MONKEYPOX: SAMPLE COLLECTION AND DIAGNOSTICS TECHNIQUES

Stefania Di Costanzo, Ph.D., Scientific Affairs
Copan Italia, Via Francesco Perotti, 10, 25125 Brescia BS

INTRODUCTION

Since May 2022, few cases of monkeypox infection have been identified in 12 states members of the World Health Organization. The cases do not seem to have travel links to countries where monkeypox is endemic [1]. This observation has put on a state of alert the World Health Organization together with different disease prevention organizations around the world (including CDC, NHS and others) who have released interim guidelines and information about the monkeypox virus and how to diagnose it. Here is briefly reported a summary of these guidelines and how Copan products can be used to effectively prevent the spread of the disease.

MONKEYPOX TRANSMISSION AND ETIOLOGY

Monkeypox (MPXV) is a double-stranded DNA virus, a member of the orthopoxvirus genus within of the Poxviridae family. Member of the same family is the variola virus, causing smallpox, which has been eradicated. Poxviruses cause disease in humans and many other animals. Specifically, the monkeypox virus can spread when a person comes into contact with the virus from an infected animal, infected person, or materials contaminated with the virus. The virus can also cross the placenta from the mother to her fetus. Monkeypox can spread through respiratory particles during prolonged face to face contact and during intimate contact between people, including sexual activity, where parts of the body with sores come into contact. It is to be clarified if monkeypox can be transmitted through vaginal fluid or semen2.

Monkeypox presentation consists of fever in the first stage, followed by rashes that develop on head and face to progress to limb and trunk. The lesions progress to vesicles and pustules that dry up in a crust and eventually fall off. Sores can develop in the mouth and genital area. Swollen lymph nodes are a distinguishing feature of monkeypox infection.

MONKEYPOX INFECTION DIAGNOSIS

Molecular diagnosis of monkeypox infection is essential to differentiate the infection from other diseases that cause similar skin lesions, such as herpes simplex virus, varicella zoster virus, molluscum contagiosum virus, enterovirus, measles, scabies, Treponema pallidum (syphilis), bacterial skin infections, medication allergies, parapoxviruses and chancroid.

Laboratory confirmation of specimens from a suspected case is done using nucleic acid amplification testing (NAAT), such as real-time or conventional polymerase chain reaction (PCR). NAAT can be generic to orthopoxvirus (OPXV) or specific to monkeypox virus (MPXV, preferable). In addition, sequencing is useful to determine virus clade and to understand epidemiology[2].

The recommended specimens for monkeypox confirmation is a swab of lesion exudate or lesion crusts. The swab can be dry or resuspended in a viral transport medium.

Collection of oropharyngeal swabs is also encouraged[2].

Serum can be used to detect IgG after confirmed infection, but it is not recommended for screening as it could result positive in individuals vaccinated for smallpox. A list of specimens and storage conditions recommended by the WHO interim guideline is shown in table 1 [2].

COMMERCIALLY AVAILABLE TESTS

Current molecular tests available for monkeypox are based on quantitative real-time PCR reactions[3]. Manufacturers launched CE-IVD or ROU tests in the last months after cases surged. Published sets of primers and probes are available as well[2].

Rapid antigen test are available as well[5].

Specimen type	Collection materials	Storage temperature	Collection purpose
Skin lesion material, including: – Swabs of lesion exudate – Lesion roofs – Lesion crusts	Dacron or polyester flocked swabs with VTM or dry swab	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	Recommended for diagnosis
Oropharyngeal swab	Dacron or polyester flocked swabs with VTM or dry swab	See above	Recommended for diagnosis if feasible, in addition to skin lesion material
Rectal and or genital swabs	Dacron or polyester flocked swabs with VTM or dry swab	See above	To be considered for research (following ethics guidelines)
Urine	Sterile collection tube	See above	To be considered for research (following ethics guidelines)
Semen	Sterile collection tube	Room temperature for <1h (then -20°C or lower)	To be considered for research (following ethics guidelines)
Whole blood	Sterile collection tube with EDTA	See above	To be considered for research (following ethics guidelines)
Serum	Serum-separating tubes	Refrigerate (2-8 °C) or freeze (-20°C or lower) within 1 hour of collection; -20°C or lower after 7 days	To be considered for serology to aid diagnosis or research (following ethics guidelines)
Plasma	collection tube with EDTA	See above	To be considered for serology to aid diagnosis or research (following ethics guidelines)

Table 1: List of specimens and storage conditions for monkeypox diagnosis. From https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385

Copan products for monkeypox sample collection

SWABS

Copan flocked swabs are the recommended gold standard device for the collection of lesions exudate[2]. The swab can be put back in the tube and stored following WHO guidelines (see Table 1) or broken and stored in a secondary tube to be analyzed. Specimens collected with Copan flocked swabs can also be resuspended in different Copan media available for viral culture/molecular testing (refer to below paragraphs). Depending on the stage of the disease, different types of specimens can be collected[6]. During the first stage (prodrome) presenting fever, body aches and myalgia or at the first appearance of macules or papules, tonsillar/nasopharyngeal swabs are recommended[7]. When skin lesions evolve to vesicles or pustules, swabbing of the lesion fluid is indicated[6,7]. Peer-reviewed studies show the effectiveness of Copan flocked swabs in detecting monkeypox from tonsils of monkeys challenged with monkeypox infection[8,9].

UTM®

Copan UTM® is a Universal Transport medium widely used for SARS-CoV-2 detection. Since its optimal capabilities in preserving viruses, it can be used to resuspend and store monkeypox after sampling and to preserve monkeypox during transport. In addition to molecular applications, it can be used if further tests need to be performed on cultured viral particles[3]. International guidelines recommend collecting the swab either dry or in viral culture media[2,6,10].

eNAT®

Copan eNAT® is a medium containing guanidine-thiocyanate. Its denaturing properties make the sample safe to handle by operators since monkeypox would be inactivated right after resuspension. Samples resuspended in eNAT® are compatible with several molecular platforms, including sequencing. Since it inhibits viral growth and amplification, it preserves the state of viral nucleic acids as a snapshot of the time of sampling. Its performance has been evaluated on several microorganisms, including DNA viruses such as Herpes Simplex Virus and Cytomegalovirus. eNAT® medium preserves nucleic acids for up to 4 weeks at room temperature and at 4°C and up to 6 months at -20°C to -80°C.

CONCLUSIONS

Monkeypox currently poses a potential threat to global healthcare, by presenting the possibility to the return of an infection of a disease like smallpox, which has been long eradicated. Vaccinations against smallpox stopped in 1972 and it is now recommended only for lab workers who work with smallpox-like viruses and those who need long-term protection, such as military personnel[11]. This means that a significant portion of the global population is currently not vaccinated against smallpox. Additionally, it is still unclear the extent of protection the smallpox vaccine offers against monkeypox and the degree of protection for individuals with compromised immune systems. As SARS-CoV-2 pandemic has taught us, it is of utmost importance to stay ahead of the case increases with novel emerging diseases by testing and isolating immediately suspected cases of infection. Copan can help in this effort by providing a line of products who are optimized for viral detection and are currently recommended by official international guidelines.

NEED MORE INFO?

Visit our web-site https://www.copangroup.com/ or contact us at info@copangroup.com.

REFERENCES

1. https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385
2. World Health Organization. Laboratory testing for the monkeypox virus. Interim guidance. May 23, 2022.
3. McCollum AM and Damon IK. Human Monkeypox. Clinical Infectious Diseases. 2014 Jan 15;58(2):260–
4. Li D, *et al.* Evaluation of the GeneXpert for Human Monkeypox Diagnosis. Am J Trop Med Hyg. 2017 Feb 8;96(2):405–10.
5. Townsend MB *et al.* Evaluation of the Tetracore Orthopox BioThreat® antigen detection assay using laboratory grown orthopoxviruses and rash illness clinical specimens. Journal of Virological Methods. 2013 Jan;187(1):37–42.
6. https://www.cdc.gov/smallpox/lab-personnel/specimen-collection/specimen-collection-transport.html
7. Hugh Adler *et al.*, Clinical features and management of human monkeypox: a retrospective observational study in the UK.
8. Graham J. *et al.* Assessment of the Protective Effect of Imvamune and Acam2000 Vaccines against Aerosolized Monkeypox Virus in Cynomolgus Macaques. Journal of Virology. 2013 July;87(14):7805-7815
9. J. A. Tree, G. *et al* Sequence of Pathogenic Events in Cynomolgus Macaques Infected with Aerosolized Monkeypox Virus. Journal of Virology. 2015 April;89(8):4335-4344.
10. https://www.gov.uk/guidance/monkeypox-diagnostic-testing
11. https://www.cdc.gov/smallpox/vaccine-basics/who-gets-vaccination.html