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Monkeypox Virus Detection Kit (Fluorescence PCR)

Instructions for Use

48 tests/kit, 96 tests/kit
REF: AMPV01/ AMPV01D
For *In Vitro* Diagnostic Use
For Professional Use Only

Version 1.1 – June 2022

[Intended Use]

The Monkeypox Virus Detection Kit (Fluorescence PCR) is a qualitative *in vitro* real-time PCR test for the detection of nucleic acid from monkeypox virus in oropharyngeal swab, nasopharyngeal swab, serum, plasma and human pustular or vesicular rash specimens from individuals suspected of monkeypox virus infectious.

Results are for the identification of monkeypox virus DNA. Positive results are indicative of the presence of monkeypox virus DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude monkeypox virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Monkeypox Virus Detection Kit (Fluorescence PCR) is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

[Test Principle]

This test is a qualitative real-time fluorescent PCR enables to amplification of monkeypox virus (MPV) from oropharyngeal swab, nasopharyngeal swab, serum, plasma and human pustular or vesicular rash specimens. Specific primers & probes are designed to detect the highly specific regions of the F3L gene and B7R gene of monkeypox virus. A pair of primers and a probe for detecting endogenous human RNase P gene are included as an internal control to monitor the whole test process and control for inhibition. The specific probes of the F3L gene and B7R gene are labeled with FAM, and the specific probe of RNase P is labeled with VIC.

The virus DNA in the sample should be purified and enriched with a nucleic acid extraction and purification reagent before detected with this kit.

Assay Target	Reporter Dye
Monkeypox virus (F3L/B7R gene)	FAM
Internal control (RNase P)	VIC

[Kit Components]

REF: AMPV01

Components	Quantity	
	48 tests/kit	96 tests/kit
MPV B-S	500μL/tube, 1 tube	1000μL/tube, 1 tube
MPV P-P	250μL/tube, 1 tube	500μL/tube, 1 tube
MPV N Ctrl	1000 μL/tube, 1 tube	1000 μL/tube, 1 tube
MPV P Ctrl	1000 μL/tube, 1 tube	1000 μL/tube, 1 tube

REF: AMPV01D

Components	Quantity	
	48 tests/kit	96 tests/kit
MPV Mix	Lyophillization, 48 tubes	Lyophillization, 96 tubes
MPV N Ctrl	400 μL/tube, 1 tube	400 μL/tube, 1 tube
MPV P Ctrl	Lyophillization, 1 tube	Lyophillization, 1 tube

[Storage]

The kit with REF: AMPV01 is stable when stored at -25°C to -15°C for 12 months. Please transport at -25℃ to -15℃.

The kit with REF: AMPV01D is stable when stored at below 35°C for 18 months.

The kits should remain sealed and away from light exposure. Do not use the kit beyond the expiration date.

[Applicable Instruments]

Recommended qPCR instruments include: Anitoa Maverick qPCR systems MQ16, MQ8, or MQ4 series. Bio-Rad CFX96 Touch Real-Time PCR Detection System; Applied Biosystems™ 7500 real-time PCR system; Applied Biosystems™ QuantStudio 5 real-time PCR system; Applied Biosystems™ QuantStudio 3 real-time PCR system; Applied Biosystems™ QuantStudio 7 Flex/Pro real-time PCR system; Applied Biosystems™ ViiA7 real-time PCR system; Applied Biosystems™ StepOnePlus™ Real-Time PCR System.

[Materials Required but Not Provided]

1. 48 or 96-well Polypropylene PCR Microplate. Or 0.2 mL Polypropylene PCR Tube Strips with Flat Cap
2. Preservation solution
3. Pipettes and Pipetting tips (10μL and 200μL tips with filters)
4. Centrifuge
5. Desktop vortex mixer
6. Disposable powder-free gloves and surgical gowns

[Specimen Requirements]

1. Sample type

Oropharyngeal swab, nasopharyngeal swab, serum, plasma and human pustular or vesicular rash specimens.

2. Specimen collection

Human pustular or vesicular rash specimens can be collected in tube containing viral transport media with a swab.

Oropharyngeal swab and nasopharyngeal swab are collected using a polypropylene fiber swab or flocked swab with

plastic shafts.
Plasma- Centrifuging the anticoagulant blood at 1000g for 15min, and transfer supernatant to a new tube.
Serum - Mix collection tube by inverting 5-8 times immediately after blood collection, then let stand for 30 minutes, centrifuge at 1500g for 10min.

3. Specimen storage conditions
The collected specimens should be sent for testing immediately. Specimens should be tested within 24 hours if stored at 2°C to 8°C. Specimens that cannot be tested within 24 hours should be stored at -70°C or below (in the absence of -70°C storage conditions, specimens can be stored at -25°C to -15°C for 10 days). Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

[Procedures]

Specimen Preparation

1. Nucleic acid extraction should be performed using an automatic nucleic acid extractor and Viral Nucleic Acid Extraction Reagents according to their package insert.

2. It is recommended to use the Viral Nucleic Acid Extraction Kit provided by xxxxAnitoa Systems, LLC, 200-300μL of sample were lysed, combined, washed twice, and finally eluted to obtain 60-100μL of DNA solution for subsequent detection.

3. The extracted DNA should be immediately tested or stored at -20℃.

NOTE:
For REF: AMPV01, the MPV N Ctrl and MPV P Ctrl should be treated simultaneously.
For REF: AMPV01D, the MPV N Ctrl and MPV P Ctrl do not need to be extracted. The MPV P Ctrl Lyophillization need to be redissolved before use. Add 200μL MPV N Ctrl to MPV P Ctrl tube and vortex 20-30s to mix thoroughly. Then stored at - 25℃ to -15℃ after re-dissolution.

PCR Reaction Preparation

For REF: AMPV01

1. Take out all components from the kit and place them at room temperature. Vortex 20s and transient centrifuge at 2000-6000rpm.

2. Prepare the MPV Master mix as shown in the table below, based on the total number of samples (including patient specimen(s), MPV P Ctrl and MPV N Ctrl). Vortex 20-30s to mix thoroughly and transient centrifuge at 2000-6000rpm.

	Component name	Volume pre test	Total of N specimens (e.g.)
MPV Master mix	MPV B-S	10 μL	10×(N+3) μL
	MPV P-P	5 μL	5×(N+3) μL

3. Aliquot ***15μL*** "MPV Master mix" into each clean PCR tube or well.
4. Pipet ***10μL*** sample (including extracted patient specimen(s), MPV P Ctrl and MPV N Ctrl) and add to the PCR tube or well containing PCR master mix, respectively. Cover PCR tubes or wells, mix and transient centrifuge.

5. Put it into the PCR instrument.

For REF: AMPV01D

1. Take out several 8-tubes-strips that containing MPV Mix from the kit based on the total number of samples (including patient specimen(s), MPV P Ctrl and MPV N Ctrl). *PCR tube(s) can be cut down from the strip.*

2. Open the "MPV Mix" PCR tube caps, then add ***23μL*** sample (including extracted DNA from patient specimen(s), MPV P Ctrl and MPV N Ctrl) to the PCR tube.

3. Cover the tubes with the new Caps of 8-strip Tube provided, mix and transient centrifuge at 2000-6000 rpm, then put them into the PCR instrument.

PCR Assay

1. Create a new experiment.
2. Select the FAM channel for Monkeypox virus (MPV) . Select the VIC channel for internal control.
3. Select "none" for Passive Reference (For ABI Real-Time PCR systems).
4. Set cycle conditions. Enter the reaction volume (25μL), and edit the PCR program as follows:

Steps	Temperature	Time	Cycle
1	50˚C	2 minutes	1
2	95˚C	2 minutes	1
3	95˚C	5 seconds	40
4*	58˚C	30 seconds	
5	37˚C	1 second	1

*: Data Collection at Step 4 (58˚C, 30S)

5. Save the file. Run the program.

[Result Analysis]

Following the completion of reactions and detection, the instrument will automatically save the results.

1. Setting analysis condition: According to the image obtained from the PCR reaction, adjust the start value, end value of baseline and threshold. The user can adjust these values according to their situation. The start value can be set at 3-15; end value can be set at 5-20.
2. Adjust the threshold to just above the curve of MPV N Ctrl.
3. Click "Analyze" icon to update the analysis.
4. Enter "Report" window and record unknown sample values (Ct):

Ct value	MPV (FAM)	Internal control (VIC)
Definition of "+" in each channel	Ct≤38.5 with typical amplification curve	Ct≤36 with typical amplification curve
Definition of "-" in each channel	Ct＞38.5 or no typical amplification curve	Ct＞36 or no typical amplification curve

Interpretation of quality control

Positive and negative controls should be examined prior to interpretation of clinical sample results.

Positive and Negative Controls	MPV (FAM)	Internal Control (VIC)	Results	Actions
MPV N Ctrl	-	-	Valid	Continue to result interpretation
MPV P Ctrl	+	+		
If controls do not meet these criteria, the samples on the plate are invalid and retest is required.				

Interpretation of clinical sample results

Assessment of clinical sample test results should be performed after the positive and negative controls have been determined to be valid. If the controls are not valid, the patient results cannot be interpreted.

MPV (FAM)	Internal Control (VIC)	Results
+	+/-	Monkeypox virus Positive
-	+	Monkeypox virus Negative
-	-	Invalid, retest[1]
[1] If the retest still invalid, resampling.		

[Limitations of the Procedure]

1. The test results of this kit are only for clinical reference. The clinical diagnosis and treatment of patients should be considered in combination with clinical observations, patient history, and epidemiological information.
2. The test results may be affected by the quality of the samples collected as well as their handling, transportation and storage. Deficiencies in these factors may lead to false negative results.
3. False positive results may occur if cross-contamination is not controlled during sample processing.
4. Amplification of internal control may fail if virus concentration in the specimen is high.
5. Viral nucleic acid extraction reagents from other companies need to be tested on several samples to verify their suitability

[Performance Index]

1. The analytical sensitivity (LoD) of the detection kit is 200 copies /mL.
2. No Cross reactivity with: human genome, Camelpox virus, Variola virus, Ectromelia virus, Vaccinia virus, Cowpox virus, Mouse hemorrhagic fever virus, Meningitis virus, Capri pox virus, Fowl pox virus, Mouse pox virus, Pigeon pox virus, Herpes simplex virus type 1&2, Varicella-zoster virus.
3. Precision:

The intra-batch/ batch precision, intra-day/ day precision and precision variation coefficients between different operators are not greater than 5%.

[Precautions]

1. Read instructions carefully prior to testing. The test must be performed according to the instructions provided.
2. Laboratory management shall be strictly in accordance with management standards of a nucleic acid test laboratory.
3. Conduct quality control for every experiment.
4. Fully thaw PCR reagents prior to use but avoid repeated freeze-thaw cycles.
5. Pipettes used with this test must be calibrated regularly.
6. The entire process should be divided into three separate areas within the laboratory: The first area for reagent preparation, the second area for specimen processing and reaction system preparation and the third area for amplification, Fluorescence

detection and result analysis. The instruments, equipment, and personal protective equipment (PPE) in each area should be used independently to prevent cross contamination.

7. Operators of the test should always avoid potential contamination of RNase and DNase. Test operators should not directly touch the reaction tube by hand. Operators must use disposable gloves and testing materials without Fluorescent properties.

8. Follow biohazard safety standard precautions. Biological safety cabinets should be used when handling specimen to ensure the safety of operators and prevent pollution. Harmful and/or toxic specimens and reagents should be properly stored and maintained by designated personnel. Waste should be properly disposed in special containers. Instruments and equipment such as operating tables, pipettes, centrifuges, and amplification instruments should be frequently wiped and disinfected with 10% sodium hypochlorite and/or 75% ethanol. Laboratory rooms and ultra-clean workbenches should be regularly treated with UV lamps and after each experiment.

9. The reagents in the centrifuge tube should be fully thawed and mixed before use. Centrifuge for a few seconds to concentrate the liquid at the bottom of the centrifuge tube. When preparing the reaction system, it should be noted that the mixing of all liquids should be carried out on the vortex mixer as much as possible. Once the reaction system is completed, centrifuge for a few seconds at a low speed.

10. Do not mix reagents from different batches.

[References]

[1] Kulesh D A , Baker R O , Loveless B M , et al. Smallpox and pan-Orthopox Virus Detection by Real-Time 3′-Minor Groove Binder TaqMan Assays on the Roche LightCycler and the Cepheid Smart Cycler Platforms[J]. Journal of Clinical Microbiology, 2004, 42(2):601-609.

[2] Jezek Z , Marennikova S S , Mutumbo M , et al. Human monkeypox: a study of 2,510 contacts of 214 patients.[J]. Journal of Infectious Diseases, 1986(4):551-555.

[3] Neubauer H , Reischl U , Ropp S , et al. Specific detection of monkeypox virus by polymerase chain reaction[J]. Journal of Virological Methods, 1998, 74(2):201.

[Index of symbols]

	Consult instructions for use	IVD	*In vitro* diagnostic medical device		Do not re-use
LOT	Batch code	REF	Catalogue number		Date of manufacture
EC REP	Authorized representative in the European Community		Use-by date		Temperature Limit
Σ	Contains sufficient for <n> tests		Manufacturer		