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Validation and implementation of an orthopoxvirus qualitative real-time PCR for the diagnosis of monkeypox in the clinical laboratory

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\textbf{ABSTRACT}

\textbf{Background:} The globally concerning outbreak of monkeypox virus (MPXV) in non-endemic countries in 2022 warranted an increased capacity of diagnostic clinical testing. In this study, we detail the Johns Hopkins analytical validation of a qualitative orthopoxvirus real-time PCR and characterize its analytical performance for MPXV testing. We also describe our first month of MPXV clinical laboratory diagnosis, including assay utilization and positivity.

\textbf{Methods:} The analytical performance of a previously characterized orthopoxvirus assay that targets the DNA polymerase gene of non-variola orthopoxviruses was determined. \textit{In silico} inclusivity analysis was performed for the primer and probe sequences. The limit of detection, reproducibility, agreement, and specificity were evaluated as well as target stability at room temperature. Clinical chart reviews were performed for patients who received testing within the first month of offering the assay for diagnosis.

\textbf{Results:} Alignment of the primer and probe binding regions of 151 sequenced genomes of MPXV from 2022 showed Sequence homology of 100%. The assay was able to detect MPXV at a concentration of at least 100 copies/mL and showed no cross reactivity with other organisms tested. Targets were stable for at least one week at room temperature. During the first month of implementation, a total of 33 patients were tested, 11 of whom were positive and presented mainly with rash in the genital region.

\textbf{Conclusions:} Increased availability of MPXV testing is essential with the progressive increase in the number of cases. The non-variola orthopoxvirus assay is a sensitive and reproducible method for diagnosing monkeypox during the current surge in cases.

\section{1. Introduction}

Monkeypox (MPX) is a disease caused by the monkeypox virus (MPXV), a member of the Orthopoxvirus (OPXV) genus and has been the most severe disease caused by OPXV since the eradication of smallpox [1]. It was first isolated from research monkeys in the 1950s, first detected in humans in 1970 [2, 3], and since then the disease has become endemic in certain countries in Africa. The first case outside of Africa was not reported until 2003 and was associated with close contact with prairie dogs [4]. A few outbreaks were reported after that including an outbreak in Nigeria in 2017, and cases in the UK with history of travel to Nigeria between 2019-2021 [5, 6].

In May 2022, multiple cases of MPX were reported from multiple non-endemic countries with no clear history of travel to endemic regions in addition to cases reported from endemic countries as well ([7] last accessed 2 Aug 2022). As of August 24, 2022, a total of 44,503 cases of MPX had been reported by the Center for Disease Control (CDC) from 96 different locations, of which only 387 (0.8%) were cases from endemic regions ([8], data as of 23 Aug 2022). Global concerns of the quick and atypical spread of the virus worldwide warranted a rapid public health response and on July 23, 2022, the WHO declared the outbreak a Public Health Emergency of International Concern [9].

In response to the 2022 MPXV outbreak, increasing the capacity of laboratory diagnostic measures have become essential. The initiative started by the CDC and public health officials’ efforts to facilitate testing through the Laboratory Response Network (LRN) [10]. With the LRN existing capacity of testing and their efforts to expand this capacity to five large commercial reference laboratories, offering on site testing...
facilitates MPXV testing as a part of a wider differential diagnosis and provides a quicker turnaround of results. In this study, we share the Johns Hopkins Health system implementation of OPXV testing, based on a qualitative real-time PCR, targeting the E9L DNA polymerase gene, originally developed by the CDC [11]. We show the data of both the analytical validation and the clinical cohort of patients diagnosed during our first month of offering the test.

2. Materials and methods

2.1. Study site and ethics

This study was performed at the Molecular Virology Laboratory, Johns Hopkins Hospital. The study was conducted with a waiver of consent after the study approval by the IRB-X committee (IRB00317591).

2.2. Materials for analytical performance experiments

Analytical performance experiments were carried out with contrived specimens consisting of universal transport medium (UTM, Copan Diagnostics, 3C047N) or viral transport media (VTM, MedSchenker, STM30) spiked with quantified synthetic MPXV DNA (ATCC, VR-3270SD™, Manassas, VA; quantified by the manufacturer at 3.8 E5 copies/µL).

Primer and probe sequences for the OPXV target were modified from Li, et al [11] (E9l-NVAR) to use FAM as the fluorophore: F: TCAACTGAAAAAGCCACTATGTA, R: GAGTATAGACGCACTTATTT CTAAATCCCA A, P: FAM-CCATGCAATATACGTACAAGATAGTAGCCAAC. The human RNAseP gene was used as an internal control when testing clinical samples (specificity and clinical patient samples). RNAseP primer and probe sequences were F: AGATTTGGACCTGCGAGCG, R: GAGCGGGTCTGTCTCCACAAGT, P: Cy5-TCTTGACCTGGAAGCTCTGCGCG.

Positive patient samples from the first month of testing were also run in-house with a research based monkeypox specific PCR, using primers and probes from Li, et al [11] targeting the B6r gene: F: ATGGGTCAATTATATGTGCAGAAC, R: GAGGTTCGTGCTTCCCAAGAT, P: Cy5-TCTTGACCTGGAAGCTCTGGCGG.

2.3. DNA extraction

Automated nucleic acid extraction was performed using the NucliSens easyMag or eMag instruments (Biomerieux, Marcy-l’Étoile, France) using software version 2.1.0.1. Samples were extracted at a volume of either 500 or 250 µL and nucleic acid was eluted at 50 or 25 µL respectively. Extraction was performed following the manufacturer’s protocol for off-board lysis procedure. Samples were processed in BSL-2 facilities using BSL-3 practices.

2.4. Real-time TaqMan chemistry PCR assay

Total reaction volume was 20 µL (5 µL of template and 15 µL of master mix). The master mix contained 5 µL of TaqPath 1-Step RT-qPCR Master Mix (Applied Biosystems, A15299, Waltham, MA), 7 µL of dH₂O, and 1 µL each of the forward and reverse primers (10 nm) and probe (5 nm) for the OPXV target. For clinical samples when the internal control was incorporated, the volume of dH₂O was reduced to 4 µL to allow for 1 µL each of the forward and reverse primers (10 nm) and probe (5 nm) for the RNAseP target. Real-time PCR was performed using Prism 7500 Sequence Detection System (Applied Biosystems) and the following cycling conditions: 1 cycle at 95.0 °C for 2 minutes and 40 cycles at 95.0 °C for 3 seconds and 60.0 °C for 31 seconds.

2.5. Limit of detection (LOD)

The LOD was determined using serial dilutions of MPXV DNA spiked into UTM/ VTM. Replicates of 3-32 were run in the range of 1 to 6 log₁₀ copies/mL. The limit of detection was defined as the concentration at which 95% of samples were detected.

2.6. Reproducibility

Reproducibility was assessed using a contrived MPXV positive sample at 100 copies/mL and negative UTM/ VTM. Intra-run precision was determined with three replicates per sample. Inter-run precision was determined by testing replicates over three separate runs, using three different thermal cyclers.

2.7. Specificity

Specificity of the assay was determined by testing patient samples positive for the following analytes: herpes simplex virus 1, herpes simplex virus 2, varicella zoster virus, chlamydia trachomatis, neisseria gonorrhoeae, trichomonas vaginalis and mycoplasma genitalium.

2.8. Agreement

Due to a lack of patient samples during the validation, a blind panel (n=88) consisting of negative UTM/ VTM (n=28) and spiked UTM/ VTM (n=60) ranging from 2-5 log₁₀ copies/mL was tested.

2.9. Results

3.1. In silico inclusivity analysis of the non-variola orthopoxvirus PCR assay primers and probe for the 2022 MPXV cases

Genomes from the 2022 MPXV outbreak in the U.S. were shown to share some unique mutations [12]. To assess if any new mutations might impact the primers or probe binding sites of the non-variola orthopoxvirus PCR assay, a total of 151 sequenced genomes of MPXV from 2022 were aligned (Fig. 1, Table S1). The sequences were obtained from NCBI and represented cases from at least 11 different countries. Alignment was done with Clustal Omega Multiple Sequence Alignment. Our analysis showed 100% identity of the primers and probe binding sites of all genomes and no mutations that could affect the primer and probe binding sites of this assay were observed.

3.2. Limit of detection

The limit of detection of the assay was determined using 83 MPXV spiked UTM/ VTM samples (Fig. 2). All replicates were detected down to a concentration of 100 copies/mL. No replicates at 10 copies/mL were detected by the assay. The defined measurable range was determined to be between 2 and 6 log₁₀ copies/mL (R² = 0.98). The limit of detection was determined to be 100 copies/mL (Table 1).

3.3. Reproducibility

Intra- and inter-assay reproducibility were determined using spiked MPXV UTM/ VTM at a concentration of 100 copies/mL and negative UTM/ VTM (Table 2). The standard deviation for the intra- and inter-assay runs was 0.35 and 0.33 cycle thresholds (Cts) respectively.

3.4. Specificity

Seven Patients’ samples previously positive for C. trachomatis, N. gonorrhoeae, T. vaginalis, M. genitalium, HSV-1, HSV-2, and VZV were randomly selected after the standard of care testing and were tested with the OPXV assay. Samples were all negatives (Table 3) consistent with what was previously reported [11].
3.5. Agreement

A blind panel of 88 positive and negative samples spiked with MPXV DNA at different concentrations were tested across three different runs on three different thermal cyclers. All negative UTM/VTM (n=28) were not detected and all spiked MPXV UTM/VTM (n=60) were detected (detailed, Table S2).

3.6. Stability of orthopoxvirus in clinical samples

To test the stability of MPXV for testing using the non-variola orthopoxvirus test, we used a clinical sample at a Ct value of 16.09 to make multiple replicates at an expected Ct of 29.29 (diluting the sample in VTM) (Table 4). Replicates were stored at room temperature and 6 replicates were tested daily for 7 days. Reproducibility between the tested replicates was 100% with an average Ct of 29.12 of the 36 tested replicates (Table 4).

3.7. First month of clinical implementation

During the first month of offering the clinical assay, a total of 33 patients were tested (Table 5). All patients had at least two body lesions. All patients’ tests performed in our laboratory were on lesion swabs collected in UTM or VTM. If more than one tube of UTM/VTM were sent, samples were pooled prior to testing. In general, the majority of providers collected more than one lesion swab from different anatomical sites (31, 93.9%). Of the 33 patients tested, 11 were positive (33.3%). All 11 samples were sent to the CDC for confirmation, of which they selected four which were confirmed as MPXV. In addition, we tested the 11 positive samples in house using MPXV specific primers and probe and all samples were positive (Table S3). The genomes of the 11 positives were sequenced as a part of multi-site validation of MPXV whole genome sequencing and were confirmed as clade II [13]. Chart review of the 22

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**Table 1**
Number of replicates tested per concentration to determine the non-variola orthopoxvirus assay’s limit of detection.

| Concentration (log10 copies/mL) | Number tested | Number detected | % Detected |
|---------------------------------|---------------|-----------------|------------|
| 6                               | 3             | 3               | 100%       |
| 5                               | 9             | 9               | 100%       |
| 4                               | 10            | 10              | 100%       |
| 3                               | 10            | 10              | 100%       |
| 2.7                             | 32            | 32              | 100%       |
| 2                               | 16            | 16              | 100%       |
| 1                               | 3             | 0               | 0%         |

**Table 2**

| Run | Sample | Replicate | Result | Ct   | Standard deviation Intra | Standard deviation Inter | % Coefficient of variation Intra | % Coefficient of variation Inter |
|-----|--------|-----------|--------|------|--------------------------|--------------------------|---------------------------------|---------------------------------|
| 1   | MPXV   | 1         | POS    | 34.92| 0.35                     | 0.33                     | 0.99                            | 0.94                            |
|     |        | 2         | POS    | 35.50|                          |                          |                                 |                                 |
|     |        | 3         | POS    | 34.67|                          |                          |                                 |                                 |
|     | UTM    | 1         | NEG    | N/A  |                          |                          |                                 |                                 |
|     |        | 2         | NEG    | N/A  |                          |                          |                                 |                                 |
|     |        | 3         | NEG    | N/A  |                          |                          |                                 |                                 |
| 2   | MPXV   | 1         | POS    | 34.57|                          |                          |                                 |                                 |
|     | UTM    | 1         | NEG    | N/A  |                          |                          |                                 |                                 |
| 3   | MPXV   | 1         | POS    | 34.78|                          |                          |                                 |                                 |
|     | UTM    | 1         | NEG    | N/A  |                          |                          |                                 |                                 |

**Table 3**
Specificity of the non-variola orthopoxvirus PCR assay. Seven patients’ samples positive for the indicated pathogens by the standard of care diagnostic assays were ND (not detected) by the OPXV PCR. Samples were previously determined positive by either the cobas 6800 (Roche Diagnostics, Basel, Switzerland) or the Aries (DiaSorin, Austin, TX).

| Analyte       | Standard of care platform | Standard of care Ct | Result with OPXV assay |
|---------------|---------------------------|---------------------|------------------------|
| C. trachomatis | cobas 4800                | 36.0                | ND                     |
| N. gonorrhoeae | cobas 4800                | 32.9                | ND                     |
| T. vaginalis  | cobas 6800                | 15.0                | ND                     |
| M. genitalium | cobas 6800                | 28.3                | ND                     |
| HSV 1         | Aries                     | 23.6                | ND                     |
| HSV 2         | Aries                     | 21.5                | ND                     |
| VZV           | Aries                     | 18.6                | ND                     |
negative patients assured the clinical specificity of the assay as alternative diagnoses (such as eczema or other infections), low clinical suspicion, and/or follow up orthopoxvirus negative testing were reported (Table S4). The main clinical indication of testing was rash (100% of the tested patients), but only 2 (9.1%) negative patients had the rash in the genital region versus 100% of the OPXV positive patients (Table 5). Of note, all the positive samples showed relatively high viral loads, with cycle thresholds (Ct) ranging from 17.43 - 23.08 and an average Ct of 19.18 (Table S3). All positive specimens were from male patients (Table 5).

4. Discussion

In this study, we provide the Johns Hopkins in house validation data of the non-variola orthopoxvirus PCR assay, previously developed by the CDC [11]. Our data showed that the primers and probe selected for the assay target regions that are conserved in genomes associated with the 2022 outbreak. The assay showed an analytical sensitivity of at least 100 copies/ mL, 100% reproducibility, agreement, and specificity. We also show that clinical samples were stable for at least one week in UTM/VTM at room temperature. As soon as we started the clinical testing with our assay, the positivity rate during the first month was 33.3%.

The change in the clinical presentation of MPXV during the current outbreak is perplexing. In contrast to the prototypic monkeypox in areas of endemicity, the clinical presentations during the current outbreak largely lack a prodrome of symptoms with primarily lesions in the genital area [14–17]. With the spectrum of infections that could associate with genital and skin lesions, the availability of laboratory diagnostics that could be offered promptly upon clinical suspicion is most valuable for proper patients’ management and infection control purposes. Even though, a capacity for testing was established by the CDC through the LRN [10], as was demonstrated by the COVID-19 pandemic, on site testing facilitates larger scale screening and quicker turnaround of the results. The remarkably high viral loads in lesion swabs emphasize the critical need for early diagnosis and patients’ isolation [18].

Transport of samples collected for OPXV/MPXV testing per CDC guidelines has been to refrigerate at 2-8°C or to store frozen at -20°C within one hour of sample collection ([19] last accessed 23 Aug 2022). As a part of our validation, we wanted to explore the specimens’ stability at room temperature to facilitate transport from certain collection locations that don’t have access to low temperature storage. Our data showed that samples were stable for at least one week at room temperature without any compromise of the relative viral loads.

Validating OPXV/MPXV tests clinically was challenged by the limited access to control materials and clinical samples from patients. Our analytical validation was followed by confirmation of our first positives by the local Department of Health (DOH) and/or the CDC using the MPXV specific PCR assay. Even though OPXV PCR is sufficient for monkeypox diagnosis in suspected cases ([20] last accessed 23 Aug 2022), we are developing a MPXV specific assay that will be multiplexed with the non-variola orthopoxvirus test.

The recommended specimen type for monkeypox molecular diagnosis has been lesion material. Scabs of lesions have been an alternative sample type that was previously shown to harbor high viral load [11]. Validation of alternative sample types that include throat swabs or rectal swabs might have clinical utility in certain situations when collecting lesion material is not possible. Studies have shown that MPXV can be isolated from other body sources that included saliva, semen, and urine.

Table 4

| Day | Replicate | Ct       | Average | SD  | Difference from Day 1 | Total Average | Total SD |
|-----|-----------|----------|---------|-----|-----------------------|---------------|----------|
| D1  | 1         | 29.11    | 29.28   | 0.12| N/A                   | 29.12         | 1.67     |
|     | 2         | 29.41    |         |     |                       |               |          |
|     | 3         | 29.44    |         |     |                       |               |          |
|     | 4         | 29.25    |         |     |                       |               |          |
|     | 5         | 29.16    |         |     |                       |               |          |
|     | 6         | 29.28    |         |     |                       |               |          |
| D2  | 1         | 28.34    | 30.13   | 3.84| -0.86                 |               |          |
|     | 2         | 38.72    |         |     |                       |               |          |
|     | 3         | 28.40    |         |     |                       |               |          |
|     | 4         | 28.46    |         |     |                       |               |          |
|     | 5         | 28.17    |         |     |                       |               |          |
|     | 6         | 28.71    |         |     |                       |               |          |
| D3  | 1         | 28.21    | 28.74   | 0.25| 0.54                  |               |          |
|     | 2         | 28.83    |         |     |                       |               |          |
|     | 3         | 28.95    |         |     |                       |               |          |
|     | 4         | 28.88    |         |     |                       |               |          |
|     | 5         | 28.84    |         |     |                       |               |          |
|     | 6         | 28.72    |         |     |                       |               |          |
| D5  | 1         | 28.46    | 28.54   | 0.50| 0.73                  |               |          |
|     | 2         | 29.00    |         |     |                       |               |          |
|     | 3         | 28.74    |         |     |                       |               |          |
|     | 4         | 27.50    |         |     |                       |               |          |
|     | 5         | 28.61    |         |     |                       |               |          |
|     | 6         | 28.96    |         |     |                       |               |          |
| D6  | 1         | 28.84    | 28.83   | 0.06| 0.45                  |               |          |
|     | 2         | 28.74    |         |     |                       |               |          |
|     | 3         | 28.80    |         |     |                       |               |          |
|     | 4         | 28.80    |         |     |                       |               |          |
|     | 5         | 28.84    |         |     |                       |               |          |
|     | 6         | 28.95    |         |     |                       |               |          |
| D7  | 1         | 29.25    | 29.21   | 0.23| 0.07                  |               |          |
|     | 2         | 29.30    |         |     |                       |               |          |
|     | 3         | 29.17    |         |     |                       |               |          |
|     | 4         | 29.26    |         |     |                       |               |          |
|     | 5         | 29.51    |         |     |                       |               |          |
|     | 6         | 28.76    |         |     |                       |               |          |
Table 5
Clinical parameters for the 33 patients tested during the first month of testing.

| OPXV positive | OPXV negative |
|---------------|---------------|
| Total (n=33)  | 11            | 22            |
| Gender        |               |               |
| Male          | 11            | 11            |
| Female        | 0             | 11            |
| Age Range (average) | 27-43 (34.9) | 2 weeks-72 (34.6) |
| Reason For Testing |           |               |
| Known Exposure | 4             | 1             |
| Rash          | 11            | 22            |
| Genitals      | 11            | 2             |
| Arms          | 8             | 8             |
| Legs          | 5             | 6             |
| Face          | 5             | 5             |
| Trunk         | 4             | 4             |
| Hands         | 3             | 5             |
| Feet          | 0             | 6             |
| Other Symptoms| 11            | 9             |
| Malaise       | 8             | 5             |
| Fever         | 5             | 6             |
| Groat Pain    | 5             | 0             |
| Rectal Bleeding | 3            | 0             |
| Pharyngitis   | 3             | 1             |
| Known Previous or Concurrent Infection | | |
| Eczema        | 0             | 2             |
| Human Immunodeficiency virus | 5 | 4 |
| Herpes simplex virus 1/2 | 1 | 0 |
| *Chlamydia trachomatis* | 6 | 0 |
| *Neisseria gonorrhoeae* | 2 | 2 |
| Treponema pallidum | 2 | 0 |
| Newly Diagnosed Infections (total tested) | | |
| Human Immunodeficiency virus | 0 (5) | 0 (2) |
| Herpes simplex virus 1/2 | 0 (3) | 0 (3) |
| Varicella zoster virus | 0 (3) | 0 (3) |
| *Chlamydia trachomatis* | 0 (7) | 0 (4) |
| *Neisseria gonorrhoeae* | 0 (7) | 0 (4) |
| Treponema pallidum | 0 (6) | 0 (5) |
| Travel History | | |
| Florida       | 1             | N/A           |
| Mexico        | 1             | N/A           |
| None          | 9             | N/A           |
| MPXV Treatment |               |               |
| Isolation     | 11            | N/A           |
| Antivirals*** | 9             | N/A           |

* Indicates travel within two weeks of symptoms
*** Indicates travel prescription of Tecovirimat (Tpoxx).

in addition to rectal and nasopharyngeal swabs [21]. A large clinical validation that evaluates samples collected from different patient populations is required for a better understanding of viral shedding in these sample types prior to utilizing them for diagnosis.

In conclusion, the in-house MPXV molecular assay facilitated an easy access to testing. The relative viral loads of the diagnosed patients' lesion swabs were consistently high and their clinical presentation was consistent with monkeypox. Incorporating an internal control human gene was instrumental in identifying potential false negative results due to insufficient sample collections [22]. We did not restrict the access to ordering and the turnaround time was less than 24 hours. With its excellent analytical performance, the non-variola orthopoxvirus PCR testing is valuable for monkeypox diagnosis.

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Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials
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References
[1] AM McCallum, IK. Damon, Human monkeypox, Clin. Infect. Dis. 58 (2014) 260–267.
[2] SS Marennikova, EB Gurvich, EM. Shelukhina, Comparison of the properties of five pox virus strains isolated from monkeys, Arch. Gesamte. Virusforsh. 33 (1971) 201–210.
[3] ID Ladnji, P Ziegler, E. Kima, A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo, Bull. World Health Organ. 46 (1972) 593–597.
[4] Centers for Disease C, Prevention, Multistate outbreak of monkeypox- Illinois, Indiana, and Wisconsin, 2003, MMWR Morb. Mortal. Wkly. Rep. 52 (2003) 537–540.
[5] EM Beer, VB. Rao, A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy, PLoS Negl. Trop. Dis. 13 (2019), e0007791.
[6] E Alakunle, U Moen, G Nchinda, M. Okeke, Monkeypox virus in Nigeria: infection biology, epidemiology, and evolution, Viruses 12 (2020).
[7] WHO, https://www.who.int/emergencies/news-events/monkeypox-oubrreak-2022.
[8] CDC, https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html.
[9] L. The. Monkeypox: a global wake-up call, Lancet 400 (2022) 337.
[10] TA Aden, P Plevin, SW York, S Rager, D Balachandran, CL Hutson, D Lowy, CN Mangal, T Wolford, A Matheny, W Davidson, K Wilkins, R Cook, RM Rolou, MK White, L Berman, J Murray, J Laurance, D Francis, N Green, AR Berumen 3rd, A Gonzalez, S Evans, M Hudzace, D Noel, M Adjie, G Hovan, P Lee, L Tate, RB Rose, G Voermans, J Crew, PR Adam, D Haydel, S Lukula, N Mastik, S Shah, J Featherstone, D Ware, D PET, E McCutchen, E Achangpong, E Batty, A Gorzalski, M Perry, R Fowler, LB Rose, R Nickla, H Ruaud, A Moore, et al., Rapid diagnostic testing for response to the monkeypox outbreak - laboratory network, United States, May 17–June 30, 2022, MMWR Morb. Mortal. Wkly. Rep. 71 (2022) 904–907.
[11] Y Li, VA Olson, T Lane, MT Laker, IK. Damon, Detection of monkeypox virus with real-time PCR assays, J. Clin. Virol. 36 (2006) 194–203.
[12] L Wang, J Shang, S Weng, SR Aliyari, C Ji, G Cheng, A. Wu, Genomic annotation and molecular evolution of monkeypox virus outbreak in 2022, J. Med. Virol. (2022), https://doi.org/10.1002/jmv.28036.
[13] NGF Chen, C Chagusa, I Gagne, M Doocette, S Smole, E Busby, J Hall, S Ash, R Harrington, S Cofsky, S Clancy, CJ Kapsak, J Sevinsky, K Libuit, DJ Park, P Hemarajata, JM Garrigues, NM Green, S Sierra-Parv, K Carpenter-Axvedo, RC Huard, C Pearson, K Inceara, C Nishimura, JP Huang, E Gagno, E Rieever, J Raneg, A Muyumbwe, V Borges, F Rehere, D Sobral, S Duarte, D Santos, L Vieria, JP Gomes, C Aquino, IM Savino, K Felton, M Bajwa, N Hayward, H Miller, A Naumann, R Allman, N Greer, A Fall, H H Mohc, MP McHugh, DM Maloney, R Dewar, et al., Multi-site validation of an amplicon-based sequencing approach for human monkeypox virus, medRxiv (2022), https://doi.org/10.1101/2022.10.14.22280783.
[14] D Philpott, CM Hughes, KA Alroy, JL Kerins, A McOwan, V Tittle, K Gedela, C Scott, SA Johnson, E Ortega, L Saathoff-Huber, A Syed, A Wills, RJ Anderson, AM Oster, A Christie, et al., Epidemiologic and Clinical Characteristics of Monkeypox Cases - United States, May 17–July 22, 2022, MMWR Morb. Mortal. Wkly. Rep. 71 (2022) 1018–1022.
[15] N Kumar, A Acharya, HE Gendelman, SN. Byrareddy, The 2022 outbreak and the pathobiology of the monkeypox virus, J. Autoimmun. 131 (2022), 102855.
[16] N Girometti, R Byrne, M Bracchi, J Heskin, A McOwan, V Tittle, K Gedela, C Scott, S Patel, J Gohl, D Nugent, T Suchak, M Dickinson, M Feeney, B Morra-Perez, K Stegmann, K Plaha, G Davies, LSP Moore, N Mughal, D Asboe, M Boffito, R Jones, G. Whitlock, 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), https://doi.org/10.1016/s1473-3099(22)00411-x.
[17] M Harril, S Jouni, MM Alibat, S Alaidi, Z. Alshehahi, The outbreak of monkeypox 2022: An overview, Ann. Med. Surg. 79 (2022) 104069. Lond.
[18] D Nierz, HT Tang, P Fennich, K Giersch, N Fischer, S Schmiedel, MM Addo, M Aepfelbacher, S Pfefferle, M Lütgeharmann, Rapid adaptation of established high-throughput molecular testing infrastructure for monkeypox virus detection, Emerg. Infect. Dis. 28 (2022) 1765–1769.
[19] CDC. https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html.

[20] WHO. https://apps.who.int/iris/bitstream/handle/10665/354488/WHO-MPX-Laboratory-2022.1-eng.pdf.

[21] A Peiro-Mestres, I Fuertes, D Campriu-Ferrer, MA Marcos, A Vilella, M Navarro, I. Rodriguez-Elena, J Riera, A Catala, MJ Martinez, JL Blanco, Hospital Clinic de Barcelona Monkeypox Study G, Frequent detection of monkeypox virus DNA in saliva, semen, and other clinical samples from 12 patients, Barcelona, Spain, May to June 2022, Euro. Surveill. 27 (2022).

[22] HH. Mostafa, Importance of internal controls to monitor adequate specimen collection: The case of orthopoxvirus real-time PCR, J. Clin. Virol. 156 (2022), 105294.