Guidelines for the Laboratory Diagnosis of Monkeypox in Korea

Ki Ho Hong, M.D., Gab Jung Kim, Ph.D., Kyoung Ho Roh, M.D., Hyukmin Lee, M.D., Ok Kyu Park, M.S., Taek Soo Kim, M.D., Jae-Seok Kim, M.D., Jaehyeon Lee, M.D., Moon-Woo Seong, M.D., So Yeon Kim, M.D., Jae-Sun Park, Ph.D., Younhee Park, M.D., Hee Jae Huh, M.D., Namhee Ryoo, M.D., Hyun Soo Kim, M.D., Heungsup Sung, M.D., and Cheon Kwon Yoo, Ph.D.; On behalf of the Committee of Management of Laboratory Tests for Infectious Diseases, Korean Society for Laboratory Medicine, and the Bureau of Infectious Disease Diagnosis Control, Korea Disease Control and Prevention Agency

While the coronavirus disease 2019 pandemic is ongoing, monkeypox has been rapidly spreading in non-endemic countries since May 2022. Accurate and rapid laboratory tests are essential for identifying and controlling monkeypox. Korean Society for Laboratory Medicine and the Korea Disease Prevention and Control Agency have proposed guidelines for diagnosing monkeypox in clinical laboratories in Korea. These guidelines cover the type of tests, selection of specimens, collection of specimens, diagnostic methods, interpretation of test results, and biosafety. Molecular tests are recommended as confirmatory tests. Skin lesion specimens are recommended for testing in the symptomatic stage, and the collection of both blood and oropharyngeal swabs is recommended in the presymptomatic or prodromal stage.

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Corresponding author:
Heungsup Sung, M.D.
Department of Laboratory Medicine
Asan Medical Center and University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea
Tel: +82-2-3010-4499
Fax: +82-2-478-0884
E-mail: sung@amc.seoul.kr

Co-corresponding author:
Cheon Kwon Yoo, Ph.D.
Bureau of Infectious Disease Diagnosis Control, Korea Disease Control and Prevention Agency, Osong Health Technology Administration Complex, 187 Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju 28159, Korea
Tel: +82-43-719-8100
Fax: +82-43-719-8149
E-mail: ckyoo@korea.kr

*These authors contributed equally to this study.

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INTRODUCTION

While the coronavirus disease 2019 pandemic is ongoing, monkeypox has been rapidly spreading in non-endemic countries worldwide since May 2022 [1–6]. As of June 22, 2022, more than 3,000 cases have been reported worldwide [7].

Accurate and rapid laboratory tests are essential for identifying and controlling monkeypox, and guidelines for laboratory testing are needed for clinical laboratories. Korean Society for Laboratory Medicine (KSLM) and the Korea Disease Control and Prevention Agency (KDCA) have drafted guidelines for the laboratory diagnosis of monkeypox in Korea based on current scientific evidence and expert opinions. Current outbreaks of monkeypox show different epidemiological and clinical characteristics from those in the past, and numerous facts remain to be elucidated [1, 3-6, 8]. Therefore, the contents of these guidelines may be revised based on future scientific evidence and expert review.

CASE DEFINITION

Case definition is based on the guidelines for the response to monkeypox released by the KDCA [9]. It may be changed based on updated information from the KDCA in the future.

Confirmed case
Cases with confirmed infection according to the laboratory criteria* and clinical symptoms consistent with monkeypox.

*Laboratory criteria: Detection of monkeypox-specific genes in human specimens (including skin swab, exudate, crust, and blood specimens).

Suspected case
Cases with suspected monkeypox presenting both clinical symptoms and epidemiological associations, but without the results that meet the laboratory criteria.

Clinical signs
Acute onset of fever (38.5°C or higher), headache, lymphadenopathy, back pain, myalgia, and asthenia (profound weakness). Centrifugal skin lesions that spread from the face to other parts of the body (palms and soles). Differential exclusion of common causes of acute rash (such as varicella zoster, herpes zoster, measles, Zika virus infection, dengue, chikungunya, herpes simplex virus infection, bacterial skin infections, disseminated gonococcus infection, primary or secondary syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale, molluscum contagiosum, and allergic reactions) that cannot fully explain the clinical status of the patient.

Epidemiological associations
In the 21 days before symptom onset: (1) contact with a confirmed or suspected case of monkeypox, (2) reported travel history to a monkeypox endemic country or a non-endemic country with identified monkeypox cases, (3) multiple or anonymous sexual partners, or (4) contact with wild animals or pets from a monkeypox endemic country.

TYPES OF TESTS

Molecular tests
Molecular tests (also known as nucleic acid amplification tests) detect monkeypox virus-specific genes and are the confirmatory tests for monkeypox [10]. Orthopoxvirus PCR, which targets genes common to other orthopoxviruses (e.g., smallpox, vaccinia), can only be used as a screening test, and not as a confirmatory test [11]. Monkeypox can also be confirmed via detection of monkeypox virus-specific genes by sequencing the PCR products [12].

The identification of other infectious diseases may be helpful for differential diagnosis of monkeypox [13]. However, the presence of other pathogens in skin lesions does not rule out monkeypox virus infection. Several cases of co-infection of monkeypox virus with other infectious agents have been reported [14-16]. Therefore, even if other pathogens are identified in suspected cases, monkeypox should not be excluded. Epidemiological associations and clinical signs should be considered before excluding monkeypox.

Virus isolation
Although virus isolation can serve as a confirmatory test, it cannot be used in medical institutions because the isolation process is complex and the sensitivity is too low. Moreover, practices and facilities with biosafety level (BSL)-3 or higher are required for virus isolation.

Antibody tests
Most antibody tests used for monkeypox detect antibodies that react with orthopoxviruses other than the monkeypox virus and cannot be used as confirmatory tests [10, 17, 18]. However, antibody tests may be considered for serosurveillance studies and
suspected cases when skin lesions have healed. The orthopoxvirus antibody test has shown cross-reactivity in smallpox-vaccinated patients [19].

**Indications for molecular tests [20, 21]**

Molecular tests for monkeypox may be performed to (1) confirm cases of suspected monkeypox, (2) decide the release of patients with confirmed monkeypox infection from the hospital, and (3) screen asymptomatic individuals in close contact with confirmed patients.

**Requirements for molecular tests**

Real-time PCR is the most widely used molecular test for monkeypox, but conventional PCR can also be used [22, 23]. Multiplex PCR, which tests for both monkeypox and other pathogens, or clade-specific PCR developed for discriminating clades of monkeypox, can also be used as confirmatory tests [24, 25].

As of June 20, 2022, no molecular test for monkeypox has been authorized for in vitro diagnostics by any government or regulation agency. Additionally, there are limited scientific data for determining the optimal protocol among various monkeypox molecular test protocols. Therefore, we do not limit the type of molecular test, location, or number of target genes used. However, the following must be considered:

- All molecular tests for confirmatory purposes must detect a target gene specific to the monkeypox virus.
- All molecular tests must be evaluated for analytical and clinical performance before use. If a molecular test that has not been evaluated for clinical performance is used as a confirmatory test, it should be authorized for emergency use by the regulatory agency of the country. It should be stated that clinical performance evaluation has not been performed and countermeasures must be provided.
- When performing tests, both positive and negative controls should be included in each run.

**SPECIMEN TYPES, SELECTION, AND COLLECTION**

**Types of specimens**

The type of specimen to be collected depends on the stage of infection (Table 1) [13, 20, 26, 27]. Collection of a sufficient amount of clinical specimens using appropriate methods is recommended. It is also recommended that specimens of multiple lesions at different sites are collected in a single container. Only one specimen type should be collected in a container. For example, swabs should be collected with swabs and crusts with crusts.

If a patient has no skin lesions, but monkeypox is suspected based on epidemiological and clinical findings, it is recommended to collect both blood (plasma) and oropharyngeal swabs (OPS). In these two specimen types, the virus can be detected during the incubation or prodromal period; however, because of their potential low viral load, it is recommended to collect both types of specimens.

More detailed information on specimen containers, transport conditions, and storage periods is provided in Table 2 [20, 28].

**Skin lesions**

Swabs, exudates, roofs, crusts, and tissue specimens can be collected.

**Blood and OPS**

If the patient has no skin lesions, both blood and OPS should have adequate viral load for detection.

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**Table 1. Recommended specimen types and laboratory tests for the diagnosis of monkeypox according to the disease stage**

| Stage (duration)       | Symptoms                                      | Specimen type                                      | Test          |
|------------------------|-----------------------------------------------|----------------------------------------------------|---------------|
| Incubation period (7–17 days) | No symptom or transient fever                  | Required: blood (plasma in EDTA tube), OPS         | PCR           |
| Prodromic stage (1–4 days) | General symptoms (fever, myalgia, lymphadenopathy) | Required: blood (plasma in EDTA tube), OPS         | PCR           |
| Symptomatic stage (14–28 days) | Skin lesions and general symptoms              | Required: skin specimens (swabs, exudate, roofs, crusts, biopsy) | PCR | Optional: blood (plasma in EDTA tube), OPS |
| Convalescent           | Loss of skin lesions                           | Blood (serum)                                      | Antibody test |

Abbreviation: OPS, oropharyngeal swabs.

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be collected. Plasma collected in an EDTA tube is recommended for blood specimens. As blood contains only a small number of virus particles, a sufficient amount of blood should be collected [29]. If the patient shows skin lesions, it is not necessary to collect blood or OPS specimens for diagnostic purposes.

Other specimens
There is limited evidence of the value of other specimens (e.g., urine and stool) for confirmatory tests. The diagnostic value and ethical issues should be carefully considered before collection of such specimens. Anorectal swabs can be considered in patients with risk factors, as recent data have shown good sensitivity among them [30, 31].

Procedures for specimen collection
Specimens should be collected by healthcare professionals [32]. When collecting specimens, personnel should wear personal protective equipment (PPE; N95 or KF94 mask, disposable gloves, gown [clean, covering the entire body, long sleeves, back closure], and eye protection [goggles or face shield]).

When collecting specimens from skin lesions, the lesion should first be disinfected with an appropriate disinfectant (e.g., 70% ethanol), and the roof of the lesion should be removed using a lancet, scalpel, scraper, or needle and collected into a sterile container [20, 33]. The base of the lesion under the roof should be collected by vigorous rubbing with a sterile swab. If the lesion has turned into a crust, the crust should be removed and collected in a sterile container. Specimens should be collected from two or more different sites and combined in sterile containers according to the specimen type (roof, swab, or crust). For example, if roofs and swabs are collected from two sites, the roofs should be collected in one container and the swabs in another.

Blood specimens and OPS should be collected using the same collection techniques used for general patients, while wearing PPE.

### Table 2. Specimen types suitable for monkeypox testing and transport and storage conditions

| Specimen type | Container | Transport conditions | Storage conditions | Comments |
|---------------|-----------|----------------------|--------------------|---------|
| Skin lesion   | Dacron or flocked swabs in VTM Sterile plastic tube (screw-capped with O-ring) | 4°C | Within 7 days: 4°C Longer than 7 days: –20°C | Inactivating agent-containing transport medium (e.g., a chaotropic agent) can be used for molecular tests, but is unacceptable for virus isolation |
| Blood         | EDTA tube  | 4°C | Within 7 days: 4°C Longer than 7 days: –20°C | Not recommended for antibody tests |
| OPS           | Dacron or flocked swabs in VTM, sterile plastic tube (screw-capped with O-ring) | 4°C | Within 7 days: 4°C Longer than 7 days: –20°C | Inactivating agent-containing transport medium (e.g., a chaotropic agent) can be used for molecular tests but is unacceptable for virus isolation |

Abbreviations: VTM: viral transport medium; OPS, oropharyngeal swabs.

### SPECIMEN PACKAGING AND TRANSPORT

Transport within medical institutions
Please refer to the Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea [28].

External transport
The triple packaging system Category B packaging instructions (UN P650) should be used for clinical specimens. Category A packaging instructions (UN P620) should be used for cultured virus isolates. For detailed guidelines, please refer to the latest version of the Guidance on Regulations for the Transport of Infectious Substances issued by the WHO (https://www.who.int/) and the guidelines for laboratory biosafety related to monkeypox from the KDCA [34, 35].

### SPECIMEN HANDLING AND TEST PROCEDURES

If smallpox vaccination for laboratory personnel is difficult to achieve, it is recommended that the specimen be inactivated in a BSL-3 laboratory or a laboratory with negative pressure. The specimen can be handled in a BSL-2 laboratory using appropriate PPE. For detailed guidelines, please refer to the Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea [28].

### TEST INTERPRETATION

General considerations
The results of both positive and negative controls should be valid for each run. In case of invalid control results, a retest is necessary regardless of the target gene and internal control amplification [28]. Negative results can only be confirmed if the internal control is amplified. If the internal control is negative, the result...
is invalid, and a retest is necessary. Positive results can be confirmed regardless of the internal control results. For molecular tests that detect multiple target genes, positive results can only be confirmed if all target genes are detected. The results are inconclusive when only some of the target genes are detected. For real-time PCR, when the threshold cycle (Ct) value of the target gene is ≤ cut-off Ct value, the target gene test should be considered positive regardless of internal control amplification. When no target gene is detected or the Ct value is > cut-off Ct value, the gene test should be considered negative regardless of internal control amplification. It should be noted that values close to the cutoff values in specimens with low viral loads may indicate false-negative or false-positive results. Thus, laboratory physicians should check the results and, if necessary, conduct a retest using residual or new specimens.

Considerations for retesting
As the monkeypox incubation period is variable, it is difficult to rule out monkeypox based on negative results from blood or OPS alone [13, 20, 26]. If a single pair of blood and OPS specimens tests negative for monkeypox, but the case is highly suspicious based on clinical and epidemiological findings, the specimens should be recollected at intervals. Even for skin lesion specimens, there is the possibility of false negatives if specimen collection is inappropriate or if the specimen was collected too early or too late.

Retesting is recommended for cases suspicious of both false-negative and false-positive results. It is not sufficient to retest the extracted nucleic acids. It is recommended to re-extract nucleic acids from clinical specimens or collect new clinical specimens. However, the procedure for collecting skin lesion specimens is invasive and it may be necessary to collect several specimens at the initial collection, including specimens for the retest.

REPORT
The final report should include the following contents. Preliminary reports or text messages might only include minimal contents, such as patient name, patient number, test order date, and results.

Basic information
Patient name, age (date of birth), sex, patient number (hospital number), specimen number, ward, test order date, specimen type, and specimen collection time should be included [34].

Results
- Negative
- Inconclusive: a retest using new specimens is recommended.
- Positive: should be reported to public health authorities within 24 hours.

Note
Any unusual findings, such as those regarding specimen quality.

Time of report
LABORATORY GUIDELINES FOR BIOSAFETY AND INFECTION CONTROL

For general information, please refer to the Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea [28].

Monkeypox biosafety measures [20, 37, 38]
The following procedures should be performed only in BSL-3 or higher facilities:
- Virus isolation from cell culture
- Manipulation of live virus isolates

The following procedures should be performed only in BSL-2 or higher facilities and in a certified Class II or higher biosafety cabinet (BSC):
- Aliquoting or diluting specimens
- Inoculating bacterial or mycological culture media
- Diagnostic testing that does not involve viral agent propagation
- Nucleic acid extraction from infectious specimens
- Preparation and chemical- or heat-fixing of smears for microscopic testing
- Rapid antigen tests on urine or respiratory specimens

The following procedures should be performed only in BSL-2 or higher facilities:
- Pathological examination of formalin-fixed or otherwise inactivated specimens
- Molecular testing of extracted nucleic acid preparations
- Electron microscopic examination of glutaraldehyde-fixed specimens
- Routine examination of bacterial and fungal cultures
- Microscopy of fixed specimens
- Final packaging of infectious specimens sealed in a secondary container
- Procedures using inactivated specimens (e.g., specimens in nucleic acid extraction buffer)

Routine tests (including hematological tests and biochemical tests):
- During routine tests (e.g., hematological, biochemical, and serological tests using blood, serum, or urine specimens), after performing a risk assessment of the infectious aerosol, the specimens can be handled in the same way as general clinical specimens in appropriate cases.
- In general, decapping is considered a low-risk procedure. However, risk determination depends on the design of the lid and container. The decision to proceed with the testing should be based on a risk assessment that considers the need for centrifuging, mixing, and aliquoting. When there is a high risk, the use of a BSC should be considered.
- Point-of-care testing, such as blood gas analysis, should be performed once safety is confirmed through risk assessment.
- Automated analyzers should be sterilized according to the laboratory manual after specimen treatment or before regular maintenance checks.
- Counting cells or making smears using body fluids other than blood (e.g., cerebrospinal fluid) should be conducted in a certified BSC.

Vaccination
Laboratory personnel performing monkeypox tests using skin lesion specimens should consider receiving the smallpox vaccine [39-41]. Vaccine types, characteristics, availability, and possible adverse effects should be considered in addition to the domestic monkeypox outbreak situation while making decisions regarding the vaccination protocol for laboratory personnel. The smallpox vaccine is not recommended for personnel handling and processing routine clinical specimens from patients with monkeypox (e.g., urine for urinalysis, blood for complete blood count, chemistry, microbiology) [38]. For the BSLs of individual procedures, please refer to the Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea [28].

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AUTHOR CONTRIBUTIONS
Hong KH and Kim GJ reviewed the literature on current general recommendations and wrote the manuscript. Sung H and Yoo CK contributed to the general concept and design of the guidelines. Roh KH, Kim J-S, and Lee H evaluated the current domestic situation of monkeypox and specimen collection. Kim TS and Park OK contributed to the recommendations for biosafety. The other authors collated protocols, interpreted the results, and contributed to the recommendations. All authors reviewed the guidelines and approved the submission of the final manuscript.

CONFLICTS OF INTEREST
No potential conflicts of interest relevant to this article were reported.

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ORCID
Kí Ho Hong https://orcid.org/0000-0002-5700-9036
Gab Jung Kim https://orcid.org/0000-0002-6284-428X
Kyoung Ho Roh https://orcid.org/0000-0002-6291-9229
Hyukmin Lee https://orcid.org/0000-0002-8523-4126
Hee Jae Huh https://orcid.org/0000-0001-8999-7561
Hyun Soo Kim https://orcid.org/0000-0002-7026-6715
Jae-Seok Kim https://orcid.org/0000-0001-6025-0341
So Yeon Kim https://orcid.org/0000-0003-1774-0382
Namhee Ryoo https://orcid.org/0000-0001-8383-709X
Younhee Park https://orcid.org/0000-0001-8458-1495
Taek Soo Kim https://orcid.org/0000-0002-2093-1721
Heungsup Sung https://orcid.org/0000-0002-6062-4451
Jaehyeon Lee https://orcid.org/0000-0003-3211-8903
Jae-Sun Park https://orcid.org/0000-0002-2746-9162
Ok Kyu Park https://orcid.org/0000-0002-3767-6835
Moon-Woo Seong https://orcid.org/0000-0003-2954-3677
Cheon Kwon Yoo https://orcid.org/0000-0002-8444-3620

REFERENCES
1. WHO. Multi-country monkeypox outbreak in non-endemic countries: update. https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON388 (Updated on May, 2022).
2. Minhaj FS, Ogale YP, Whitehill F, Schultz J, Foote M, Davidson W, et al. Monkeypox outbreak — nine states, May 2022. MMWR Morb Mortal Wkly Rep 2022;71:764-9.
3. Antinori A, Mazzotta V, Vita S, Carletti F, Tacconi D, Lapini LE, et al. Epi-
demographical, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, May 2022. Euro Surveill 2022;27:2000421.

4. Hammerschlag Y, MacLeod G, Papadakis G, Adan Sanchez A, Druce J, Taiaroa G, et al. Monkeypox infection presenting as genital rash, Australia, May 2022. Euro Surveill 2022;27:2000411.

5. Duque MP, Ribeiro S, Martins JV, Casaca P, Leite PP, Tavares M, et al. Ongoing monkeypox virus outbreak, Portugal, 29 April to 23 May 2022. Euro Surveill 2022;27:2000424.

6. Vivancos R, Anderson C, Blomquist P, Balasesgaram S, Bell A, Bishop L, et al. Community transmission of monkeypox in the United Kingdom, April to May 2022. Euro Surveill 2022;27:2000422.

7. Mathieu E, Spooner F, Dattani S, Ritchie H, Roser M. Monkeypox. https://ourworldindata.org/monkeypox (Updated on Jun, 2022).

8. WHO. Surveillance, case investigation and contact tracing for monkeypox: interim guidance, 24 June 2022. https://www.who.int/publications/i/item/WHO-MPX-surveillance-2022-2 (Updated on Jun, 2022).

9. KDCA. Guidelines on response to monkeypox. 1st ed. (for local governement). https://www.kdca.go.kr/board/board.es?mid=a20507020000&bid=0019&act=view&list_no=719914 (Updated on Jun, 2022).

10. Dubois ME and Stilfka MK. Retrospective analysis of monkeypox infection, Emerg Infect Dis 2008;14:592-9.

11. Li Y, Meyer H, Zhao H, Damon IK. GC content-based pan-panx universal PCR assays for poxvirus detection. J Clin Microbiol 2010;48:268-76.

12. Kulesh DA, Loveless BM, Norwood D, Garrison J, Whitehouse CA, Hartmann C, et al. Monkeypox virus detection in rodents using real-time 3’-minor groove binder TaqMan assays on the Roche LightCycler. Lab Insec 2004;84:1200-8.

13. McCollum AM and Damon IK. Human monkeypox. Clin Infect Dis 2014;58:260-7.

14. Bížová B, Veselý D, Trojánek M, Rob F. Coinfection of syphilis and monkeypox in HIV positive man in Prague, Czech Republic. Travel Med Infect Dis 2022;49:102368.

15. Hughes CM, Liu L, Davidson WB, Radford KW, Wilkins K, Monroe B, et al. A tale of two viruses: coinfections of monkeypox and varicella zoster virus in the Democratic Republic of Congo. Am J Trop Med Hyg 2020;104:604-11.

16. Hoff NA, Morier DS, Kisalu NK, Johnston SC, Doshi RH, Hensley LE, et al. Varicella coinfection in patients with active monkeypox in the Democratic Republic of Congo. Ecohealth 2017;14:564-74.

17. Kareem KL, Reynolds M, Braden Z, Lou G, Bernard N, Patton J, et al. Characterization of acute-phase humoral immunity to monkeypox: use of immunoglobulin M envelope-linked immunosorbent assay for detection of monkeypox virus infection during the 2003 North American outbreak. Clin Diagn Lab Immunol 2005;12:867-72.

18. Sejvar JJ, Chowdry Y, Schomoggi M, Stevens J, Patel J, Kareem K, et al. Human monkeypox infection: a family cluster in the midwestern United States. J Infect Dis 2004;190:1833-40.

19. Hammarlund E, Lewis MW, Carter SV, Amanna I, Hansen SG, Strelow LJ, et al. Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. Nat Med 2005;11:1005-11.

20. WHO. Laboratory testing for the monkeypox virus: interim guidance. https://www.who.int/publications/i/item/WHO-MPX-laboratory-2022-1 (Updated on May, 2022).

21. UK Health Security Agency. De-isolation and discharge of monkeypox-infected patients: interim guidance. https://www.gov.uk/guidance/de-isolation-and-discharge-of-monkeypox-infected-patients-interim-guidance (Updated on May, 2022).

22. Li Y, Olson VA, Laue T, Laker MT, Damon IK. Detection of monkeypox virus with real-time PCR assays. J Clin Virol 2006;36:194-203.

23. Shchelkunov SN, Gavrilova EV, Babiinn IV. Multiplex PCR detection and species differentiation of orthopoxviruses pathogenic to humans. Mol Cell Probes 2005;19:1-8.

24. Maksyutov RA, Gavrilova EV, Shchelkunov SN. Species-specific differentiation of variola, monkeypox, and varicella-zoster viruses by multiplex real-time PCR assay. J Virol Methods 2016;236:215-20.

25. Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. J Virol Methods 2010;169:223-7.

26. Sklenovská N. Monkeypox virus. In: Malik YS, Singh RK, et al., eds. Animal-origin viral zoosizes. Springer, 2020;39-68.

27. Centers for Disease Control and Prevention. Monkeypox. Preparation and collection of specimens. https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html (Updated on Jun, 2022).

28. Hong KH, Lee SW, Kim TS, Huh HJ, Lee J, Kim SY, et al. Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea. Ann Lab Med 2020;40:351-60.

29. Adler H, Gould S, Hine P, Snell LB, Wong W, Howlian CF, et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. Lancet Infect Dis 2022;22:1153-62.

30. Peiró-Mestres A, Fuertes I, Campurbi-Ferrer D, Marcos MA, Villella A, Navarro M et al. Frequent detection of monkeypox virus DNA in saliva, semen, and other clinical samples from 12 patients, Barcelona, Spain, May to June 2022. Euro Surveill. 2022 Jul;27(28):2200503.

31. Tarín-Vicente EJ, Alemany A, Agud-Dios M, Ubals M, Sufer C, Antón A et al. Clinical presentation and virological assessment of confirmed human monkeypox virus cases in Spain: a prospective observational cohort study. Lancet. 2022 Aug 27;400(10353):661-669.

32. Centers for Disease Control and Prevention. Infection prevention and control of monkeypox in healthcare settings. https://www.cdc.gov/poxvirus/monkeypox/infectious-control-healthcare.html (Updated on May, 2022).

33. Miller JM and Miller SA. A guide to specimen management in clinical microbiology. 3rd ed. Hoboken: John Wiley & Sons, 2017.

34. WHO. Guidance on regulations for the transport of infectious substancies 2021-2022. https://www.who.int/publications/i/item/9789240019720 (Updated on Feb, 2021).

35. KDCA. Guidance for laboratory biosafety related to monkeypox. 1st ed. (Korean). https://www.kdca.go.kr/board/board.es?mid=a20302111500&bid=0065&act=view&list_no=719904 (Updated on Jun, 2022).

36. CLSI. Molecular diagnostic methods for infectious diseases. 3rd ed. CLSI MM03. Wayne, PA: Clinical and Laboratory Standards Institute, 2015.

37. Meechan PJ and Potts J, eds. Biosafety in microbiological and biomedical laboratories (BMBL). 6th ed. United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health, 2020. https://www.cdc.gov/labs/BMBL.html (Updated on Nov, 2021).

38. Centers for Disease Control and Prevention. Monkeypox. Laboratory procedures and biosafety guidelines. https://www.cdc.gov/poxvirus/monkeypox/lab-personnel/lab-procedures.html (Updated on Nov, 2021).

39. WHO. Vaccines and immunization for monkeypox: interim guidance, 14 June 2022. https://www.who.int/publications/i/item/who-mpx-immunization-2022.1 (Updated on Jun, 2022).

40. Rao AK, Petersen BW, Whitehill F, Razeq JH, Isaac KS, Merchnlinsky MJ, et al. Use of JYNNEOS (smallpox and monkeypox vaccine, live, nonreplicating) for preexposure vaccination of persons at risk for occupational exposure to orthopoxviruses: recommendations of the Advisory Committee on Immunization Practices – United States, 2022. MMWR
41. UK Health Security Agency. Guidance. Monkeypox vaccination recommendations. Recommendations for the use of pre and post-exposure vaccination during a monkeypox incident. https://www.gov.uk/government/publications/monkeypox-vaccination (Updated on Jun, 2022).