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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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## 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 gonococ-

cus 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.

- When performing tests, an internal quality control should be included to evaluate the test process.
- For rapid molecular tests, which are automated cartridge-based and usually do not include positive and negative controls, an internal control must be added to each reaction well containing a specimen.

## 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

**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) Optional: blood (plasma in EDTA tube), OPS	PCR
Convalescent	Loss of skin lesions	Blood (serum)	Antibody test

Abbreviation: OPS, oropharyngeal swabs.

**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.

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.

## 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  $\leq$  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 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, chemistries, 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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