion specimens) is discouraged in order to avoid inadvertent propagation of MPXV. Rather than performing culture of lesion specimens, laboratories are encouraged to use diagnostic techniques which extract nucleic acids, thus rendering the viral DNA non-infectious.

Handling of Lesion Specimens from Patients under Evaluation or with Confirmed Monkeypox Infection

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 also perform MPXV risk assessments.

1. **Principles of Biosafety: Primary and Secondary Protection Barriers**
   Biosafety incorporates a combination of primary and secondary barriers between the laboratory personnel and the biologic agent, facility practices, and other safety equipment such as personal protective equipment (PPE). Physical safety equipment such as biological safety cabinets (BSCs) are primary barriers that directly protect a person from the hazard. Additional primary containment devices include enclosed containers, centrifuge safety cups, and other sealed containers. Facility design can offer a secondary barrier to hazards and includes effective ventilation, anterooms, and airlocks. PPE is also often considered a secondary barrier. Use of PPE varies according to tasks performed in the laboratory and specimens encountered. In general, however, PPE includes protective laboratory coats or gowns, eye and face protection (e.g., safety glasses, goggles, face shield), and gloves. It is a tenet of biosafety that laboratories should rely more heavily on primary equipment or facility barriers to shield workers from hazards, as compared to relying solely on PPE to provide protection (BMBL).

2. **MPXV Biosafety during Specimen Processing and NAAT**
   The CDC recommends use of a Class II biological safety cabinet (BSC) or other shielding or containment device as a primary barrier when manipulating specimens suspected of containing MPXV. Lesion specimens should be worked up in BSL-2 facilities. The biohazard risk assessment performed by each laboratory should guide further risk mitigation efforts or safety practices. If procedures cannot be performed within a BSC, the CDC recommends use of a containment device (e.g., glove box) plus PPE. Viral inactivation is recommended by the CDC for testing lesions from suspected MPXV patients; refer to "Inactivation of Specimens Prior to Analysis" section. In general, whether or not laboratory staff are vaccinated against MPXV, BSCs should be used for manipulation of primary specimens, and laboratory aerosolization procedures should be avoided, if possible.

3. **Laboratory Aerosolizing Procedures**
   Aerosolization can lead to suspended droplets. When aerosolizing procedures cannot be avoided, it is best to perform these activities inside a BSC. Examples of laboratory aerosol-generating activities include but are not limited to centrifugation, vortexing, diluting, capping/uncapping, aliquoting/pouring/decanting, tissue cutting/grossing, shaking, mixing, stirring, blending, homogenizing, sonicating, lyophilizing, grinding, microtomy/cryotomy, sawing, drilling, harvesting tissues, using sharps/blades/needles, aspirating, pipetting, and roughly discarding specimens into receptacles.

   To mitigate the potential of aerosol production, pipetting may be performed using filter tips. Pipette contents may be expelled close to the targeted surface or by allowing the fluid to flow down the inside of the container. Centrifuge buckets should be opened inside a BSC. If a BSC is not available, splash barrier protections such as a bench or instrument shield may be used (10, 16). Alternatively, inactivation of MPXV from specimens may be pursued (see "Inactivation of Specimens Prior to Analysis" section).

4. **Bacterial and Fungal Cultures of Lesion Specimens**
   Microbiology laboratories may receive lesion specimens sent for routine bacterial and fungal cultures from patients suspected of MPXV infection. When manipulating bacterial and fungal culture plates from patients with confirmed or suspected MPXV, laboratory personnel should avoid procedures that could generate infectious aerosols, wear appropriate PPE, and adhere to standard precautions.

5. **Disinfection**
   Laboratory areas and equipment where specimens from suspect or confirmed MPXV cases are handled should be appropriately disinfected. According to the Environmental Protection Agency (EPA), MPXV is a tier 1 enveloped virus and is easily inactivated by approved disinfectants that damage the lipid envelope. A list of disinfectants approved for use in hospital settings is available on the EPA website (17).

6. **Laboratory Notification of Suspect Specimens**
   Microbiology laboratories may request to be notified if a suspect lesion specimen will be submitted from inpatient and outpatient locations. Laboratories may also consider working with their information technology departments to assist in creating special labeling on patient specimens if a "suspect MPXV" flag has been added to the patient's record or when a MPXV test order has been submitted. It may be helpful to have specimens submitted for regular bacterial and fungal cultures specifically labelled to indicate special specimen handling. While laboratory notification can supplement routine laboratory practices, given the potential delay in recognizing infections, we recommend that laboratories do not rely solely on notification. Instead, specimen type, collection devices, and accompanying test orders can be used to identify increased probability that a specimen may contain MPXV. The laboratory can use this information to label the derivatives of the primary specimen to indicate special specimen handling, according to the laboratory's biohazard risk assessment.

7. **Inactivation of Specimens Prior to Analysis**
   Clinical laboratories implementing LDTs for MPXV and/or performing *in vitro* diagnostics (IVD) HSV or VZV testing on specimens from patients with suspicion of MPXV infection may be interested in performing an inactivation step prior to clinical testing.

   On August 22, 2022, the UK Health Security Agency published reports summarizing assessments of various inactivation reagents, including cobas PCR Media (Roche), PrimeStore Molecular Transport Medium (Longhorn Vaccines & Diagnostics), Buffer AVL (QIAGEN), 70% ethanol, InhibiSURE Viral Inactivation Medium (Thermo Scientific), L6 Buffer (Severn Biotech), Nuclisens Lysis Buffer (bioMérieux), MagBead Viral RNA Lysis Buffer (Neuromics), Panther Fusion Specimen Lysis Tubes (HOLOGIC), and Molecular Sample Solution (MMS; E&O Laboratories) (18). In general, all tested chemical agents significantly (≥99.9%) reduced infectious viral titers when cultured virus was incubated with reagents for various amounts of time. Preliminary data suggest that the Buffer ATL (QIAGEN) does not inactivate MPXV and laboratories should consider alternative options until additional data are available for this reagent. Studies are underway to determine the efficacy of other reagents, as well as heat, by research teams such as those from the NIH/National Institute of Allergy and Infectious Diseases (NIAID) Rocky Mountain Laboratories and the Indiana University School of Medicine. Preliminary data from these groups indicate that heat treatment for 15-30 minutes at 65°C and 15 minutes at 95°C will inactivate MPXV in specimens. Additional studies are underway to confirm these findings, and data will be published soon.

   If a laboratory decides an inactivation step is needed for specimens that may contain MPXV, the entire testing process should be evaluated for any downstream effect the inactivation process may have on results (for MPXV testing and other NAAT testing, such as HSV/VZV). The inactivation process should be a

component of the initial test validation, when possible. However, if the inactivation process is being added to an already established test, whether LDT or IVD, a formal evaluation of the effect of this separate inactivation step should be undertaken and the test validated prior to clinical use. For a recommended protocol, see ASM's Monkeypox Virus Inactivation Guidance for Nucleic Acid Testing Validation.

Summary and Approaches to Risk Assessment during the Monkeypox Outbreak

When performing a laboratory risk assessment and developing laboratory-specific procedures during the current MPXV outbreak, we recommend that laboratories consider key points discussed in this document. Elements to evaluate may include, but are not limited to:

- Determining the biosafety level of current laboratory practices for the handling of any lesion sample that might contain MPXV.
- Ensuring biosafety containment to limit exposure to MPXV using enhanced PPE when handling lesion specimens collected for NAAT or culture.
- Creating mechanisms to identify specimens with increased likelihood of containing MPXV, through specimen-type risk determination and/or laboratory notification (physician communication or electronic medical record (EMR)-based).
- Minimizing aerosol production during pre-analytic and analytic specimen processing.
- Selecting and using disinfectants expected to inactivate MPXV for routine laboratory surface decontamination.
- Assessing need and availability of vaccination for laboratory staff.
- Validating and implementing MPXV inactivation methods before NAAT testing for MPXV and other analytes from lesion specimens.

Acknowledgements: We thank Randal Fowler, PhD, D(ABMM), for his input and ASM's Laboratory Practices Subcommittee for their critical review.

References

1. Von Magnus P, Andersen EK, Petersen BK, Birch-Andersen A. 1959. A pox-like disease in cynomolgus monkeys. *Acta Pathol Microbiol Scand.* 46:156.
2. Parker S, Buller RM. 2013. A review of experimental and natural infections of animals with monkeypox virus between 1958 and 2012. *Future Virol.* 8(2):129-157.
3. World Health Organization. 2022. Monkeypox. https://www.who.int/news-room/fact-sheets/detail/monkeypox. Accessed 22 Aug 2022.
4. SA Grams SA Grams - 2022 | Resources | Federal Select Agent Program (selectagents.gov), Accessed Sep 28.
5. Centers for Disease Control and Prevention. 2022. About Monkeypox. https://www.cdc.gov/poxvirus/monkeypox/about.html. Accessed 22 Aug 2022.
6. Centers for Disease Control and Prevention. 2022. Monkeypox Treatment. https://www.cdc.gov/poxvirus/monkeypox/treatment.html. Accessed 22 Aug 2022.

7. Centers for Disease Control and Prevention. 2022. Monkeypox Vaccines. https://www.cdc.gov/poxvirus/monkeypox/vaccines.html. Accessed 22 Aug 2022.
8. Centers for Disease Control and Prevention. 2022. 2022 Monkeypox Outbreak Global Map. https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html. Accessed 22 Aug 2022.
9. Centers for Disease Control and Prevention. 2022. Monkeypox Information For Laboratory Personnel. https://www.cdc.gov/poxvirus/monkeypox/lab-personnel/index.html. Accessed 13 Aug 2022.
10. Centers for Disease Control and Prevention. 2022. Monkeypox Laboratory Procedures and Biosafety Guidelines. https://www.cdc.gov/poxvirus/monkeypox/lab-personnel/lab-procedures.html. Accessed 23 Aug 2022.
11. Centers for Disease Control and Prevention/National Institutes of Health (NIH). Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. Revised 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf. Accessed 21 Sep 2022.
12. Centers for Disease Control and Prevention. 2022. Test Procedure: Monkeypox virus Generic Real-Time PCR Test. https://www.cdc.gov/poxvirus/monkeypox/pdf/PCR-Diagnostic-Protocol-508.pdf. Accessed 23 Aug 2022.
13. Centers for Disease Control and Prevention. 2022. Test Procedure: Non-variola Orthopoxvirus Generic Real-Time PCR Test. https://www.cdc.gov/poxvirus/monkeypox/pdf/Non-variola-Orthopoxvirus-Generic-Real-Time-PCR-Test.pdf. Accessed 23 Aug 2022.
14. U.S. Food and Drug Administration. 2022. For Monkeypox Testing, Use Lesion Swab Samples to Avoid False Results: FDA Safety Communication. https://www.fda.gov/medical-devices/safety-communications/monkeypox-testing-use-lesion-swab-samples-avoid-false-results-fda-safety-communication. Accessed 23 Aug 2022.
15. Thornhill JP, Barkati S, Walmsley S, Rockstroh J, Antinori A, Harrison LB, et al. Monkeypox virus infection in humans across 16 countries - April-June 2022. *N Engl J Med.* 387:679-691.
16. Dubuis ME and Duchaine C. 2021. Aerosol production during blood and urine pre-analytical processing and handling in a hospital biochemistry clinical laboratory during the COVID-19 pandemic. *Front Public Health.* 9:643724.
17. United States Environmental Protection Agency. 2022. Pesticide Registration. Disinfectants for Emerging Viral Pathogens (EVPs): List Q. https://www.epa.gov/pesticide-registration/disinfectants-emerging-viral-pathogens-evps-list-q#-search. Accessed 29 Aug 2022.
18. UK Health Security Agency. 2022. Monkeypox: UKHSA Laboratory Assessments of Inactivation Methods. https://www.gov.uk/government/publications/monkeypox-ukhsa-laboratory-assessments-of-inactivation-methods. Accessed 27 Sep 2022.

Citation: Mitchell S, Bateman A, Relich, R, Munster V, Antonara S, Humphries R, Anderson N, She R, Anglewicz C, Filkins L, Schuetz, A. 30 Sep 2022. Monkeypox Virus Biosafety and Testing Recommendations. American Society for Microbiology. https://asm.org/Guideline/Monkeypox-Virus-Biosafety-and-Testing-Recommendati