



DHS SCIENCE AND TECHNOLOGY

Master Question List for Monkeypox Virus (MPXV)

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Science and
Technology

Monkeypox Virus – Master Question List

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FOREWORD

The following Master Question List (MQL) was developed by the Department of Homeland Security Science and Technology Directorate (DHS S&T) to present the current state of available information to government decision makers in the operational response to the 2022 monkeypox virus (MPXV) outbreak. This MQL quickly summarizes what is known and what additional information is needed to address fundamental questions such as, “What is the infectious dose?” and “How long does the virus persist in the environment?” The information provided is a succinct summary to allow structured and scientifically guided discussions across the federal government without burdening them with the need to review scientific reports, and to prevent duplication of efforts by highlighting and coordinating research.

SITUATION OVERVIEW

Monkeypox virus is a zoonotic virus (a virus that originates in animals) that causes symptoms similar to but less severe than smallpox, which was eradicated in 1980. The virus is native to Africa, but cases are occasionally exported to Europe and North America. Since May 13, 2022, monkeypox cases have been reported in multiple countries, including the United States. The virus that is causing the present outbreak is a member of a subgroup of monkeypox viruses associated with less severe disease than other groups of monkeypox viruses. Historically, this subgroup of monkeypox viruses has not generally been associated with significant human-to-human transmission. However, this has not been the case in the current outbreak. To date, most but not all cases have been reported in men who have sex with men (MSM), and the location of the lesions suggests that sexual transmission has had a significant role in the spread of the disease. There is reason to suspect that this previously unseen mode of transmission may be contributing to increased transmission rates.

The cutoff date for information-gathering related to this document was 07/11/2022.

Major Findings by Topic Area	
Topic	Overview of Current Knowledge
VIRUS BACKGROUND	<ul style="list-style-type: none"> • Monkeypox virus (MPXV) belongs to the same group of viruses as the Variola major virus, which causes the human disease known as smallpox and was declared eradicated in 1980. This group also includes vaccinia virus (an attenuated poxvirus used in the smallpox vaccine), horsepox virus, and cowpox virus. These viruses, known as orthopoxviruses (OPVs), are very large viruses with DNA genomes. • MPXV causes a disease very similar to, but generally less severe than smallpox. • There are at least two broad groups (clades) of MPXVs. <ul style="list-style-type: none"> • Virologists use the term “clade” to describe a group of viruses that have very similar genetic sequences and are different enough from other viruses of the same species that they form a distinct genetic cluster. • The Congo Basin clade is found in Central Africa. About 10% of cases are fatal. • The West African clade includes the virus responsible for the current outbreak, and is associated with a lower death rate, about 1%. In the past, viruses of this clade have also been less transmissible than Congo Basin clade viruses.
CLINICAL PRESENTATION	<ul style="list-style-type: none"> • Early presentation consists of fever, fatigue, headache, backache, mild to severe pulmonary lesions, anorexia, dyspnea, conjunctivitis, nasal discharge, swollen lymph nodes, chills and/or sweats, sore throat, cough, and shortness of breath. • Rash presents within 1-4 days of onset of symptoms and lasts from 2-4 weeks. • Rash is typically confined to the trunk but may appear on the palms and soles of feet. Lesions can develop on mucous membranes, in the mouth, on the tongue, and on the genitalia. • In the current outbreak, lesions on the genitalia and perianal region have been more common due to the role of sexual transmission, and early presentation has not always included fever or other typical early symptoms of monkeypox. • Some patients in the current outbreak have presented without fever. • These unusual presentations can lead to misdiagnosis as a more common sexually transmitted infection such as syphilis, chancroid, or herpes. • Swollen lymph nodes (lymphadenitis) are a feature of monkeypox disease not seen in smallpox.
INFECTIOUS DOSE	<ul style="list-style-type: none"> • The infectious dose of monkeypox virus in humans is unknown. • Based upon studies in non-human primates (NHPs), the infectious dose via inhalation is estimated to be between 10 and 10,000 infectious viral particles. Most of these studies were conducted with the Congo Basin MPXVs. • West African MPXVs have generally been found to be less infectious than Congo Basin MPXVs.

Major Findings by Topic Area	
Topic	Overview of Current Knowledge
TRANSMISSIBILITY	<ul style="list-style-type: none"> • The virus enters the body through broken skin, the respiratory tract, and other non-respiratory mucous membranes. • Human-to-human transmission is thought to occur primarily through respiratory droplets, direct contact with body fluids/lesion material, and fomites contaminated with lesion material. <ul style="list-style-type: none"> • Based on available evidence, rates of droplet transmission in this outbreak do not appear to be different from prior outbreaks. • New research suggests that the current outbreak strain may have accumulated mutations that increase its transmissibility. However, there is not yet enough evidence to suggest that these dramatically increase the R_0 of the virus. • The basic reproductive number (R_0) of monkeypox is generally estimated to be between 0.57 to a maximum of 1.25. However, an R_0 for the current outbreak has not been determined. • Evidence suggests that the transmission rate of MPXV has increased over time due to declining immunity in the population after the end of smallpox vaccination in the general public. • Direct contact among men who have sex with men (MSM) currently has been cited as a source of a significant number of the infections in the current outbreak.
HOST RANGE	<ul style="list-style-type: none"> • MPXV is a zoonotic disease and outbreaks are initiated by human contact with animals. • The primary reservoir of the virus is unknown but is likely one or more species of rodent. • NHPs can be intermediate hosts but are not likely to be reservoir hosts. • It is not known if rodent species native to the United States could serve as reservoir hosts, though several can become infected.
INCUBATION PERIOD	<ul style="list-style-type: none"> • The interval between exposure and the development of symptoms ranges from 1-31 days, with 7-17 days being the typical range. • Patients are contagious during the first week of the rash and may continue shedding virus for weeks after symptoms have dissipated.
CLINICAL DIAGNOSIS	<ul style="list-style-type: none"> • Although no fully US FDA-approved MPXV-specific assays exist, the US CDC has developed an FDA-cleared test for provisional use via the public health Laboratory Response Network. Currently this capacity includes 67 laboratories in 48 states. • Five commercial laboratory companies have received authorization to use the CDC test and have begun receiving testing materials. • Within the United States, culture-based diagnostics should only be performed by the Centers for Disease Control and Prevention (CDC) per CDC guidelines. • Quantitative polymerase chain reaction (qPCR)-based detection is recommended by CDC, and optimal samples include scab and lesion material.

Major Findings by Topic Area	
Topic	Overview of Current Knowledge
	<ul style="list-style-type: none"> • Tests based on detection of antibodies against MPXV (enzyme-linked immunosorbent assays [ELISA]) can be performed but are of limited utility in the early phase of the disease. • The current CDC case definition requires that a patient be PCR, sequencing, or culture positive to be considered a confirmed case.
MEDICAL TREATMENT	<ul style="list-style-type: none"> • Tecovirimat and brincidofovir have been approved by the U.S. FDA for treatment of smallpox virus under the animal rule. • In a published case study, tecovirimat was found to reduce hospitalization time by more than half in a treated patient with monkeypox. • Cidofovir is effective against other poxviruses (including vaccinia and smallpox) in <i>in vitro</i> and animal studies. • Vaccinia immune globulin (Ig) is a potential treatment option. • Cidofovir, tecovirimat, and vaccinia Ig administered intravenously are treatment options in the Strategic National Stockpile (SNS). • When used as post-exposure prophylaxis, smallpox vaccination should be administered within 3 days of exposure for maximal effect but may still attenuate disease if administered as late as 2 weeks post-exposure.
VACCINES	<ul style="list-style-type: none"> • Vaccination with smallpox vaccine (vaccinia virus) is reported to provide protection against 85% of MPXV infections. • Two OPV vaccines are licensed by the FDA for use in the United States. • ACAM2000 (Emergent) is a live vaccinia virus vaccine that can be used for MPXV although it is not specifically licensed by the FDA for this indication. • JYNNEOS (Bavarian Nordic A/S) is a two-dose non-replicating Modified Vaccinia Virus Ankara (MVA) vaccine that can be given to people for whom live vaccinia vaccines are not safe. • JYNNEOS is specifically licensed for MPXV by the FDA in addition to licensure for smallpox. • ACAM2000 and JYNNEOS are maintained in the SNS, along with Aventis Pasteur Smallpox Vaccine, another live vaccinia vaccine that would be used under an emergency use authorization (EUA) or as an investigational new drug (IND) in an emergency.
ENVIRONMENTAL STABILITY	<ul style="list-style-type: none"> • MPXV, like all OPVs, is very stable in the environment. These viruses can be stable for days to weeks under some circumstances. • MPXV can survive in scabs for months to years. • MPXV is resistant to desiccation in hot and cold environments. • MPXV may be stable for days to weeks in water, soil, and on refrigerated food. • MPXV is susceptible to inactivation under acidic conditions.
DECONTAMINATION	<ul style="list-style-type: none"> • U.S. Environmental Protection Agency recommends the use of bleach and a number of quaternary ammonium reagents for use against emerging viral pathogens.

Major Findings by Topic Area	
Topic	Overview of Current Knowledge
	<ul style="list-style-type: none"> Data demonstrating effectiveness against MPXV are not available for most common disinfectants, however testing with vaccinia virus (a close relative) suggests that bleach, Virkon, Dettol, and Sanytex, are effective.
PERSONAL PROTECTIVE EQUIPMENT (PPE)	<ul style="list-style-type: none"> Optimal PPE for clinicians caring for infected patients includes disposable gown and gloves, National Institute for Occupational Safety and Health (NIOSH)-certified N95 (or comparable) filtering disposable respirator, and face shield or goggles. Laboratory studies with MPXV require Biosafety Level-3 precautions. These laboratories have enhanced safety precautions (such as the use of respirators) and higher levels of containment (e.g. HEPA filtration of all exhaust air) to avoid laboratory staff exposure or accidental release of a pathogen.
GENOMICS	<ul style="list-style-type: none"> MPXV is a DNA virus with a genome more than 10X larger than that of SARS-CoV-2. WHO is currently considering a nomenclature change in which the Congo Basin clade would be renamed “Clade 1”, with the West African clade split into “Clade 2” and “Clade 3.” Like the other viruses it is related to, MPXV evolves very slowly. Its genome changes 100-1,000 times slower than that of SARS-CoV-2. There is some recent research that suggests that the virus responsible for the current outbreak may be evolving slightly faster than normal, but still significantly slower than other viruses like influenza and SARS-CoV-2. This faster evolutionary rate may be responsible for the accumulation of mutations that slightly enhance transmission of the virus, but more work is needed to clearly demonstrate this. Congo Basin clade MPXVs are regulated as U.S. Department of Health and Human Services (HHS) Select Agents by the CDC Federal Select Agents Program. West African clade MPXVs are not Select Agents due to their lower severity/lethality.

Infectious Dose – How much agent will make a healthy individual ill?

What do we know?

- The infectious dose of MPXV in humans by any route is unknown.
- The estimated infectious dose (the dose required to cause any infection, not necessarily death) is $<10^1$ - 10^4 infectious viral particles (plaque-forming units, PFU) in various animal models via intravenous, oral, intranasal, inhalation/aerosol, intradermal, and cutaneous routes. However, only aerosol infectious doses have been tested in non-human primates.¹⁻²
- The estimated median dose required to cause a lethal infection (median lethal dose or LD₅₀) is variable and has been estimated to be 10^5 - 10^7 PFU in NHPs, depending on route of exposure.¹⁻⁴
 - Across multiple animal models and exposure routes, the Congo Basin/Central Africa clade MPXV has generally been shown to have a lower lethal dose and higher mortality rate than the West Africa clade MPXV. Exposure doses in these studies ranged from 10 - 10^7 PFU.⁵
 - In an aerosol study with cynomolgus macaques, the inhalation LD₅₀ for the ZaireV79-I-005 strain (Congo Basin clade) of MPXV was determined to be between 10^4 and 10^5 PFU.⁶
- Cynomolgus macaques infected with monkeypox are considered to currently be the best animal model for studying human smallpox and its treatments.⁷

What do we need to know?

- While cynomolgus macaques infected with monkeypox are considered to currently be the best animal model for smallpox in humans, no NHP model exists that perfectly represents human disease arising from OPV infection.⁷
- The infectious dose in humans by relevant routes.
- Additional studies are needed to develop improved animal models and an understanding of their correlation to human infection and disease.

Transmissibility – How does it spread from one host to another? How easily is it spread?

What do we know?

- Transmission of MPXV occurs when a person comes into contact with the virus from an animal, human, or materials contaminated with the virus. The virus may enter the body through broken skin, the respiratory tract, or other mucous membranes such as the rectum, eyes, genitals and oral cavity.⁸⁻¹¹
- Human-to-human transmission is thought to occur primarily through respiratory droplets, direct contact with body fluids or lesion material, and indirect contact with lesion material via contaminated fomites.⁸⁻¹¹
 - Rates of droplet transmission in this outbreak do not appear to be different from prior outbreaks.^{8-10, 12}
- MPXV can be spread by direct contact with infected animals and humans,¹³⁻¹⁵ including a bite, scratch, or handling of an infected animal and exposure to excretion or secretion from an infected animal.¹⁶
- Person-to-person transmission has been reported in previous outbreaks but was not common.¹⁷⁻¹⁹
 - West African MPXV infection has historically been rarely associated with human-to-human transmission.²⁰ However, up to six sequential person-to-person Congo Basin MPXV transmissions have been documented prior to this outbreak.^{9, 21} Approximately 3-10% of those in close contact with MPXV cases have been infected in prior outbreaks.^{17, 22-24}

- Person-to-person transmission has been more common in the current outbreak than in the past. The mechanism for this is unknown, but may be related to population-specific behaviors that increase risk.²⁵
- The basic reproduction number (R_0) for MPXV, indicating the average number of new infections arising from one infectious individual in an entirely susceptible population, is estimated to range from 0.57 to 0.96.²⁶⁻²⁷
 - R_0 is a mathematical estimate of the average number of new individuals that a single infected individual will infect in a population without immunity. If this number is less than one, the infection will quickly burn out. If it is greater than one, the infection can spread and cause an epidemic if not controlled.
 - A maximum R_0 value of 1.25 was estimated from data on smallpox cross-immunity in the Democratic Republic of the Congo.¹⁰
- It is believed that transmission rate of MPX has increased through time, coincident with reductions in the fraction of the population with vaccine-derived immunity to smallpox.¹⁰
- The R_0 of the current outbreak is not currently known. Several aspects of the current outbreak, including transmission between MSM, are expected to complicate efforts to estimate R_0 and may make previous estimates unreliable for this outbreak.²⁵
- Mutations have been identified in the current outbreak strain that may enhance transmissibility relative to previous West African clade viruses, but these are unlikely to dramatically increase the R_0 of the virus.²⁸
- Monkeypox is transmissible between both animals and humans. Humans can be contagious before a visible rash appears and can continue shedding the virus weeks after symptoms have dissipated.^{8-11, 25, 29-30}
- Sources of laboratory-acquired infections include exposure to aerosols, environmental samples, naturally or experimentally infected animals, infectious cultures, or clinical samples, including lesion fluid or crusted scabs, tissue specimens, excretions, and respiratory secretions.³¹
- A case study of 81 healthcare workers reported no signs or symptoms consistent with monkeypox following exposure to infected patients, indicating transmission in a healthcare setting is unlikely when infection control measures are taken.³²

What do we need to know?

- What is the R_0 of the current outbreak and how it is impacted by the association with the MSM community?
- Is transmissibility affected by route of exposure? That is, would an individual infected via the aerosol route be more infectious than a person infected via direct contact?
- Is the apparent increase in person-to-person transmission in the current outbreak related to viral factors, sociological factors, or both?
- Do these factors impact decisions about the types of public health interventions needed to stop an outbreak?
- Improve understanding of the ultimate impact of cessation of smallpox vaccination on the rate of person-to-person transmission.¹⁴
- What is the role of droplet/respiratory transmission in the current outbreak vs. other routes?
- Improve surveillance and epidemiological analysis to better assess public health burden and develop strategies to reduce widespread disease.¹⁴
- Is there is a clade-dependent difference in transmission?³³

Host Range – How many species does it infect? Can it transfer from species to species?

What do we know?

- Monkeypox is a zoonotic disease with transmission from animals (e.g., NHPs and rodents) to humans.^{13, 34}
 - Primary reservoir is unknown but is likely rodents. The Gambian pouched rat and rope squirrels are of particular interest.^{16, 24, 34-38}
 - The name monkeypox comes from the fact that the virus was first isolated from a monkey. Although NHPs are often intermediate hosts, they are not likely to be the reservoir.^{19, 34, 39}
 - Other potential hosts include multiple rodent species (prairie dogs, rabbits, porcupines, hamsters), opossums, anteaters, and hedgehogs.^{13, 24, 39-42}
- In 2003, humans became infected with West African monkeypox after having contact with infected prairie dogs purchased as pets;⁴³ these animals had been housed near a number of infected African rodents prior to being sold as pets. Touching a sick animal, receiving a bite or scratch that broke the skin, and cleaning the bedding of a sick animal led to higher likelihood of human infection. Symptoms in animals included fever, cough, discharge from the eyes, enlarged lymph nodes, lack of eating/drinking, and lethargy.⁴³
- CDC instructs veterinarians to consider all mammals susceptible to monkeypox due to the wide variety of animals shown to exhibit monkeypox infection, and the lack of information regarding the types of animals that may become ill.³⁰

What do we need to know?

- Which animal hosts (including new animal reservoirs) outside of Central Africa are capable of harboring disease and what is the ease of transmission?¹⁴
- What is the potential for monkeypox to become an ongoing disease outside of West and Central Africa, such as by establishing a reservoir within native North American species?
- Are household pets (i.e., cats, dogs, guinea pigs), agricultural animals or zoo animals susceptible to MPXV?

Incubation Period – How long after infection do symptoms appear? Are people infectious during this time?

What do we know?

- Mean incubation period is 7-17 days (range of 1-31 days).^{9, 18, 36, 44}
 - Time from exposure to fever onset ranges from 10-14 days, and from exposure to rash onset ranges from 12-16 days.³⁷
 - In the 2003 U.S. monkeypox outbreak (West African clade), the median incubation period was 12 days (range 2-26 days).^{18, 45}
 - In the 2017-2018 human monkeypox outbreak in Nigeria (West African), the time from first contact to disease onset ranged from 3 days to 34 days (mean 13 [SD 9]; median 9.5 days [interquartile range 11]).⁴⁶
 - Black-tailed prairie dogs infected via intranasal and intradermal routes with MPXV at $10^{4.5}$ PFU continued shedding viable virus for up to 21 days post-infection.³³
- Patients are contagious during the first week of the rash,^{36, 44} and direct contact should be prevented until lesions have crusted.⁴⁷
- Humans can be contagious before a visible rash appears and can continue shedding the virus weeks after symptoms have dissipated.^{8-11, 25, 29-30}

What do we need to know?

- What is the degree of infectivity of host during incubation prior to onset of symptoms?
- Does the incubation period depend on route of exposure or exposure dose?

Clinical Presentation – What are the signs and symptoms of an infected person?

What do we know?

- Human disease associated with West African MPXV infection is less severe and is associated with <1% mortality, whereas Congo Basin MPXV infection has a 10% case fatality rate in unvaccinated persons.²⁰ Case fatality rate can range from 0-17%, depending on vaccination status and age.^{19, 23, 36-37, 48}
- The disease typically presents with a short prodromal phase with influenza-like illness before classical MPXV disease symptoms such as rash appear.^{9, 16, 19, 44, 49-50}
- Prodromal period (lasts 1-4 days): fever, fatigue, headache, backache, mild to severe pulmonary lesions, anorexia, dyspnea, conjunctivitis, nasal discharge, swollen lymph nodes, chills and/or sweats, sore throat, cough, and shortness of breath.^{9, 16, 19, 44, 49-50}
- Following the prodromal phase, mild to severe rash/lesions may appear, lasting 2-4 weeks. The rash is typically confined to the trunk but may appear on the palms and soles of feet. Lesions can develop on mucous membranes, in the mouth, on the tongue, and on the genitalia.^{9, 16, 19, 44, 50}
- In the current outbreak, lesions on the genitalia and perianal lesions have been more common due to the outsized role of sexual transmission.^{25, 51}
- In the current outbreak, disease has not always been accompanied by classical prodromal symptoms and may present as a rash with typical lesions with or without perceptible fever. In combination with the genital and perianal presentation of lesions in many cases, this may lead to misdiagnosis via confusion with sexually transmitted infections such as syphilis, chancroid, and herpes.^{25, 51}
- The major clinical features of human monkeypox are similar to those of smallpox;¹⁹ however, lymphadenopathy (lymph nodes with abnormal size, number, or consistency) is a key distinguishing feature of monkeypox.^{35, 44}
- In prior outbreaks, children (less than 10 years of age) have been affected more frequently than adults.^{17, 19}

What do we need to know?

- Why are atypical clinical presentations being observed in some patients during the current outbreak?
- How is clinical presentation affected by route of exposure?
- Assess mortality and complications associated with MPXV infection.¹⁴
- Can the current monkeypox disease case definition be refined to improve the ability of health care workers and others to differentiate monkeypox disease from other diseases caused by poxviruses, including smallpox?

Clinical Diagnosis – Are there tools to diagnose infected individuals? When during infection are they effective?

What do we know?

- Although no fully US FDA-approved MPXV-specific assays exist, the US CDC has developed an FDA-cleared test for provisional use via the public health Laboratory Response Network. Currently this capacity includes 67 laboratories in 48 states.⁵²
 - Five commercial laboratory companies have received authorization to use the CDC test and have begun receiving testing materials.⁵²
- Within the United States, local or state health departments should be contacted to inquire about diagnostic testing prior to contacting the CDC.^{11, 53}
- Within the United States, culture-based diagnosis should only be attempted by the CDC.^{11, 53}
- The current US CDC case definition requires that a patient be PCR, sequencing, or culture positive to be considered a confirmed case.⁵⁴

- Polymerase chain reaction (PCR)-based diagnostics are the most reliable diagnostic tools for MPXV.^{11, 53}
 - PCR-based methods can be used on scab or vesicle material samples with or without virus isolation or propagation.^{14, 16, 41, 48, 55-57}
 - Additionally, PCR-based methods are effective during acute illness¹⁵ and can differentiate between MPXV Congo Basin and MPXV West African strains.⁵⁸
 - Collaboratively, Roche and TIB Molbiol developed three LightMix Modular Virus kits to detect MPXV using quantitative PCR (qPCR) after nucleic acid extraction. The first kit detects OPVs including, but not limited to, MPXV. The second kit is specific for detecting only the West African and Central African MPXV strains, while the third kit provides a simultaneous detection of OPVs and specific identification of MPXV. These kits have not been fully validated, and are not approved for clinical use.⁵⁹
- ELISA-based serological diagnostics can be used to determine if exposure has occurred after a patient is PCR negative, but cannot differentiate between clades, and may not be able to differentiate vaccinated from infected individuals.⁶⁰
 - IgM titers may be positive as early as day 2 after rash onset; it is recommended that samples be collected at least 5 days after onset of rash.⁶⁰
 - IgG titers may be positive as early as 1-2 days after rash onset; it is recommended that samples be collected after day 14 following onset of rash.⁶⁰
 - IgM ELISA can distinguish between recent MPXV infection and previous smallpox vaccination.⁶⁰
 - MPXV-specific IgG was detected in human serum as long as one year post-infection.¹⁵
- Confirmation is possible through MPXV isolation and immunohistochemistry from human samples¹⁶ and animal samples.⁶¹⁻⁶²
- Intracellular cytokine staining analysis has been used to quantify OPV-specific CD4+ and CD8+ T-cell responses based on gamma interferon and tumor necrosis factor- α production.¹⁵
- Confirmation possible through morphological identification consistent with an OPV by electron microscopy.¹⁶
- Antibody-based tests and electron microscopy are only sufficient for “probable” case status under the current case definition.⁵⁴

What do we need to know?

- Are there rapid and sensitive tests (field or laboratory) that are able to differentiate MPXV from other OPVs?
- How quickly does a patient become PCR positive after exposure to MPXV?
- How can tests to detect MPXV without requiring virus isolation or amplification, for use in resource-limited settings, be improved?

Medical Treatment – Are there effective treatments?

What do we know?

- Cidofovir, brincidofovir, and tecovirimat have proven activity against poxviruses in *in vitro* and animal studies. Only limited efficacy data are available for these drugs. In at least one small case study, tecovirimat decreased hospitalization time from more than 3 weeks to 10 days in the one patient who received the drug.⁶³⁻⁶⁴
- Cidofovir, Tecovirimat, and Vaccinia Immune Globulin Intravenous (VIGIV) are in the Strategic National Stockpile (SNS).⁶⁴
- For post-exposure prophylaxis, smallpox vaccines should be administered within 3 days post-exposure to provide maximal benefit, but may still attenuate the disease and prevent death when given up to 2 weeks post-exposure.^{31, 62, 65-67}

What do we need to know?

- What is the efficacy of antiviral drugs against MPXV specifically?
- What is the effectiveness of post-exposure vaccination against MPXV relative to its efficacy against smallpox?
- Is JYNNEOS suitable for use in post-exposure vaccination? If so, what is its efficacy compared to the live vaccinia vaccine?

Vaccines – Are there effective vaccines?

What do we know?

- Vaccination with smallpox vaccine (vaccinia virus) is reported to provide protection against 85% of MPXV infections. CDC recommends revaccination after 3 years for individuals with likely exposure.^{14, 24, 68}
- Two OPV vaccines are licensed by the FDA for use in the United States. ACAM2000 (Emergent) is a live vaccinia virus vaccine that can be used for MPXV, although it has not been specifically licensed for this use. JYNNEOS (Bavarian Nordic A/S) is a two-dose non-replicating MVA vaccine that is appropriate for use in individuals for whom the live vaccine is contraindicated. This vaccine is specifically licensed for MPXV by the FDA in addition to licensure for smallpox.⁶⁹
- Both FDA-approved vaccines are maintained in the SNS, along with Aventis Pasteur Smallpox Vaccine, another live vaccinia vaccine that would be used under an EUA or as an IND in an emergency.⁶⁹
- There has been a significant increase in human monkeypox cases over the decades following the end of smallpox vaccinations in rural areas of the Congo.¹⁴

What do we need to know?

- Which vaccine provides the best protection and carries the least health burden on the patient?
- Would it be advantageous to make the smallpox vaccine routinely available to those in MPXV-endemic regions with an increased risk of exposure?
- What factors, other than immune status, should be considered when deciding to give a patient a particular vaccine?
- Preclinical and clinical trials of live vaccinia smallpox vaccines for human MPXV are needed to better establish efficacy and obtain specific FDA licensure for MPXV.

Environmental Stability – How long does the agent live in the environment?

What do we know?

- Poxviruses are known to remain infectious in the scabs of patients for months to years,⁷⁰ and viral DNA present in lesion material is stable for a long period of time if kept in a relatively dark, cool environment.⁹
- Poxviruses are stable for days to weeks in storm water and soil, lasting longer at cold temperatures.⁷⁰
- After 14 days of storage at 4°C, MPXV was detected via PCR on food at titers of 200–300 TCID₅₀/100 µL.⁷⁰ Under the same conditions and methods, MPXV was also observed on gauze at ~200 TCID₅₀/100 µL.⁷⁰
- MPXV-contaminated material including clothes, paper, and dust may remain contagious for years if not disinfected.⁷¹
- As an OPV, MPXV is expected to be quite stable in the environment:⁷²
 - MPXV is relatively resistant to desiccation both in heat and cold.⁷²
 - Repeated freezing and thawing of undiluted MPXV-infected tissue culture fluid up to 12 times produced a 1.5 to 4 fold loss of infectious virus.⁷²

- After 6 months of storage of infected tissue culture fluid at 4°C, the infectivity titer of stocks remained unchanged from the original; however, at -20°C there was a 100 fold loss of infectious virus, and at -70°C there was a 30 fold loss of infectious virus.⁷²
- After 15 months of storage, the loss in viability was about 500 fold at 4°C, 1000 fold at -70°C, and more than 10,000 fold at -20°C.⁷²
- MPXV-infected tissue culture fluid adjusted to pH 2 completely inactivated 10⁵ PFU/mL virus; 10 fold loss was observed when starting fluid was adjusted to pH 10.⁷²

What do we need to know?

- How long does virus remain viable in dried scabs on patient and after specimen collection?
- Does MPXV stability follow the same general trends as other OPVs?
 - If it does not, what is the stability of MPXV on various common surfaces and on food items?

Decontamination – What are effective methods to kill the agent in the environment?

What do we know?

- Effective means of home disinfection include standard laundry and dish washing methods while utilizing personal protective equipment and observing proper hand hygiene. Hot water cycle achieving 140°F is recommended for laundering bed linens and clothing.⁷³ Do not shake soiled laundry, as it may cause the virus to become airborne. Clean and disinfect surfaces with U.S. Environmental Protection Agency (EPA)-registered chemicals with emerging viral pathogens claim.⁷⁴⁻⁷⁶
- EPA lists emerging viral pathogens into Tiers (1-3), denoting increasing difficulty of inactivation. MPXV is Tier 1, meaning that it is among the easiest to destroy (chemically), with inactivation of virus upon destruction of its envelope.⁷⁶
 - List Q contains all EPA-registered disinfectants for use on emerging viral pathogens.⁷⁶
 - Common household disinfectants on List Q include many with active ingredients of sodium hypochlorite (bleach) or quaternary ammonium (compounds). Bleach at 0.1% concentration (1:50 dilution) is recommended for use in household disinfection of MPXV,⁷³ and 0.5% freshly made bleach for work in hospitals and laboratories.^{74, 76}
 - List Q also identifies hospital-grade disinfectants.⁷⁶⁴ Hospital disinfection is similar to above recommendations, with added notes to avoid sweeping, dry dusting, and vacuuming in the room of a patient with MPXV.^{76,75}
- Ultraviolet (UV) light at 254 nm for 20 seconds inactivates vaccinia virus in water.⁷⁰
- Heating MPXV for 20 minutes at 40°C caused no significant loss of infectivity, while heating for 20 minutes at 50 or 56°C resulted in significant (87%) or complete loss of infectivity.⁷²
- Vaccinia virus is commonly used as a surrogate for OPV inactivation. The disinfectants Virkon® and Dettol® were found to inactivate vaccinia virus (starting titers 10⁹-10¹⁰ TCID₅₀/mL).
 - 5% Virkon (disinfectant containing potassium peroxymonosulfate, sodium dodecylbenzenesulfonate, sulfamic acid, and inorganic buffers) completely inactivated the virus on contact in presence and absence of fetal bovine serum (FBS).⁷⁷
 - Dettol (household disinfectant containing 4.8% chloroxylenol and isopropanol and castor oil soap) completely or near completely inactivated the virus on contact. Complete inactivation was achieved with contact time of 30 minutes in presence or absence of FBS.⁷⁷
- Vaccinia virus in tissue culture at 10^{7.98} TCID₅₀/mL was completely inactivated within 5 minutes using 80% ethanol and 5% isopropanol.⁷⁸

- Sanytex (3-10%) (a non-corrosive commercial solution containing quaternary ammonium, aldehydes, alcohol, and detergent) reduced virus >10⁴-fold in suspension containing protein after a 3-minute incubation. The higher concentration Sanytex was required to decontaminate higher protein concentrations in the suspension. Vaccinia virus (with protein) dried on a surface was reduced >10⁴-fold with 30% Sanytex after 30 minutes.⁷⁹

What do we need to know?

- What viral titers of MPXV can be effectively decontaminated with sodium hypochlorite?
- What is the minimum inhibitory concentration of decontaminating agent and duration of required contact time in samples relevant to MPXV, such as scabs, blood, etc.?
- What are the inactivation kinetics of germicidal UV and other UV light types against MPXV?

Personal Protective Equipment (PPE) – What PPE is effective and who should be using it?

What do we know?

- Precautions should be taken against direct contact with lesions until the lesions have crusted.⁴⁷
- For clinicians, PPE should be donned before entering the patient's room and used for all patient contact. All PPE should be disposed of prior to leaving the isolation room where the patient is admitted. Optimal PPE include disposable gown and gloves, NIOSH-certified N95 (or comparable) filtering disposable respirator, and face shield or goggles.⁸⁰
- The CDC recommends pre-exposure vaccination every 3 years for several groups:^{31, 44}
 - Persons who are investigating animal or human monkeypox cases.
 - Healthcare workers who are caring for patients with MPXV.
 - Anyone who has direct contact with suspected MPXV-infected animals.
 - Laboratory workers who handle specimens that may contain MPXV.
- All persons working in or entering laboratory or animal care areas where activities with MPXV are being conducted. All procedures involving handling potentially infectious material should be performed in laboratories utilizing Biosafety Level 2 or 3 practices, depending on the risks involved in the procedure.³¹

What do we need to know?

- What additional precautions are required for immunosuppressed or other populations that may have prolonged contact with an infected individual?
- Is there a need for everyday use of PPE among the general population? If there is, what type of PPE is appropriate?

Genomics – How does the disease agent compare to previous strains?

What do we know?

- MPXV is a DNA virus with a 197kb genome, about 10X larger than that of SARS-CoV-2.³⁵
- There are two clades of MPXV that correspond to the Congo River Basin and coastal West Africa.^{24, 56-58, 81}
 - WHO is currently considering a nomenclature change in which the Congo Basin clade would be renamed “Clade 1”, with the West African clade split into “Clade 2” and “Clade 3.”⁸²⁻⁸³
- The strains belonging to the Western African clade tend to be less pathogenic and human-transmissible than the Central African strains.^{48, 58, 81, 84} The current outbreak strain is a West African clade virus.²⁵
- Like all OPVs, MPXV has an extremely low evolutionary rate, estimated at 6.5 x 10⁻⁶ substitutions/per site/per year, which is approximately 2-3 orders of magnitude slower than RNA viruses like SARS-CoV-2 or Ebola virus.⁸⁵

- This evolutionary rate is so low that the inherent error rate of some sequencing technologies can create noise that may artificially inflate apparent evolutionary rates.⁸⁶
- The evolutionary rate may be affected by recombination between strains during coinfections.⁸⁷
- Recent work has found that the virus associated with the current outbreak may have an accelerated evolutionary rate relative to other OPVs, potentially as much as an order of magnitude greater.²⁸
 - More corroboration is needed before definitive conclusions are drawn.
 - This enhanced evolutionary rate appears to have led to accumulation of mutations that would be expected to enhance the transmissibility of the virus.²⁸
 - Although it is possible that this represents a change in the biology of the virus, it is more likely that it is the result of increased editing by the host RNA editing enzyme APOBEC3, which may be the result of the extensive human-to-human transmission experienced by this strain.²⁸
- Four distinct lineages have been detected in the Congo Basin clade, and a deletion that resulted in gene loss appears to correlate with human-to-human transmission.⁴⁸
- A constellation of interdependent virulence factors appears to be responsible for the difference in virulence between West African clade and Congo Basin clade viruses.^{57, 88-90}
- Congo Basin clade MPXVs are regulated as U.S. Department of Health and Human Services (HHS) Select Agents by the CDC Federal Select Agents Program. West African clade MPXVs are not Select Agents due to their lower severity/lethality.⁹¹

What do we need to know?

- Are there changes due to genomic destabilization and gene loss that may pose a potential threat for accelerated adaptation to humans?
- Has the progressive loss of non-essential genes enabled MPXV to adapt to human-to-human transmission? A study demonstrated that gene copy number variation might be a crucial factor for modulating virus fitness.^{48, 92}
- Determine if further adaptation of MPXV to humans could occur through gene gain or through nucleotide changes resulting in optimization of non-equivalent, redundant pathways (convergent evolution).³⁸
- Determine whether and how MPXV circulated in human populations prior to the current outbreak.

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TECHNICAL INFORMATION REGARDING MONKEYPOX VIRUS (MONKEYPOX)

Acronym/Term	Definition	Description
CDC	Centers for Disease Control and Prevention	N/A
DHS S&T	U.S. Department of Homeland Security Science and Technology Directorate	N/A
ELISA	Enzyme-Linked Immunosorbent Assay	An assay used to detect the presence of antibodies to a specific protein.
EPA	U.S. Environmental Protection Agency	N/A
EUA	Emergency Use Authorization	Provisional FDA approval granted for pharmaceuticals and other medical products under emergency conditions.
FBS	Fetal Bovine Serum	A media additive used in tissue culture to facilitate cell growth.
FDA	U.S. Food and Drug Administration	N/A
HHS	U.S. Department of Health and Human Services	N/A
Ig	Immunoglobulin	Antibodies (glycoprotein molecules produced by white blood cells).
IND	Investigational New Drug	An FDA designation allowing for limited/controlled use of an unapproved pharmaceutical under specific conditions.
LD ₅₀	Median Lethal Dose	The dose required to cause a lethal effect in 50% of subjects.
MPXV	Monkeypox Virus	N/A
MQL	Master Question List	N/A
MSM	Men who have Sex with Men	N/A
MVA	Modified Vaccinia Virus Ankara	A vaccinia virus that cannot replicate in normal cells.
NHP	Non-Human Primate	N/A
NIOSH	National Institute for Occupational Safety and Health	N/A
OPV	Orthopoxvirus	The group of viruses containing smallpox, monkeypox, vaccinia virus, and others.

TECHNICAL INFORMATION REGARDING MONKEYPOX VIRUS (MONKEYPOX)

PFU	Plaque Forming Unit	A unit representing a single infectious viral particle derived from viral quantification via plaque assay.
PPE	Personal Protective Equipment	Equipment intended to protect individuals against hazardous environments.
qPCR	Qualitative Polymerase Chain Reaction	An assay used to determine the number of RNA or DNA molecules representing a specific sequence target are present in a sample.
R_0	Basic Reproductive Number	The average number of new infections that each case is expected to generate in a population where all individuals are susceptible to infection.
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2	N/A
SNS	Strategic National Stockpile	The stockpile of drugs, tests, vaccines, and equipment maintained by the federal government for pandemic and biothreat response.
TCID ₅₀	Median Tissue Culture Infectious Dose	The dose necessary to infect 50% of tissue cells. Used as a standard measure of infectivity (e.g., it required 10 ³ TCID ₅₀ to produce clinical signs in exposed chickens).
UV	Ultraviolet	Light with wavelength in the 100-400 nm range.

References

1. Nalca, A.; Livingston, V. A.; Garza, N. L.; Zumbun, E. E.; Frick, O. M.; Chapman, J. L.; Hartings, J. M., Experimental Infection of Cynomolgus Macaques (*Macaca fascicularis*) with Aerosolized Monkeypox Virus. *PLoS One* **2010**, *5* (9), 1-12.
2. Moss, B., Smallpox Vaccines: Targets of Protective Immunity. *Immunol Rev* **2011**, *239* (1), 8-26.
3. Stittelaar, K. J.; Neyts, J.; Naesens, L.; van Amerongen, G.; van Lavieren, R. F.; Holy, A.; De Clercq, E.; Niesters, H. G.; Fries, E.; Maas, C.; Mulder, P. G.; van der Zeijst, B. A.; Osterhaus, A. D., Antiviral Treatment is More Effective than Smallpox Vaccination upon Lethal Monkeypox Virus Infection. *Nature* **2006**, *439* (7077), 745-8.
4. Huggins, J.; Goff, A.; Hensley, L.; Mucker, E.; Shamblin, J.; Wlazlowski, C.; Johnson, W.; Chapman, J.; Larsen, T.; Twenhafel, N.; Karem, K.; Damon, I. K.; Byrd, C. M.; Bolken, T. C.; Jordan, R.; Hruby, D., Nonhuman Primates are Protected from Smallpox Virus or Monkeypox Virus Challenges by the Antiviral Drug ST-246. *Antimicrob Agents Chemother* **2009**, *53* (6), 2620-5.
5. Hutson, C. L.; Damon, I. K., Monkeypox Virus Infections in Small Animal Models for Evaluation of Anti-poxvirus Agents. *Viruses* **2010**, *2* (12), 2763-76.
6. Barnewall, R. E.; Fisher, D. A.; Robertson, A. B.; Vales, P. A.; Knostman, K. A.; Bigger, J. E., Inhalational Monkeypox Virus Infection in Cynomolgus Macaques. *Front Cell Infect Microbiol* **2012**, *2*, 117.
7. Schmitt, A.; Mätz-Rensing, K.; Kaup, F. J., Non-Human Primate Models of Orthopoxvirus Infections. *Vet Sci* **2014**, *1* (1), 40-62.
8. Graham, M. B.; Gunkel, J. L.; Fairley, J., Monkeypox. <http://emedicine.medscape.com/article/1134714-overview#showall> (accessed 5/29/2014).
9. McCollum, A. M.; Damon, I. K., Human Monkeypox. *Clin Infect Dis* **2014**, *58* (2), 260-7.
10. Grant, R.; Nguyen, L.-B. L.; Breban, R., Modeling Human-to-Human Transmission of Monkeypox. *Bulletin of the World Health Organization* **2020**, *98* (9), 638-640.
11. Centers for Disease Control and Prevention (CDC), 2022 Monkeypox: Information for Healthcare Professionals. <https://www.cdc.gov/poxvirus/monkeypox/response/2022/hcp/index.html> (accessed 06/13/2022).
12. Centers for Disease Control and Prevention (CDC), CDC Monkeypox Response: Transmission. Centers for Disease Control and Prevention (CDC),; 2022. <https://www.cdc.gov/media/releases/2022/0509-monkeypox-transmission.html>
13. Guarner, J.; Johnson, B. J.; Paddock, C. D.; Shieh, W. J.; Goldsmith, C. S.; Reynolds, M. G.; Damon, I. K.; Regnery, R. L.; Zaki, S. R., Monkeypox Transmission and Pathogenesis in Prairie Dogs. *Emerg Infect Dis* **2004**, *10* (3), 426-31.
14. Rimoin, A. W.; Mulembakani, P. M.; Johnston, S. C.; Lloyd Smith, J. O.; Kitalu, N. K.; Kinkela, T. L.; Blumberg, S.; Thomassen, H. A.; Pike, B. L.; Fair, J. N.; Wolfe, N. D.; Shongo, R. L.; Graham, B. S.; Formenty, P.; Okitolonda, E.; Hensley, L. E.; Meyer, H.; Wright, L. L.; Muyembe, J. J., Major Increase in Human Monkeypox Incidence 30 Years After Smallpox Vaccination Campaigns Cease in the Democratic Republic of Congo. *Proc Natl Acad Sci U S A* **2010**, *107* (37), 16262-7.
15. Hammarlund, E.; Lewis, M. W.; Carter, S. V.; Amanna, I.; Hansen, S. G.; Strelow, L. I.; Wong, S. W.; Yoshihara, P.; Hanifin, J. M.; Slifka, M. K., Multiple Diagnostic Techniques Identify Previously Vaccinated Individuals with Protective Immunity against Monkeypox. *Nat Med* **2005**, *11* (9), 1005-11.
16. Reynolds, M. G.; Davidson, W. B.; Curns, A. T.; Conover, C. S.; Huhn, G.; Davis, J. P.; Wegner, M.; Croft, D. R.; Newman, A.; Obiesie, N. N.; Hansen, G. R.; Hays, P. L.; Pontones, P.;

- Beard, B.; Teclaw, R.; Howell, J. F.; Braden, Z.; Holman, R. C.; Karem, K. L.; Damon, I. K., Spectrum of Infection and Risk Factors for Human Monkeypox, United States, 2003. *Emerg Infect Dis* **2007**, *13* (9), 1332-9.
17. Jezek, Z.; Grab, B.; Szczeniowski, M. V.; Paluku, K. M.; Mutombo, M., Human Monkeypox: Secondary Attack Rates. *Bull World Health Organ* **1988**, *66* (4), 465-70.
 18. Centers for Disease Control and Prevention (CDC), Multistate Outbreak of Monkeypox - -- Illinois, Indiana, and Wisconsin, 2003. *Morbidity and Mortality Weekly Report* **2003**, *52*(23), 537-540.
 19. Breman, J. G.; Kalisa, R.; Steniowski, M. V.; Zanotto, E.; Gromyko, A. I.; Arita, I., Human Monkeypox, 1970-79. *Bull World Health Organ* **1980**, *58* (2), 165-82.
 20. Hutson, C. L.; Carroll, D. S.; Self, J.; Weiss, S.; Hughes, C. M.; Braden, Z.; Olson, V. A.; Smith, S. K.; Karem, K. L.; Regnery, R. L.; Damon, I. K., Dosage Comparison of Congo Basin and West African Strains of Monkeypox Virus using a Prairie Dog Animal Model of Systemic Orthopoxvirus Disease. *Virology* **2010**, *402* (1), 72-82.
 21. Learned, L.; Reynolds, M.; Wassa, D.; Li, Y.; Olson, V.; Karenm, K.; Stempora, L.; Braden, Z.; Kline, R.; Likos, A.; Libama, F.; Moudzeo, H.; Bolanda, J.; Tarangonia, P.; Boumandoki, P.; Formenty, P.; Harvey, J.; Damon, I., Extended Interhuman Transmission of Monkeypox in a Hospital Community in the Republic of Congo, 2003. *Am J Trop Med Hyg* **2005**, *73* (2), 428-434.
 22. Jezek, Z.; Gromyko, A. I.; Szczeniowski, M. V., Human monkeypox. *J Hyg Epidemiol Microbiol Immunol* **1983**, *27* (1), 13-28.
 23. Jezek, Z.; Grab, B.; Paluku, K. M.; Szczeniowski, M. V., Human Monkeypox: Disease Pattern, Incidence and Attack Rates in a Rural Area of Northern Zaire. *Trop Geogr Med* **1988**, *40* (2), 73-83.
 24. Hutson, C. L.; Lee, K. N.; Abel, J.; Carroll, D. S.; Montgomery, J. M.; Olson, V. A.; Li, Y.; Davidson, W.; Hughes, C.; Dillon, M.; Spurlock, P.; Kazmierczak, J. J.; Austin, C.; Miser, L.; Sorhage, F. E.; Howell, J.; Davis, J. P.; Reynolds, M. G.; Braden, Z.; Karem, K. L.; Damon, I. K.; Regnery, R. L., Monkeypox Zoonotic Associations: Insights from Laboratory Evaluation of Animals Associated with the Multi-state US Outbreak. *Am J Trop Med Hyg* **2007**, *76* (4), 757-68.
 25. Centers for Disease Control and Prevention (CDC), *Monkeypox Virus Infection in the United States and Other Non-endemic Countries—2022*; CDCHAN-00466; CDC (Centers for Disease Control and Prevention),: Atlanta, Georgia, 5/20/2022, 2022.
https://emergency.cdc.gov/han/2022/han00466.asp?ACSTrackingID=USCDC_511-DM82529&ACSTrackingLabel=HAN%20466%20-%20General%20Public&deliveryName=USCDC_511-DM82529
 26. Lloyd-Smith, J. O., Vacated Niches, Competitive Release and the Community Ecology of Pathogen Eradication. *Philosophical Transactions of the Royal Society B: Biological Sciences* **2013**, *368* (1623), 20120150.
 27. Shah, S., The Jump. In *Pandemic: Tracking Contagions, from Cholera to Ebola and Beyond*, Sarah Crichton Books: New York, NY, 2016; pp 27-28.
 28. Isidro, J.; Borges, V.; Pinto, M.; Sobral, D.; Santos, J. D.; Nunes, A.; Mixao, V.; Ferreira, R.; Santos, D.; Duarte, S.; Vieira, L.; Borrego, M. J.; Nuncio, S.; de Carvalho, I. L.; Pelerito, A.; Cordeiro, R.; Gomes, J. P., Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. *Nat Med* **2022**.
 29. Centers for Disease Control and Prevention (CDC), Monkeypox
<https://www.cdc.gov/poxvirus/monkeypox/transmission.html> (accessed 7/29/2020).
 30. Centers for Disease Control and Prevention (CDC), Monkeypox Transmission (for Veterinarians). <https://www.cdc.gov/poxvirus/monkeypox/veterinarian/transmission.html>.
 31. U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories. 5th ed.; Chosewood, L. C., and Wilson, Deborah E., Ed. U.S.

Department of Health and Human Services: 2009.

<http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf>.

32. Fleischauer, A. T.; Kile, J. C.; Davidson, M.; Fischer, M.; Karem, K. L.; Teclaw, R.; Messersmith, H.; Pontones, P.; Beard, B. A.; Braden, Z. H.; Cono, J.; Sejvar, J. J.; Khan, A. S.; Damon, I.; Kuehnert, M. J., Evaluation of human-to-human transmission of monkeypox from infected patients to health care workers. *Clin Infect Dis* **2005**, *40* (5), 689-94.
33. Hutson, C. L.; Olson, V. A.; Carroll, D. S.; Abel, J. A.; Hughes, C. M.; Braden, Z. H.; Weiss, S.; Self, J.; Osorio, J. E.; Hudson, P. N.; Dillon, M.; Karem, K. L.; Damon, I. K.; Regnery, R. L., A Prairie Dog Animal Model of Systemic Orthopoxvirus Disease using West African and Congo Basin Strains of Monkeypox Virus. *J Gen Virol* **2009**, *90* (Pt 2), 323-33.
34. Mutombo, M.; Arita, I.; Jezek, Z., Human Monkeypox Transmitted by a Chimpanzee in a Tropical Rain-Forest Area of Zaire. *Lancet* **1983**, *1* (8327), 735-7.
35. *Fields Virology*. Sixth ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2013.
36. Di Giulio, D. B.; Eckburg, P. B., Human Monkeypox: An Emerging Zoonosis. *Lancet Infect Dis* **2004**, *4* (1), 15-25.
37. Damon, I. K., Status of Human Monkeypox: Clinical Disease, Epidemiology and Research. *Vaccine* **2011**, *29* Suppl 4, D54-9.
38. Reynolds, M. G.; Carroll, D. S.; Karem, K. L., Factors Affecting the Likelihood of Monkeypox's Emergence and Spread in the Post-Smallpox Era. *Curr Opin Virol* **2012**, *2* (3), 335-43.
39. Kulesh, D. A.; Loveless, B. M.; Norwood, D.; Garrison, J.; Whitehouse, C. A.; Hartmann, C.; Mucker, E.; Miller, D.; Wasieloski, L. P., Jr.; Huggins, J.; Huhn, G.; Miser, L. L.; Imig, C.; Martinez, M.; Larsen, T.; Rossi, C. A.; Ludwig, G. V., Monkeypox Virus Detection in Rodents using Real-Time 3'-Minor Groove Binder TaqMan Assays on the Roche LightCycler. *Lab Invest* **2004**, *84* (9), 1200-8.
40. Khodakevich, L.; Jezek, Z.; Messinger, D., Monkeypox Virus: Ecology and Public Health Significance. *Bull World Health Organ* **1988**, *66* (6), 747-52.
41. Ropp, S. L.; Jin, Q.; Knight, J. C.; Massung, R. F.; Esposito, J. J., PCR Strategy for Identification and Differentiation of Smallpox and other Orthopoxviruses. *J Clin Microbiol* **1995**, *33* (8), 2069-76.
42. Silva, N. I. O.; de Oliveira, J. S.; Kroon, E. G.; Trindade, G. S.; Drumond, B. P., Here, There, and Everywhere: The Wide Host Range and Geographic Distribution of Zoonotic Orthopoxviruses. *Viruses* **2020**, *13* (1).
43. Centers for Disease Control and Prevention (CDC), Update: Multistate Outbreak of Monkeypox: Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. *MMWR Morb Mortal Wkly* **2003**, *52* (27), 642-646.
44. Nalca, A.; Rimoim, A. W.; Bavari, S.; Whitehouse, C. A., Reemergence of Monkeypox: Prevalence, Diagnostics, and Countermeasures. *Clin Infect Dis* **2005**, *41* (12), 1765-71.
45. Huhn, G. D.; Bauer, A. M.; Yorita, K.; Graham, M. B.; Sejvar, J.; Likos, A.; Damon, I. K.; Reynolds, M. G.; Kuehnert, M. J., Clinical Characteristics of Human Monkeypox and Risk Factors for Severe Disease. *Clin Infect Dis* **2005**, *41* (12), 1742-51.
46. Yinka-Ogunleye, A.; Aruna, O.; Dalhat, M.; Ogoina, D.; McCollum, A.; Disu, Y.; Mamadu, I.; Akinpelu, A.; Ahmad, A.; Burga, J.; Ndoreraho, A.; Nkuzimana, E.; Manneh, L.; Mohammed, A.; Adeoye, O.; Tom-Aba, D.; Silenou, B.; Ipadeola, O.; Saleh, M.; Adeyemo, A.; Nwadiutor, I.; Aworabhi, N.; Uke, P.; John, D.; Wakama, P.; Reynolds, M.; Mauldin, M. R.; Doty, J.; Wilkins, K.; Musa, J.; Khalakdina, A.; Adedeji, A.; Mba, N.; Ojo, O.; Krause, G.; Ihekweazu, C.; C. D. C. Monkeypox Outbreak Team, Outbreak of Human Monkeypox in Nigeria in 2017-18: A Clinical and Epidemiological Report. *The Lancet. Infectious Diseases* **2019**, *19* (8), 872-879.
47. Siegel, J. D.; Rhinehart, E.; Jackson, M.; Chiarello, L., 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. *Am J Infect Control* **2007**, *35* (10 Suppl 2), S65-164.

48. Kugelman, J. R.; Johnston, S. C.; Mulembakani, P. M.; Kivalu, N.; Lee, M. S.; Koroleva, G.; McCarthy, S. E.; Gestole, M. C.; Wolfe, N. D.; Fair, J. N.; Schneider, B. S.; Wright, L. L.; Huggins, J.; Whitehouse, C. A.; Wemakoy, E. O.; Muyembe-Tamfum, J. J.; Hensley, L. E.; Palacios, G. F.; Rimoin, A. W., Genomic Variability of Monkeypox Virus Among Humans, Democratic Republic of the Congo. *Emerg Infect Dis* **2014**, *20* (2), 232-9.
49. Stittelaar, K. J.; van Amerongen, G.; Kondova, I.; Kuiken, T.; van Lavieren, R. F.; Pistor, F. H.; Niesters, H. G.; van Doornum, G.; van der Zeijst, B. A.; Mateo, L.; Chaplin, P. J.; Osterhaus, A. D., Modified Vaccinia Virus Ankara Protects Macaques Against Respiratory Challenge with Monkeypox Virus. *J Virol* **2005**, *79* (12), 7845-51.
50. Croft, D. R.; Sotir, M. J.; Williams, C. J.; Kazmierczak, J. J.; Wegner, M. V.; Rausch, D.; Graham, M. B.; Foldy, S. L.; Wolters, M.; Damon, I. K.; Karem, K. L.; Davis, J. P., Occupational Risks during a Monkeypox Outbreak, Wisconsin, 2003. *Emerg Infect Dis* **2007**, *13* (8), 1150-7.
51. Girometti, N.; Byrne, R.; Bracchi, M.; Heskin, J.; McOwan, A.; Tittle, V.; Gedela, K.; Scott, C.; Patel, S.; Gohil, J.; Nugent, D.; Suchak, T.; Dickinson, M.; Feeney, M.; Mora-Peris, B.; Stegmann, K.; Plaha, K.; Davies, G.; Moore, L. S. P.; Mughal, N.; Asboe, D.; Boffito, M.; Jones, R.; Whitlock, G., Demographic and clinical characteristics of confirmed human monkeypox virus cases in individuals attending a sexual health centre in London, UK: an observational analysis. *Lancet Infect Dis* **2022**.
52. U.S. Department of Health and Human Services, HHS Expanding Monkeypox Testing Capacity to Five Commercial Laboratory Companies. U.S. Department of Health and Human Services, 2022. <https://www.hhs.gov/about/news/2022/06/22/hhs-expanding-monkeypox-testing-capacity-five-commercial-laboratory-companies.html>
53. World Health Organization, Laboratory testing for the monkeypox virus: Interim guidance. <https://apps.who.int/iris/bitstream/handle/10665/354488/WHO-MPX-Laboratory-2022.1-eng.pdf> (accessed 06/09/2022).
54. Centers for Disease Control and Prevention (CDC), Updated Case-finding Guidance: Monkeypox Outbreak—United States, 2022. Centers for Disease Control and Prevention (CDC), Ed. 2022. <https://emergency.cdc.gov/han/2022/han00468.asp>
55. Li, Y.; Olson, V. A.; Laue, T.; Laker, M. T.; Damon, I. K., Detection of Monkeypox Virus with Real-Time PCR Assays. *J Clin Virol* **2006**, *36* (3), 194-203.
56. Likos, A. M.; Sammons, S. A.; Olson, V. A.; Frace, A. M.; Li, Y.; Olsen-Rasmussen, M.; Davidson, W.; Galloway, R.; Khristova, M. L.; Reynolds, M. G.; Zhao, H.; Carroll, D. S.; Curns, A.; Formenty, P.; Esposito, J. J.; Regnery, R. L.; Damon, I. K., A Tale of Two Clades: Monkeypox Viruses. *J Gen Virol* **2005**, *86* (Pt 10), 2661-72.
57. Chen, N.; Li, G.; Liszewski, M. K.; Atkinson, J. P.; Jahrling, P. B.; Feng, Z.; Schriewer, J.; Buck, C.; Wang, C.; Lefkowitz, E. J.; Esposito, J. J.; Harms, T.; Damon, I. K.; Roper, R. L.; Upton, C.; Buller, R. M., Virulence Differences Between Monkeypox Virus Isolates from West Africa and the Congo Basin. *Virology* **2005**, *340* (1), 46-63.
58. Li, Y.; Zhao, H.; Wilkins, K.; Hughes, C.; Damon, I. K., Real-Time PCR Assays for the Specific Detection of Monkeypox Virus West African and Congo Basin Strain DNA. *J Virol Methods* **2010**, *169* (1), 223-7.
59. Roche, Roche develops unique PCR tests to detect the monkeypox virus. 2022. <https://www.roche.com/media/releases/med-cor-2022-05-25>
60. Karem, K. L.; Reynolds, M.; Braden, Z.; Lou, G.; Bernard, N.; Patton, J.; Damon, I. K., Characterization of Acute-phase Humoral Immunity to Monkeypox: Use of Immunoglobulin M Enzyme-Linked Immunosorbent Assay for Detection of Monkeypox Infection during the 2003 North American Outbreak. *Clin Diagn Lab Immunol* **2005**, *12* (7), 867-72.
61. Zaucha, G. M.; Jahrling, P. B.; Geisbert, T. W.; Swearingen, J. R.; Hensley, L., The Pathology of Experimental Aerosolized Monkeypox Virus Infection in Cynomolgus Monkeys (*Macaca fascicularis*). *Lab Invest* **2001**, *81* (12), 1581-600.

62. Smith, S. K.; Self, J.; Weiss, S.; Carroll, D.; Braden, Z.; Regnery, R. L.; Davidson, W.; Jordan, R.; Hruby, D. E.; Damon, I. K., Effective Antiviral Treatment of Systemic Orthopoxvirus Disease: ST-246 Treatment of Prairie Dogs Infected with Monkeypox Virus. *J Virol* **2011**, *85* (17), 9176-87.
63. Andrei, G.; Snoeck, R., Cidofovir Activity against Poxvirus Infections. *Viruses* **2010**, *2* (12), 2803-30.
64. Centers for Disease Control and Prevention (CDC), Monkeypox Treatment. <https://www.cdc.gov/poxvirus/monkeypox/treatment.html> (accessed 5/31/2022).
65. Massoudi, M. S.; Barker, L.; Schwartz, B., Effectiveness of Postexposure Vaccination for the Prevention of Smallpox: Results of a Delphi Analysis. *J Infect Dis* **2003**, *188* (7), 973-6.
66. Mortimer, P. P., Can Postexposure Vaccination Against Smallpox Succeed? *Clin Infect Dis* **2003**, *36* (5), 622-9.
67. Centers for Disease Control and Prevention (CDC), 2003 U.S. Outbreak. <https://www.cdc.gov/poxvirus/monkeypox/outbreak.html> (accessed 06/16/2017).
68. Fine, P. E.; Jezek, Z.; Grab, B.; Dixon, H., The Transmission Potential of Monkeypox Virus in Human Populations. *Int J Epidemiol* **1988**, *17* (3), 643-50.
69. Centers for Disease Control and Prevention (CDC), Monkeypox and Smallpox Vaccine Guidance. <https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html> (accessed 06/06/2022).
70. Essbauer, S.; Meyer, H.; Porsch-Ozcurumez, M.; Pfeffer, M., Long-lasting Stability of Vaccinia Virus (Orthopoxvirus) in Food and Environmental Samples. *Zoonoses Public Health* **2007**, *54* (3-4), 118-24.
71. Rheinbaben, F. v.; Gebel, J.; Exner, M.; Schmidt, A., Environmental resistance, disinfection, and sterilization of poxviruses. In *Poxviruses*, Mercer, A. A.; Schmidt, A.; Weber, O., Eds. Birkhäuser Basel: Basel, 2007; pp 397-405.
72. Rouhandeh, H.; Engler, R.; Taher, M.; Fouad, A.; Sells, L. L., Properties of Monkeypox Virus. *Arch Gesamte Virusforsch* **1967**, *20* (3), 363-73.
73. ECDC, Monkeypox multi-country outbreak. <https://www.ecdc.europa.eu/sites/default/files/documents/Monkeypox-multi-country-outbreak.pdf> (accessed 06/07/2022).
74. Centers for Disease Control and Prevention (CDC), Interim Guidance for Household Disinfection of Monkeypox Virus. <https://www.cdc.gov/poxvirus/monkeypox/pdf/Monkeypox-Interim-Guidance-for-Household-Disinfection-508.pdf> (accessed 06/07/2022).
75. Centers for Disease Control and Prevention (CDC), Infection Prevention and Control of Monkeypox in Healthcare Settings. <https://www.cdc.gov/poxvirus/monkeypox/clinicians/infection-control-healthcare.html> (accessed 06/08/2022).
76. Environmental Protection Agency, Disinfectants for Emerging Viral Pathogens (EVPs): List Q. <https://www.epa.gov/pesticide-registration/disinfectants-emerging-viral-pathogens-evps-list-q#evps> (accessed 06/08/2022).
77. Butcher, W.; Ulaeto, D., Contact Inactivation of Orthopoxviruses by Household Disinfectants. *J Appl Microbiol* **2005**, *99* (2), 279-84.
78. van Engelenburg, F. A.; Terpstra, F. G.; Schuitemaker, H.; Moorer, W. R., The Virucidal Spectrum of a High Concentration Alcohol Mixture. *J Hosp Infect* **2002**, *51* (2), 121-5.
79. Ferrier, A.; Garin, D.; Crance, J. M., Rapid Inactivation of Vaccinia Virus in Suspension and Dried on Surfaces. *J Hosp Infect* **2004**, *57* (1), 73-9.
80. Centers for Disease Control and Prevention (CDC), Infection Control: Hospital. <https://www.cdc.gov/poxvirus/monkeypox/clinicians/infection-control-hospital.html> (accessed 06/16/2017).
81. Reynolds, M. G.; Damon, I. K., Outbreaks of Human Monkeypox After Cessation of Smallpox Vaccination. *Trends Microbiol* **2012**, *20* (2), 80-7.

82. World Health Organization, WHO Director-General's opening remarks at the COVID-19 media briefing– 14 June 2022. 2022. <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-covid-19-media-briefing--14-june-2022>
83. Happi, C.; Adetifa, I.; Mbala, P.; Njouom, R.; Nakoune, E.; Happi, A.; Ndodo, N.; Ayansola, O.; Mboowa, G.; Bedford, T.; Neher, R.; Roemer, C.; Hodcroft, E.; Tegally, H.; O'Toole, A.; Rambaut, A.; Pybus, O.; Kraemer, M.; Wilkinson, E.; Isidro, J.; Borges, V.; Pinto, M.; Gomes, J. P.; Baxter, C.; Lessells, R.; Ogbwell, A.; Kebede, Y.; Tessema, S.; de Oliveira, T., *Urgent need for a non-discriminatory and non-stigmatizing nomenclature for monkeypox virus*; 6/10/2022, 2022. <https://virological.org/t/urgent-need-for-a-non-discriminatory-and-non-stigmatizing-nomenclature-for-monkeypox-virus/853>
84. Hendrickson, R. C.; Wang, C.; Hatcher, E. L.; Lefkowitz, E. J., Orthopoxvirus Genome Evolution: The Role of Gene Loss. *Viruses* **2010**, *2* (9), 1933-67.
85. Zehender, G.; Lai, A.; Veo, C.; Bergna, A.; Ciccozzi, M.; Galli, M., Bayesian reconstruction of the evolutionary history and cross-species transition of variola virus and orthopoxviruses. *J Med Virol* **2018**, *90* (6), 1134-1141.
86. Rambaut, A., Discussion of on-going MPXV genome sequencing. <https://virological.org/t/discussion-of-on-going-mpxv-genome-sequencing/802/2> (accessed 6/21/2022).
87. Qin, L.; Evans, D. H., Genome scale patterns of recombination between coinfecting vaccinia viruses. *J Virol* **2014**, *88* (10), 5277-86.
88. Estep, R. D.; Messaoudi, I.; O'Connor, M. A.; Li, H.; Sprague, J.; Barron, A.; Engelmann, F.; Yen, B.; Powers, M. F.; Jones, J. M.; Robinson, B. A.; Orzechowska, B. U.; Manoharan, M.; Legasse, A.; Planer, S.; Wilk, J.; Axthelm, M. K.; Wong, S. W., Deletion of the Monkeypox Virus Inhibitor of Complement Enzymes Locus Impacts the Adaptive Immune Response to Monkeypox Virus in a Nonhuman Primate Model of Infection. *J Virol* **2011**, *85* (18), 9527-42.
89. Hudson, P. N.; Self, J.; Weiss, S.; Braden, Z.; Xiao, Y.; Girgis, N. M.; Emerson, G.; Hughes, C.; Sammons, S. A.; Isaacs, S. N.; Damon, I. K.; Olson, V. A., Elucidating the Role of the Complement Control Protein in Monkeypox Pathogenicity. *PLoS One* **2012**, *7* (4), e35086.
90. Kindrachuk, J.; Arsenaault, R.; Kusalik, A.; Kindrachuk, K. N.; Trost, B.; Napper, S.; Jahrling, P. B.; Blaney, J. E., Systems Kinomics Demonstrates Congo Basin Monkeypox Virus Infection Selectively Modulates Host Cell Signaling Responses as Compared to West African Monkeypox Virus. *Mol Cell Proteomics* **2012**, *11* (6), M111 015701.
91. Federal Select Agents Program, Select Agents and Toxins. In *42 CFR Part 73*, United States Department of Health and Human Services, Ed. 2005.
92. Elde, N. C.; Child, S. J.; Eickbush, M. T.; Kitzman, J. O.; Rogers, K. S.; Shendure, J.; Geballe, A. P.; Malik, H. S., Poxviruses Deploy Genomic Accordions to Adapt Rapidly Against Host Antiviral Defenses. *Cell* **2012**, *150* (4), 831-41.