Monkeypox Virus Antigen Rapid Test
(Whole Blood/Serum/Plasma/Oropharyngeal Swab/Exudate of Blain)

A rapid test for the qualitative detection of human monkeypox virus antigens in human whole blood/serum/plasma, oropharyngeal swab, and exudate of blain specimen.
For professional in vitro diagnostic use only.
Please read the package insert carefully before using.

【SPECIFICATION】
25 Tests/Kit

【INTENDED USE】
The iCARE Monkeypox Virus Antigen Rapid Test is a lateral flow immunoassay intended for the in vitro rapid, simultaneous qualitative detection of human monkeypox virus antigens in human whole blood/serum/plasma, oropharyngeal swab, and exudate of blain swab specimen to aid in the diagnosis of Monkeypox Virus infection.

【SUMMARY】
Monkeypox is a zoonotic orthopoxvirus that incidentally causes disease in humans similar to smallpox, although with notably lower mortality. This virus is clinically relevant because it is endemic to western and central Africa, with outbreaks in the Western Hemisphere related to the exotic pet trade and international travel.
Transmission can occur through contact with bodily fluids, skin lesions, or respiratory droplets of infected animals directly or indirectly via contaminated fomites. Although human-to-human transmission has previously been limited, mathematical modeling in the context of decreasing herd immunity to orthopoxviruses reflects an increasing threat of disease spread between humans. The Centers for Disease Control and Prevention (CDC) recommends isolation in a negative pressure room and standard, contact, and droplet precautions in the healthcare setting with escalation to airborne precautions if possible.
Following viral entry from any route (oropharynx, nasopharynx, or intradermal), the monkeypox virus replicates at the inoculation site then spreads to local lymph nodes. Next, an initial viremia leads to viral spread and seeding of other organs. This represents the incubation period and typically lasts 7to 14 days with an upper limit of 21 days.

【TEST PRINCIPLE】
The iCARE Monkeypox Virus Antigen Rapid Test is a qualitative, membrane-based immunoassay for the detection of Monkeypox Virus antigen in human whole blood/serum/plasma, oropharyngeal swab, and exudate of blain swab specimen.
The membrane is pre-coated with specific monoclonal antibodies in the test line region (T). During testing, the specimen reacts with the particle coated with anti-Monkeypox virus antibodies to form an antigen-antibody-gold complex. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-Monkeypox virus antibodies immobilized in the membrane and produce a colored line. The presence of this colored line in the test line region (T), indicates a positive result, while its absence indicates a negative result.
An internal quality control is included in the test, in the form of a colored line appearing in the control line region (C), indicating that the test is functional, and proper and sufficient volume of specimen has been applied to enable migration through the test and control lines, regardless of whether there is a test line or not. If the control line (C) does not appear within the testing time, test result is invalid and the test should be repeated with a new test card and specimen.

【MATERIALS PROVIDED】
- 25 x Test card individually foil pouched with a desiccant
- 25 x Extraction solution
- 25 x Sterile swab
- 25 x Dropper
- 1 x Package insert

【OPTIONAL MATERIALS】
- Blood lancet
- Alcohol pad

【MATERIALS REQUIRED BUT NOT PROVIDED】
Timer, Biohazard Container

【WARNINGS AND PRECAUTIONS】
1. For *in vitro* diagnostic use only.
2. Do not reuse the test.
3. Do not freeze the test kit or its components.
4. These instructions must be carefully read and strictly followed by a trained healthcare professional to achieve accurate results. All users should read the instructions before performing test.
5. Fresh samples are recommended for use to ensure optimal performance. Freshly collected specimens should be tested immediately.
6. Inadequate or inappropriate specimen collection, storage, and transportation are likely to result in false negative test results.
7. Do not eat, drink or smoke in the area where handling specimens or performing the test.
8. Do not use the test kit beyond its expiration date.
9. Do not mix components from different kit lots.
10. Leave test card sealed in its foil pouch until just before use. Do not use the test card if the pouch is damaged or the seal is broken.
11. To avoid contamination or inaccurate test result, do not touch the absorbent tip of swab or reaction area of test card when performing the test.
12. Dispose of all used test devices and potentially contaminated materials in a biohazard container as if they were infectious waste and dispose according to applicable local laws and regulations.

【STORAGE AND STABILITY】
1. The test kit should be stored at a temperature between 2-30°C, away from direct sunlight. Do not freeze the kit or its components.
2. The shelf life of the kit is as indicated on the outer package (24 months from date of manufacture).
3. This test kit is stable until the expiration date marked on the outer package and foil pouch. Ensure all test components are at room temperature (15-30°C) before use.

4. Perform the test immediately after taking out the test card from the foil pouch.

【Sample Collection and Preparation】

1. Plasma/Serum

- Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
- To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
- To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C if not tested immediately. Specimens can be stored at 2-8°C for up to 3 days, and should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles (no more than 3 times). Prior to testing, equilibrate frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

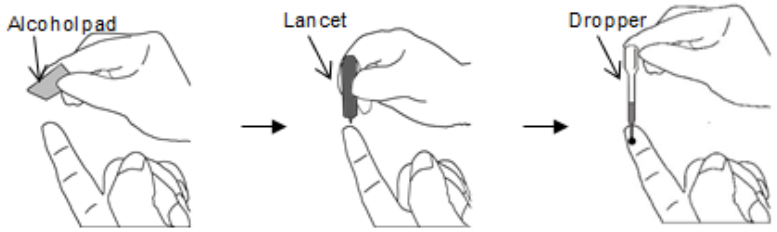
Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity so as to avoid interference on result interpretation.

2. Whole Blood

Collect whole blood by either fingertip puncture or by venipuncture into collection tube containing EDTA, citrate or heparin for plasma.

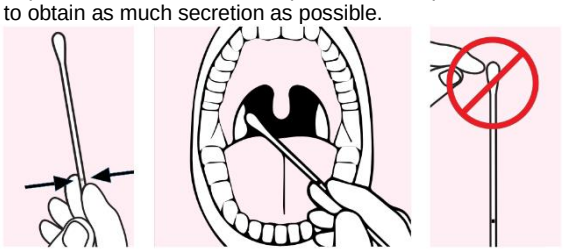
Do not use any hemolyzed blood for testing.

Do not freeze a whole blood specimen, otherwise the red blood cell will break, which may cause hemolysis. Whole blood specimens should be stored in refrigeration (2-8°C) if not tested immediately. The specimens must be tested within 24 hours after collection.



3. Oropharyngeal Swab

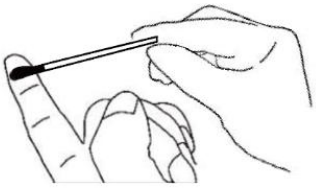
To achieve accurate test result, good sample collection is the most important first step. Therefore, carefully follow the instructions below to collect oropharyngeal swab specimens to obtain as much secretion as possible.



To collect the specimen, hold the swab with hand. Insert the swab into the mouth and collect the specimen from the bilateral posterior pharynx, both tonsils and the uvula. Withdraw the swab carefully.
Note: Do not touch the absorbent tip of swab before placing it into the collection tube.

4. Exudate of Blain

Have the patient leak the affected area of the monkeypox infection. Take the swab and rub it back and forth along the affected area four times, then turn the swab to the opposite side, and using the opposite side of the swab, rub back and forth along the affected area four times.



Specimen Storage

If the specimen cannot be tested immediately after collection, properly store the specimen in a Viral Transport Media (VTM) or Universal Transport Media (UTM) contained in a lidded storage container. The specimens contained in VTM or UTM can be stored for up to 72 hours when refrigerated (2~8℃) or frozen (-20℃).

【TEST PREPARATION】

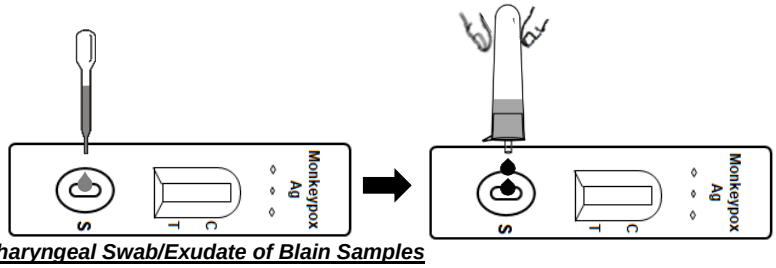
Before testing, open the package and equilibrate the test card, extraction solution and specimens to room temperature, and shake the extraction solution gently before use. The most suitable temperature condition to perform the test is room temperature (15~30℃). If the test kit is stored at room temperature, it can be opened and used immediately.

【TEST PROCEDURES】

For Whole blood/Serum/Plasma Samples

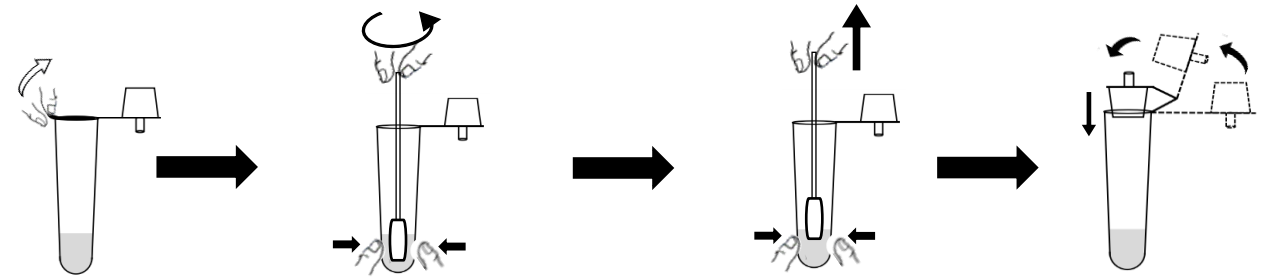
1. Take out the test device from sealed foil pouch and place on a dry, clean and level surface.
2. Fill the pipette dropper with the specimen. Hold the dropper vertically and transfer one drop of whole blood/serum/plasma specimen (approximately 30μL) into the well (S) making sure that there are no air bubbles. Then add two drops of extraction solution to the well (S) immediately. See illustration below.
3. Start the timer.

4. Read the results at 15 minutes, and the result after 30 minutes is no longer valid.



For Oropharyngeal Swab/Exudate of Blain Samples

1. Tear off the sealing film of the extraction solution tube.
2. Place the swab in the extraction solution tube, rotate the swab for about 10 times and no less than 15 seconds, and press the swab tip against the tube wall to release the antigen in the swab.
3. Squeeze the swab over the swab tip to withdraw the swab so as to extract as much liquid as possible from the swab. Dispose of used swabs according to biohazard waste disposal method and local regulations.
4. Install the dripper of the tube and shake well the tube.
5. Take out the test card from sealed foil pouch and place on a dry, clean and level surface. Add two drops of extracted specimen to the specimen well(S) on the test card, and start the timer.
6. Read the results at 15 minutes, and the result after 30 minutes is no longer valid.

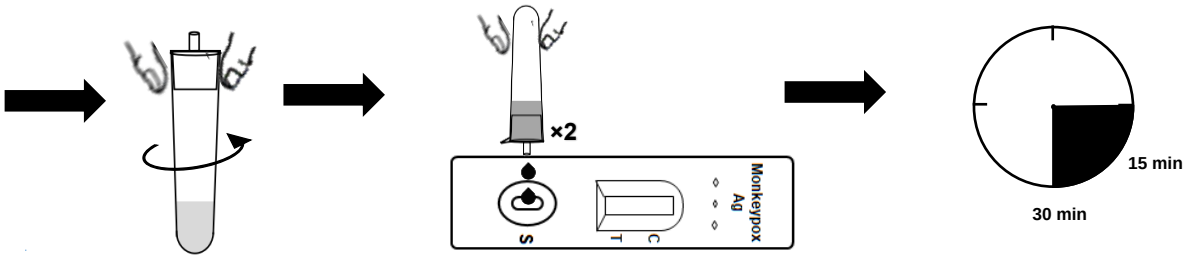


Tear off the sealing film.

Place the swab in the extraction solution tube, rotate the swab and press the swab tip against the tube wall to release the antigen in the swab.

Squeeze the swab over the swab tip to withdraw the swab so as to extract as much liquid as possible from the swab.

Install the dripper of the tube.



Shake well the tube.

Add 2 drops of extracted specimen to the specimen well(S) on the test card, and start the timer.

Read the results at 15-30 minutes, and the result after 30 minutes is no longer valid.

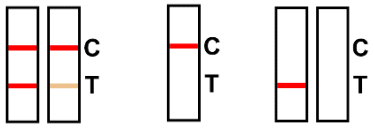
【INTERPRETATION OF TEST RESULTS】

(Please refer to the illustrations below)

POSITIVE: Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

NEGATIVE: One colored line appears in the control line region (C). No line appears in the test line region.

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, stop using the test kit immediately and contact your local distributor.



Positive **Negative** **Invalid**

【LIMITATIONS】

1. The test is only used for the qualitative detection of Monkeypox Virus antigen by healthcare professionals. The intensity of the test line does not have linear correlation with the antigen titer in the specimen.
2. The test does not indicate the quantity of Monkeypox Virus antigen in the specimen, and should not be used as the sole criteria for the diagnosis of infection with Monkeypox Virus.
3. A negative test result may occur if quantity of the Monkeypox Virus antigen present in a specimen is below the detection limits of the test, or if the antigens that are detected are not present during the stage of disease in which a sample is collected.
4. A negative or non-reactive result indicates that the Monkeypox Virus antigen is not present in the specimen. However, a negative or non-reactive result at any time does not preclude the possibility of exposure to or infection with Monkeypox Virus.
5. Infection may develop rapidly. If symptoms are suspicious or persist while test result from the iCARE Monkeypox Virus Antigen Rapid Test is negative or non-reactive, additional testing using alternative clinical methods is recommended.

6. Test results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.
7. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor (RF) may affect expected results. Even if the test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.

【PERFORMANCE CHARACTERISTICS】

1. Clinical Performance

The iCARE Monkeypox Virus Antigen Rapid Test has been tested with clinical specimens and compared with PCR as reference. Test results are presented in the table below.

iCARE Monkeypox Virus Antigen Rapid Test	PCR		
	Positive	**Negative**	**Total**
Positive	108	1	109
Negative	1	309	310
Total	109	310	419

Sensitivity (Positive Percent Agreement): 99.08% = 108/109 (95% CI: 94.99%~99.84%)
Specificity (Negative Percent Agreement): 99.67% = 309/310 (95% CI: 98.20%~99.94%)
Accuracy (Overall Percent Agreement): 99.52% = (108+309)/419 (95% CI: 98.28%~99.87%)

2. Cross-Reactivity

No cross-reactivity was observed by testing the following positive specimens respectively: HAMA, HBsAg, HBeAg, HBeAb, HBcAb, HCV, HIV, H. pylori, RF (≤2,500 IU/ml), and Syphilis.

3. Interference

The following potentially interfering substances were added to Monkeypox Virus negative and positive specimens, respectively. Test results demonstrate that performance of the iCARE Monkeypox Virus Antigen Rapid Test was not affected by the listed potentially interfering substances at the concentrations tested.

Acetaminophen	20 mg/dl	Caffeine	20 mg/dl
Ascorbic acid	20 mg/dl	Creatinine	200 mg/dl
Acetylsalicylic acid	20 mg/dl	Gentistic acid	20 mg/dl
Albumin	10.5 g/dl	Hemoglobin	1,000 mg/dl
Bilirubin	1,000 mg/dl	Oxilic acid	600 mg/dl
Cholesterol	800 mg/dl	Triglycerides	1,600 mg/dl

4. Precision

Intra Assay

Within‐run precision has been determined by using 10 replicates of twelve specimens containing negative, low positive and high positive samples. The negative and positive values were correctly identified >99% of the time.

Inter Assay

Between‐run precision has been determined by using the same twelve specimens of negative, low positive and high positive of 10 independent assays and with three different lots of iCARE Monkeypox Virus Antigen Rapid Test. The negative and positive values were correctly identified >99% of the time.

【REFERENCES】

1. Moore M, Zahra F. Monkeypox. 2022 May 22. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 34662033.

【INDEX OF SYMBOLS】

	Consult instruction for use	IVD	For *in vitro* diagnostic use only	REF	Catalog number		Temperature limit
LOT	Lot number		Use by		Do not reuse		Contains sufficient for <X> tests
	Keep dry		Manufacturer		Date of manufacture		Keep away from sunlight
	Do not use if package is damaged	CE	CE mark		Importer		Distributor
EC REP	Authorized Representative in the European Community		Country of manufacture	UDI	Unique device identifier		

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