Elsevier has created a Monkeypox Information Center in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its monkeypox related research that is available on the Monkeypox Information Center - including this research content - immediately available in publicly funded repositories, with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the Monkeypox Information Center remains active.
Monkeypox virus (MPXV) belongs to the subfamily Chordopoxvirinae of Poxviridae and is assigned to the genus Orthopoxvirus [1]. Orthopoxviruses contain double-stranded DNA (dsDNA) genomes, are roughly 200 to 400 nm in size, and are often characterized by a brick-like shape when observed via electron microscopy [2].

To date, five orthopoxviruses have been reported to infect humans: MPXV, Akkemite virus, Cowpox virus, Vaccinia virus, and Variola virus (smallpox) [2]. In 1970, 12 years after being discovered in captive cynomolgus monkeys, the first case of zoonotic MPXV transmission was recorded in the Central African Democratic Republic of the Congo [3]. Additional cases of human MPXV infections have been reported in West Africa, but since the 1980s, outbreaks have been largely concentrated in the Congo basin of Central Africa [4]. Recently, transmission of MPXV has expanded beyond historic regions of endemicity in Africa and the Western Hemisphere. This long-standing sporadic infectivity may be due in part to the fact that MPXV can utilize numerous host reservoirs [5]. Currently, human MPXV infections can be delineated into two distinct clades of the virus: clade I (former Congo Basin clade) and clade II (former West African clade), with clade I typically causing more severe disease, possibly through mutations in a small number of open reading frames [6,7].

Given the degree of homology between smallpox virus and MPXV, smallpox vaccines have been reported to provide some degree of coverage against infection with MPXV, although it remains unclear how long this protection lasts [8,9]. Since the eradication of smallpox, declared in May 1980, vaccination has all but ceased, rendering individuals born in the past 4 decades more vulnerable to MPXV infection [10]. Presenting as a rash illness, clinical distinction between MPXV; varicella zoster virus (VZV); herpes simplex virus (HSV); hand, foot, and mouth disease (HFMD), caused by enterovirus (ENV); and other common rash illnesses can be difficult, underscoring the necessity for robust clinical and diagnostic
have been proposed to minimize negative outcomes associated with the vertical transmission of MPXV [27,30].

There is also a theoretical risk of transmission via aerosolization. In three severe adult cases now at Los Angeles County plus University of Southern California (LAC+USC), the route of transmission has been suspected to be through aerosols. All three patients had AIDS, and they presented with oropharyngeal mucosal lesions and concerns about airway edema and compromise. Several cases of health care-associated transmission have occurred, most related to unsafe practices in specimen collection [31].

Determinants
In a study of more than 500 MPX infections spanning 16 countries between April and June 2022, it was determined that the current outbreak of MPXV clade IIB disproportionately affected men who had sex with men (MSM) [26]. To this point, further investigation is required to determine if MXPV can be transmitted via semen or vaginal secretions or if transmission is a consequence of sustained physical contact during sexual encounters [15,26]. The epidemiological determinants from the clade IIB outbreak differ from those in historic areas of endemicity, given the propensity of the former to spread through sexual networks, such as MSM communities. It is difficult to determine the cause of the recent and prolific MPXV resurgence, with potential explanations ranging from waning smallpox immunity post-vaccine cessation to widespread deforestation in historic regions of endemicity [11,32,33].

Prevention
Preventing human-to-human spread of MPXV begins with educating the public about the risks associated with MPXV transmission and strategies to mitigate exposure to the virus. Caution should be taken for those living in the same household as an individual infected with MPXV due to the risk for contaminated-fomite-driven infection. Although MPXV DNA has been detected on household surfaces 3 weeks following symptom onset, the viability of MPXV under these conditions remains unclear [25]. In these instances, avoiding close contact with those presenting with rash illness, avoiding contact with potentially contaminated objects, and hand hygiene are key strategies to mitigate the risk of MPXV transmission. Due to limited vaccine availability, the Advisory Committee on Immunization Practices is currently recommending vaccination only for clinicians caring for patients with MPXV, those administering MPXV vaccines, and laboratory personnel performing MPXV testing [24,34]. Unfortunately, these recommendations fall short in protecting laboratory workers who handle specimens with suspected MPXV infection yet are not eligible for vaccination if their laboratory does not perform testing for MPXV. This point was further evidenced by the first documented case of laboratory-acquired MPX in the U.S., reported in Los Angeles County, CA [35]. Expanded vaccine access may be available, depending on state and local public health guidelines.

There are currently two smallpox vaccines licensed in the U.S. providing coverage for MPXV: ACAM2000 and JYNNEOS. ACAM2000 is a live, replicating, attenuated vaccinia virus vaccine administered percutaneously using a bifurcated needle. The
attenuated strain of vaccinia virus used in the ACAM2000 vaccine is replication competent, and those receiving the vaccine must take caution not to spread the attenuated vaccine strain to others through contact with skin lesions that form at the site of administration. Individuals are not considered vaccinated until 1 month following inoculation [34]. Moreover, the replication competency of ACAM2000 vaccinia virus gives rise to contraindication under the following circumstances: children less than 1 year of age, pregnancy, history of atopic dermatitis, exfoliative skin conditions, heart disease, and immunocompromising conditions [34]. These contraindication criteria also apply in circumstances where an otherwise eligible individual cannot sufficiently isolate from household contacts with one of the preceding conditions [34]. In contrast, JYNNEOS is a replication-deficient live vaccinia virus vaccine administered subcutaneously in two doses spaced 1 month apart. Since JYNNEOS is a non-replicating virus vaccine, there is no risk of spreading the vaccine strain of vaccinia virus to others. Individuals receiving JYNNEOS are considered fully vaccinated 2 weeks after the second dose is administered. Due to its replication deficiency, contraindications for receiving the JYNNEOS vaccine are less extensive and reserved for individuals with a serious allergy to one or more of the vaccine components [34]. Vaccination is not indicated if an individual has recovered from MPX, as they are considered immune following infection. In an effort to maximize the number of available vaccine doses, an alternative regimen may be used for individuals \( \geq 18 \) years old under emergency use authorization (EUA), in which inoculation is performed intradermally with 20% of the initial dose [24]. It is important to note that current protocols for MPX vaccination are largely experimental, as there is a dearth of clinical data demonstrating the efficacy of smallpox vaccines such as ACAM2000 and JYNNEOS for the prevention of MPX.

### Clinical Presentation and Management

#### Case definitions

In light of the recent outbreak of MPXV within the U.S., the Centers for Disease Control and Prevention (CDC) have updated case definitions as outlined in \( \text{Table 1.} \) Factors contributing to case definition include epidemiologic criteria, such as travel or recent exposure to MPXV, symptomology, and laboratory test results. Based on these criteria, the CDC categorizes MPXV cases as suspected, probable, confirmed, or excluded.

#### Clinical recognition

The classic clinical syndrome for MPXV includes prodromal symptoms such as fever and lymphadenopathy followed by a generalized pustular rash [11,36]. The most common symptoms in the 2022 U.S. outbreak include rash (98%), fever (76%), malaise (74%), chills (71%), enlarged lymph nodes (68%), and myalgia (65%) (CDC demographics). Historically, the rash was described as monomorphic, presenting at the same stage in development, first appearing as macules, then papules, vesicles, and finally pustules [11]. The rash was historically described as starting in the

---

**Table 1. CDC MPX case definitions**

| Criterion type       | Definition                                                                 |
|----------------------|---------------------------------------------------------------------------|
| Clinical             | **Suspected:** At least one of the following:                             |
|                      | 1. New rash illness                                                       |
|                      | 2. High clinical suspicion for MPXV and meets at least one epidemiological criterion |
|                      | **Probable:** No suspected recent vaccination and at least one of the following: |
|                      | 1. Orthopoxvirus DNA detected by PCR from a clinical specimen             |
|                      | 2. Orthopoxvirus detected by immunohistochemical or electron microscopy   |
|                      | 3. Anti-orthopoxvirus IgM antibody detected 4 to 56 days after rash onset  |
|                      | **Confirmed:** At least one of the following from a clinical specimen:    |
|                      | 1. MPXV DNA detected by PCR                                              |
|                      | 2. MPXV DNA detected by NGS\(^a\)                                       |
|                      | 3. Isolation of MPXV in culture                                           |
| Epidemiological      | At least one of the following within 21 days of symptom onset:           |
|                      | 1. Contact with individuals with similar rash illness and received a diagnosis of confirmed or probable MPX |
|                      | 2. Had close or intimate contact with individuals within a disproportionately affected social network |
|                      | 3. Traveled outside the U.S. to a country with confirmed cases of MPX or where MPXV is endemic |
|                      | 4. Had contact with a wild animal, animal product, or exotic pet that is an African endemic species |
| Exclusion            | At least one of the following:                                           |
|                      | 1. An alternative diagnosis is confirmed.                                 |
|                      | 2. A rash does not develop within 5 days from the onset of prodromal symptoms. |
|                      | 3. The presence of Orthopoxvirus or MPXV is not detected in high-quality specimens. |
|                      | 4. The absence of Orthopoxvirus antibodies following suspected infection    |

\(^a\)NGS, next-generation sequencing.
mouth and spreading outward to the face, trunk, and extremities, including the palms and soles of the feet [11].

After an outbreak of clade IIA MPXV in the U.S. from pet prairie dogs imported from West Africa in 2003, strains from West Africa and the Central Congo basin were compared, and two distinct clades were identified using molecular analysis [6]. Clinical symptoms of clade I were more severe, with 73.7% of unvaccinated patients presenting with ≥100 lesions and 32.5% of unvaccinated patients becoming severely ill. Clinical symptoms of clade IIA were less severe, with only 13% of unvaccinated patients presenting with ≥100 lesions and 3.8% of unvaccinated patients becoming severely ill. Severe illness was seen only in children in both clades [6].

The 2022 outbreak of clade IIB has unique characteristics that diverge from our previous understanding of the clinical disease (Table 2). Data reported from more than 500 international cases of clade IIB MPXV indicate 95% of patients presented with new-onset rash illness, 64% of whom had less than 10 lesions [26]. Primary genital, anal, and oral mucosal lesions were common in this case series and were thought to represent infection at the inoculation site. The prodromal symptoms could be absent, and lesions in different stages of progression could be seen side by side [26]. Severe presentations have included proctitis, urethritis, pharyngitis, myocarditis, lymphadenopathy, and bacterial super-infection [26]. Historically, clade I severe infections have included encephalitis, septicemia, bronchopneumonia, keratitis, vomiting/diarrhea/dehydration, and death [37].

From 28 May to 14 July 2022, a group of Spanish dermatologists performed a cross-sectional prospective study to describe the cases of MPXV in the current outbreak [38]. Of the 185 patients enrolled, they reported the initial cutaneous lesions occurred at the probable inoculation area and were localized, homogeneous, and specifically described as “pseudopustular.” This unique description meant the initial lesions looked pustular, but it was impossible to unroof them and obtain purulent material. Instead, histological evaluation showed keratinocytic debris and inflammation, but no liquid. Over time, the center became necrotic, unbilicated, and ulcerative. The lesions could be single or multiple, and if multiple, they could become confluent. They were often painful, with surrounding erythema and edema. Some patients also had mucosal ulcers, whitlows, or ocular involvement. Additionally, a secondary, generalized eruption could occur later in disease, typically consisting of small vesicles with an erythematous halo leading to a pustular eruption, but it could be heterogeneous, with papules and, less often, macules reported. This generalized rash tended to be asymptomatic or slightly pruritic and healed in a few days without a scar [38].

MPX rash can look like other infections. Local genital ulcer diseases that could present similarly to the MPX rash include HSV, syphilis, chancroid, and lymphogranuloma venereum (LGV). Proctitis could be caused by gonorrhea, chlamydia (including LGV), and HSV. A diffuse rash could be caused by syphilis, VZV, disseminated HSV, molluscum contagiosum, HFMD, disseminated gonococcal infection, disseminated fungal infection, or measles.

**Isolation and monitoring**

In addition to standard precautions, special considerations should be implemented in a clinical setting to prevent MPXV transmission. Patients with suspected or confirmed MPXV infection should be placed in single-patient rooms with the door closed when

---

**Table 2. Comparison of clinical presentations**

| Parameter                  | Clade I                          | Clade IIA                         | Clade IIB                         |
|----------------------------|----------------------------------|-----------------------------------|----------------------------------|
| Initial geography          | Central Congo basin              | West Africa                       | International                    |
| Transmission               | Unknown animal reservoir (likely  | Unknown animal reservoir (likely   | Human to human [26]              |
|                           | rodents) to humans and human to  | rodents) to humans and human to    |                                   |
|                           | human [11]                        | human [58]                         |                                   |
| Presumed mode of transmission | Close contact with infected person or animal, contaminated objects, respiratory droplets, consuming infected meat [36] | Close contact with infected person or animal, contaminated objects, respiratory droplets, consuming infected meat [36] | Sexual intercourse, possibly through saliva and semen; close contact with an infected person; contaminated objects; respiratory droplets [59] |
| Clinical presentation      | Fever, lymphadenopathy, and rash [14] | Fever, lymphadenopathy, and vesiculopustular rash; 12% during 2017 outbreak presented without fever [60]. | Fever, lymphadenopathy, and localized lesions; can present with only localized or single lesion without fever [34] |
| Rash distribution          | Lesions develop on the face, then across the body, hands, legs, and feet, including the palms and soles of the feet [36]. | Lesions develop on the face, then across the body, hands, legs, and feet, including the palms and soles of the feet [60]. | Primary localized pseudopustular-ulcerative lesions at the site of inoculation, with secondary generalized vesiculopustular eruption [38] |
| Rash description           | Numerous lesions, 100s to 1,000s; macules → papules → vesicles → pustules → scabs [6] | Numerous lesions, <100; macules → papules → vesicles → pustules → scabs [6,37] | Majority have <25 lesions, localized at the site of inoculation [26,38]. |
possible [24]. Moreover, patient transport should be reserved for medically essential purposes, and active lesions should be covered during transport (e.g., skin lesions covered with a sheet, oral lesions covered with a medical mask, etc.) [24]. Oral mucosal lesions pose additional risk for aerosolized transmission during intubation and extubation; thus, these procedures should be performed in a room designed to isolate airborne infections (i.e., a negative-pressure room) [24]. Resuspension of dried lesion material from activities such as shaking soiled linens, vacuuming, dry sweeping, and portable fan use should be avoided when possible [24]. Patient-facing clinicians should wear appropriate personal protective equipment (PPE), including gloves, gown, goggles or a face shield, and a respirator with at least N95 filters [23,24]. Patients with confirmed MPX should self-isolate until all lesions have formed a crust, the crust has separated, and new skin is covering the site [24].

Patients with rash illness consistent with MPXV should be monitored daily for 21 days following exposure until MPX is either confirmed or excluded (Table 1) [24]. If an individual has a high risk of exposure to someone with MPXV, self-monitoring for prodromal symptoms and development of rash illness is recommended for 21 days, with post-exposure prophylaxis implemented where appropriate [23,24].

Clinical management

MPXV clade IIb infections are generally mild and self-limiting; thus, supportive care is the mainstay of case management [15,23,24]. Secondary bacterial skin infections and ocular complications associated with MPXV should be treated when indicated, as the latter can lead to permanent scarring of the cornea and subsequent blindness [39,40]. For severe cases of MPXV infection, compassionate use of antiviral therapies including tecovirimat, cidofovir, or brincidofovir, may prove beneficial, although there are limited clinical data describing antiviral therapy for MPXV infection [24,41]. Additionally, vaccinia immune globulin intravenous (VIGIV) has been made available in the U.S. through the Strategic National Stockpile and may be used for prophylaxis in severely immunocompromised patients or for treatment of severe MPXV infections under the CDC’s expanded protocol [24]. However, there are currently no data describing the efficacy of VIGIV for the treatment of MPXV. Tecovirimat is the preferred agent, given its favorable side effect profile from initial studies, but a large multicenter clinical trial (ACTG A5418 STOMP) is currently ongoing to characterize the pharmacokinetics, safety, and efficacy of tecovirimat in adults, children, and pregnant women [42].

Pediatric considerations

Although there is a paucity of data on clade IIb pediatric MPXV infections, risk factors for severe disease caused by clade I within pediatric populations have included immunocompromised status; skin disorders, such as eczema; and age of <8 years [24,43]. Children infected with clade I and clade IIa virus had more severe disease than adults, with death occurring primarily in children [37,43]. MPX may be confused with more common rash illnesses in children, including varicella, HFMD, measles, herpes, scabies, molluscum contagiosum, and idiopathic or allergic urticaria. Depending on the care setting, laboratory testing for the etiologic agents of these more common rash illnesses may be available and even encouraged as part of the differential diagnosis. When pharmacologic intervention is necessary and warranted, tecovirimat is currently being used under an investigational protocol to treat infections with MPXV in high-risk pediatric populations [24,44]. In a single case report, a 28-month-old child was successfully treated for Orthopoxvirus infection with a combination of cidofovir, tecovirimat, and VIGIV; however, larger clinical studies are necessary to determine the safety and efficacy of these treatments in pediatric populations [45]. Close monitoring of renal function is recommended for pediatric patients treated with tecovirimat, especially for those under 2 years of age, as renal toxicity caused by exposure to a component of tecovirimat, hydroxypropyl-β-cyclodextrin, has been reported in animal models [46].

Pregnancy considerations

Current literature on MPXV in pregnancy raises concerns for miscarriage and intrauterine fetal demise. A systematic review of 7 cases found that miscarriage occurred in 2/7 cases and intrauterine fetal demise occurred in 3/7 cases [47]. The review is limited by the small number of cases but highlights the need for thorough surveillance of pregnant women with MPXV. Tecovirimat and vaccinia immune globulin can be considered as therapy, but both cidofovir and brincidofovir have shown teratogenicity in animal models [48].

Los Angeles County and University of Southern California experience

In the LAC+USC patient population, the clinical observations have been consistent with the current international reported literature for the 2022 clade IIb outbreak. An overwhelming majority of confirmed cases have been among HIV-positive MSM between ages 20 and 50. There have been no cases in children or women. Several co-infections have been present in a significant proportion of cases, particularly syphilis and HSV. In cases where MPXV testing has been negative, alternative diagnoses, including HSV, syphilis, and gonorrhea, have been made. Unlike the diffuse pox-like lesions characteristic of clade I infection, most patients with clade IIb disease have presented with less typical lesions: some like impetigo, some ulcerative, and some vesicular. The lesions do not appear to heal in the same stage as the classic teaching. Mild cases have been seen with perigenital involvement. Severe disease requiring hospitalization and refractory to oral tecovirimat has occurred in several patients with advanced AIDS. Intravenous tecovirimat can be considered when there is suspicion for poor oral drug absorption suggested by rectal lesions, diarrhea, and failure to improve on oral tecovirimat.

Laboratory Testing

Laboratory safety

Clinical specimens received in the laboratory from patients infected with MPX are considered infectious, and laboratory personnel are at risk of possible transmission during specimen handling and testing. Current best practices include the use of...
biosafety level 2 (BSL-2) facilities with BSL-3 work practices if BSL-3 facilities are not available [24]. These practices include conducting all specimen manipulation within a class II biosafety cabinet. Laboratory personnel must don appropriate PPE, including a gown and/or lab coat with cuffed sleeves, double gloves, eye protection, or face shield, and a respirator with at least N95 level filters, when manipulating clinical specimens suspected to contain MPXV [24,49]. Additional engineering controls should include manipulation of specimens within a room designed for unidirectional airflow. When possible, vaccinated laboratorians should perform specimen handling prior to nucleic acid extraction, at which point the viral DNA is considered non-infectious and can be manipulated using BSL-2 practices. All waste generated from specimen collection, nucleic acid extraction, and diagnostic testing should be decontaminated via autoclaving or chemical disinfection prior to on-site disposal [24]. In addition to the above-mentioned precautions, each laboratory should perform a site-specific risk assessment to determine the need for additional safety controls where appropriate.

 Diagnostic testing

Considering the expansive implementation of molecular diagnostic assays in the past 3 decades, it is no surprise that real-time PCR (RT-PCR) detection of MPXV has surpassed other testing methodologies in light of recent outbreaks (Table 3). For RT-PCR MPXV testing, the most appropriate clinical specimen is a lesion swab. Acceptable swabs may be dry or stored in universal or viral transport medium (VTM), depending on the testing facility. To increase diagnostic sensitivity, two swabs from two separate lesions should be collected. Lesions must be swabbed vigorously, de-roofing the lesion if possible, to ensure adequate transfer of DNA-containing material. Inclusion of an endogenous internal control, such as RNase P DNA, allows clinical laboratories to determine the appropriateness of collection, as RNase P is found in mammalian cells.

**Table 3. Summary of MPX laboratory testing options**

| Test | Principle | Specimen type | Utility | Limitations |
|------|-----------|---------------|---------|-------------|
| Antibody testing | Detection of anti-orthopoxvirus IgG via ELISA/LFI | Plasma | May aid in determining previous exposure to an orthopoxvirus or smallpox vaccination | Not specific to MPXV exposure; subject to large inter-individual responses in antibody titer at time of testing |
| | Detection of anti-orthopoxvirus IgM via ELISA/LFI | Plasma | May aid in determining acute response to a recent orthopoxvirus exposure, ideally prior to vaccination | Not specific to MPXV exposure; subject to large inter-individual responses in antibody titer at time of testing |
| Electron microscopy | Visualization of poxvirus following negative staining | Lesion biopsy specimen | Useful for identification of viral particles consistent with orthopoxvirus directly from histology specimens | Requires highly specialized equipment and personnel; high cost barrier to entry for equipment; resolution limited to Orthopoxvirus genus level |
| Immunohistochemistry | Enzyme- or fluorophore-linked antibodies bind to orthopoxvirus antigens. | Lesion biopsy specimen | Can be used to identify orthopoxvirus antigen directly from histology specimens | Not specific to MPXV; interpretation and reporting require highly trained personnel. |
| Point of care | Antibody capture and detection of orthopoxvirus antigens via LFI | Lesion swabs, scabs, or tissue | May prove useful in the context of limited diagnostic resources | Poor sensitivity and negative predictive value |
| PCR<sup>a</sup> | Amplification and detection of MPXV DNA in real time | Lesion swab or rectal swab, depending on laboratory (specific to clade IIb outbreaks) | Highly sensitive and specific; can be multiplexed to detect non-MPXV orthopoxvirus DNA or other viral targets | Limited sites performing testing; high cost barrier to entry for equipment |
| Viral culture | Isolation and propagation of MPXV from clinical specimens in vitro | Lesion swab | No utility for routine clinical diagnostic purposes; may prove useful in epidemiological or research settings | Not currently recommended as a routine diagnostic procedure in clinical laboratories; should be performed only by vaccinated personnel under BSL-3 conditions |

<sup>a</sup>Adapted from McCollum and Damon [61]. ELISA, enzyme-linked immunosorbent assay; LFI, lateral-flow immunoassay.

<sup>b</sup>Currently considered the gold standard testing methodology.
The CDC’s published protocol for MPXV testing is based on two publications by Li et al. [50,51]. In the former study, the authors designed a TaqMan-based RT-PCR assay with primer/probe sequences targeting the non-variola orthopoxvirus DNA polymerase gene, E9L, in addition to MPXV-specific primer/probe sequences targeting the extracellular enveloped virus protein gene, B6R [50]. This assay was later revised to differentiate between clade I and clade II MPXV—a non-trivial task, considering the two clades share 99% sequence identity [51]. The authors were able to achieve clade-specific resolution by targeting the tumor necrosis factor (TNF) receptor gene located within the terminal inverted-repeat region, which contains many single nucleotide polymorphisms and insertions/deletions between the two clades [51]. A recent study describes 100% sensitivity and specificity from lesion crusts and 98.3% sensitivity with 100% specificity from lesion vesicles when comparing the CDC-developed assay to the GeneXpert platform (Cepheid, Sunnyvale, CA), although performance may vary by institution [52].

Additional considerations for RT-PCR LDTs include designing primers to avoid primer dropout in rare cases of TNF receptor gene deletion—the target of the CDC’s published protocol [24,50,51,53]. Although this deletion is rare, at least three cases of TNF receptor gene deletion have been reported in California. Fortunately, these cases were still correctly diagnosed, as testing was performed using the CDC protocol, which targets non-variola orthopoxvirus in addition to the TNF receptor gene of MPXV. Consequently, LDTs should ideally be multiplexed to include primers and probes targeting non-variola orthopoxviruses, MPXV genes essential for viral genes less susceptible to mutation, multiple viral genes, and/or primer/probe sequences that distinguish between clade I and clade II MPXV.

To rule in or out more common rash illnesses, the diagnostic workup for MPXV should ideally include concomitant tests for HSV, VZV, and ENV. If nucleic acid amplification assays, such as RT-PCR, are not available, additional testing methodologies may be considered, depending on the clinical indication and availability of laboratory resources (Table 3). For example, although antibody detection alone should not be used for confirmatory diagnosis, IgM detection from acutely ill patients or IgG detected in paired serum samples collected more than 21 days apart may aid in diagnosis should other testing methodologies prove inconclusive or not be available [49]. As with any antibody test, recent vaccination may interfere with the interpretation of test results. In the interest of diagnostic stewardship, a high index of clinical suspicion for MPXV infection should precede laboratory testing, regardless of the testing methodology. To this point, and in an effort to prevent reporting of false-positive results, the CDC currently recommends re-extraction and re-testing when a cycle threshold value of ≥34 is encountered in patients failing to meet epidemiological criteria for MPX [54].

**Regulatory requirements**

Testing requirements vary by country and region; this review aims to summarize the current regulatory requirements within the U.S. On 7 September 2022, the U.S. Food and Drug Administration (FDA) issued a guidance document for laboratories and commercial manufacturers regarding regulatory requirements for MPXV testing [55]. According to the document, Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories are allowed to develop and perform in vitro diagnostic testing for MPXV so long as the following requirements are met: (i) the assay utilizes PCR technology, (ii) the assay utilizes lesion swabs, (iii) the assay has been appropriately validated, and (iv) the laboratory notifies the FDA of validation within 5 business days of offering the test or within 5 days of the FDA guidance document issuance if testing was already performed [55]. So long as the preceding criteria are met, the FDA does not currently object to MPXV testing by CLIA-certified laboratories, and thus, EUA is not required at this time. Large commercial laboratories performing high-throughput MPXV testing are currently receiving prioritized review of EUA requests.

The CDC MPXV RT-PCR protocol mentioned above is currently the only FDA-cleared diagnostic test for non-variola orthopoxviruses, including MPXV. However, this protocol is limited by restrictive acceptable specimen types, including dry swabs or swabs collected in VTM from lesions or lesion crusts [24]. Consequently, additional specimen types in transport media must be independently validated by each laboratory performing MPXV testing. To date, only one laboratory has been granted FDA EUA for MPXV testing in the U.S. [56].

The CDC’s Division of Select Agents and Toxins has issued guidance on the regulatory status of MPXV stating that MPXV is considered an HHS-only select agent unless there is an applicable exclusion, such as clade II identification [57]. If material within the laboratory has been identified as being or containing MPXV but the clade has not been determined or the MPXV belongs to clade I, the laboratory must be registered with the Federal Select Agent Program (FSAP) and be approved to possess MPXV [57]. Moreover, materials confirmed to contain *Orthopoxvirus* that are presumptively, but not definitively, identified as MPXV are not regarded as select agents by the FSAP until identification of MPXV is confirmed [57]. These abstruse guidelines must be considered when developing laboratory tests to detect MPXV, as an assay designed to detect *Orthopoxvirus* without clade differentiation may be subject to select agent regulations. The regulatory guidelines for select agent designation, and more broadly those of diagnostic testing in general, exist as dynamic entities with ever-changing recommendations, requirements, and legal status, placing a burden on laboratories to quickly interpret and adapt to such requirements.

**Conclusions**

The recent outbreaks of MPXV clade IIIb infections continue to test the clinical, epidemiological, and public health knowledge gained from the omnipresent SARS-CoV-2 pandemic. Clinical laboratories have adapted quickly in the wake of the MPXV public health emergency, developing LDTs and modifying their workflows to detect MPXV. However, the limited availability of testing and vaccines is reminiscent of that experienced during the early
days of the SARS-CoV-2 pandemic. As the outbreaks continue, there are several remaining points of inquiry:

- The extent to which pregnant women and children are at risk of severe MPXV infection, as has been reported from clade I infections
- The transmissibility of MPXV through oral and respiratory secretions, urine, feces, semen, and vaginal secretions
- The transmissibility of MPXV from asymptomatic MPXV cases
- The efficacy of various pharmacological antiviral therapies in the clinical management of severe MPXV cases in both adult and pediatric population
- The efficacy of smallpox vaccination for the prevention of MPX, particularly when one-fifth of a typical dose is administered intradermally.

References

[1] International Committee on Taxonomy of Viruses. Current ICTV taxonomy release, https://ictv.global/taxonomy; n.d. [accessed 23 August 2022].

[2] Loeffelholz MJ, Hodinka RL, Young SA, Pinsky BA. Clinical virology manual. 5th ed. Washington, DC: ASM Press; 2016.

[3] Ladhnyi JD, Ziegler P, Kim E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. Bull World Health Organ 1972;46:593-7.

[4] Reynolds MG, Damon IK. Outbreaks of human monkeypox after cessation of smallpox vaccination. Trends Microbiol 2012;20:80-7. https://doi.org/10.1016/j.tib.2011.12.001.

[5] Liu L. Fields virology, 6th edition. Clin Infect Dis 2014;59:613. https://doi.org/10.1093/cid/ciu346.

[6] Likos AM, Sammons SA, Olson VA, Frace AM, Li Y, Olsen-Rasmussen M, et al. A tale of two clades: monkeypox viruses. J Gen Virol 2005;86:2661-72. https://doi.org/10.1099/vir.0.81215-0.

[7] World Health Organization. Monkeypox: expert gives virus variants new names, https://www.who.int/news/item/12-08-2022-monkeypox-experts-give-virus-variants-new-names; n.d. [accessed 23 August 2022].

[8] Fine PE, Jezek Z, Grab B, Dixon H. The transmission potential of monkeypox virus in human populations. Int J Epidemiol 1988;17:643-50. https://doi.org/10.1093/ije/17.3.643.

[9] Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, et al. The changing epidemiology of human monkeypox—a potential threat? A systematic review. PLOS Negl Trop Dis 2022;16:e0010141. https://doi.org/10.1371/journal.pntd.0010141.

[10] Jezek Z, Khodakevich LN, Wickett JF. Smallpox and its post-eradication surveillance. Bull World Health Organ 1987;65:425-34.

[11] Petersen E, Kantele A, Koopmans M, Asogun D, Yinka-Ogunleye A, Ihekweazu C, et al. Human monkeypox: epidemiologic and clinical characteristics, diagnosis, and prevention. Infect Dis Clin North Am 2019;33:1027-43. https://doi.org/10.1016/j.idc.2019.03.001.

[12] Durski KN. Emergence of monkeypox—West and Central Africa, 1970-2017. MMWR Morb Mortal Wkly Rep 2018;67:306-10. https://doi.org/10.15585/mmwr.mm6710a5.

[13] Sklenovská N, Van Ranst M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. Front Public Health 2018;6:241. https://doi.org/10.3389/fpubh.2018.00241.

[14] Rimoin AW, Mulembakani PM, Johnston SC, Lloyd Smith JO, Kisalu NK, Kinkelaa TL, et al. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. Proc Natl Acad Sci 2010;107:16262-7. https://doi.org/10.1073/pnas.1005769107.

[15] World Health Organization. Monkeypox, https://www.who.int/news-room/fact-sheets/detail/monkeypox; n.d. [accessed August 17, 2022].

[16] Formenty P, Muntasir MO, Damon IK, Chowdhary V, Opoka ML, Monimart C, et al. Human monkeypox outbreak caused by novel virus belonging to Congo Basin clade, Sudan, 2005. Emerg Infect Dis J 2010;16:1539-45. https://doi.org/10.3201/eid1610.100713.

[17] Giulio DBD, Eckburg PB. Human monkeypox: an emerging zoonosis. Lancet Infect Dis 2004;4:15-25. https://doi.org/10.1016/S1473-3099(03)00856-9.

[18] World Health Organization. World Health Organization best practices for the naming of new human infectious diseases, https://www.who.int/publications-detail-directive/WHO-HSE-FOS-15.1; n.d. [accessed 24 August 2022].

[19] Kraemer MUG, Tegally H, Pigott DM, Dasgupta A, Sheldon J, Wilkinson E, et al. Tracking the 2022 monkeypox outbreak with epidemiological data in real-time. Lancet Infect Dis 2022;22:941-2. https://doi.org/10.1016/S1473-3099(22)00359-0.

[20] Department of Health and Human Services, Assistant Secretary for Public Affairs. Biden-Harris administration bolsters monkeypox response; HHS Secretary Becerra declares public health emergency. https://www.hhs.gov/about/news/2022/08/04/biden-harris-administration-bolsters-monkeypox-response-hhs-secretary-becerra-declares-public-health-emergency.html; 2022 [accessed 30 August 2022].

[21] World Health Organization. WHO director-general declares the ongoing monkeypox outbreak a public health emergency of international concern. https://www.who.int/news/item/23-07-2022-who-director-general-declares-the-ongoing-monkeypox-outbreak-a-public-health-event-of-international-concern; n.d. [accessed 30 August 2022].

[22] Ferré VM, Bachelard A, Zaidi M, Armand-Lefevre L, Descamps D, Charpentier C, et al. Detection of monkeypox virus in anorectal swabs from asymptomatic men who have sex with men in a sexually transmitted infection screening program in Paris, France. Ann Intern Med 2022;175:1491-2. https://doi.org/10.7326/M22-2183.

[23] Titanji BK, Tegomoh B, Nematollahi S, Konomos M, Kulkarni PA. Monkeypox: a contemporary review for healthcare professionals. Open Forum Infect Dis 2022;9:ofac310. https://doi.org/10.1093/ofid/ofac310.

[24] Centers for Disease Control and Prevention. Monkeypox in the U.S., https://www.cdc.gov/poxvirus/monkeypox/transmission.html; 2022 [accessed 24 August 2022].

[25] Pfeiffer JA. High-contact object and surface contamination in a household of persons with monkeypox virus infection—Utah, June 2022. MMWR Morb Mortal Wkly Rep 2022;71:1091-4. https://doi.org/10.15585/mmwr.mm7134e1.

[26] 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 2022;387:679-91. https://doi.org/10.1056/NEJMoaa2207323.

[27] Dashraath P, Nielsen-Saines K, Mattar C, Musso D, Tambyah P, Baud D. Guidelines for pregnant individuals with monkeypox virus infection screening program in Paris, France. COVID-19 vaccine and monkeypox virus infection screening program in Paris, France. Lancet 2022;8:400-21.2. https://doi.org/10.1016/S1473-3099(22)00363-7.

[28] Mhala PK, Huggins JW, Riu-Rovira T, Ahuka SM, Mulembakani P, Rimoin AW, et al. Maternal and fetal outcomes among pregnant women with human monkeypox infection in the Democratic Republic of Congo. J Infect Dis 2017;216:824-8. https://doi.org/10.1093/infdis/jix260.
[59] Peiró-Mestres A, Fuertes I, Camprubi-Ferrer D, Marcos MÁ, Vilella A, Navarro M, et al. Frequent detection of monkeypox virus DNA in saliva, semen, and other clinical samples from 12 patients, Barcelona, Spain, May to June 2022. Euro Surveill 2022;27:1-5. https://doi.org/10.2807/1560-7917.ES.2022.27.28.2200503.

[60] Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, et al. Outbreak of human monkeypox in Nigeria in 2017-18: a clinical and epidemiological report. Lancet Infect Dis 2019;19:872-9. https://doi.org/10.1016/S1473-3099(19)30294-4.

[61] McCollum AM, Damon IK. Human monkeypox. Clin Infect Dis 2014;58:260-7. https://doi.org/10.1093/cid/cir703.