INSTRUCTIONS FOR USE

QuantiVirus™ MPXV Test Kit

For Real Time RT-qPCR test

Rx Only

IVD

For Use Under an Emergency Use Authorization (EUA) only

Current Version:	Rev. 4, January 2023
Previous Versions:	Rev. 3, December 2022
	Rev. 2, December 2022
	Rev. 1, December 2022
	Rev. 0

CATALOG NUMBER

DC-04-0009 (24 Reactions)
DC-04-0005 (48 Reactions)
DC-04-0006 (480 Reactions)

COMPANY

DiaCarta, Inc.
4385 Hopyard Rd, Suite 100
Pleasanton, CA 94588, USA

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CONTENTS

This Emergency Use Authorization (EUA) package insert must be read carefully prior to use and must be followed accordingly. Reliability of EUA assay results cannot be guaranteed if there are any deviations from the instructions in this Instruction for Use document.

PART 1. INTENDED USE

The QuantiVirus™ MPXV Test Kit is a real-time multiplex polymerase chain reaction (PCR) test intended for the qualitative detection of DNA from monkeypox virus (clade I/II) in human lesion swab specimens, (i.e., swabs of acute pustular or vesicular rash) from individuals suspected of mpox by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.

Results are for the identification of monkeypox virus (clade I/II) DNA, which is generally detectable in human pustular or vesicular lesion specimens during the acute phase of infection. Positive results are indicative of the presence of monkeypox virus (clade I/II) 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 obtained with this device do not preclude MPXV (clade I/II) infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Laboratories within the United States and its territories are required to report test results to the appropriate public health authorities.

The QuantiVirus™ MPXV Test Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

The QuantiVirus™ MPXV Test Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

PART 2. PRODUCT DESCRIPTION

The monkeypox genome is a large single linear molecule of dsDNA, about 197 kilobase-pairs (kbp) in length. The genome consists of about 190 non-overlapping open reading frames (>180 bp long) containing 60 or more amino acid residues. There are two clades of MPXV: the West African clade (clade II) and the Congo Basin clade (clade I). The QuantiVirus™ MPXV Test Kit detects DNA from both clade I and clade II of MPXV in lesion swab specimens (i.e., swabs of acute pustular or vesicular rash). It uses a fluorescent probe with specific primer sets to detect the J2L and B6R genes within the genome of MPXV. Primers and probe for an internal control, RNase P are also integrated in the assay to validate the assay quality.

Lesion swab specimens are collected in Viral Transport Media (VTM). A total of 200 µL of specimen is used for DNA isolation by a MGISP-960 High-throughput Automated Sample Preparation System. Detection of PCR amplicons is accomplished using TaqMan chemistry on the ABI QuantStudio 5, ABI 7500, Bio-Rad CFX 384 or Roche LightCycler 480 II. The assay detects the two gene targets within the MPXV multiplexed in one tube, along with human RNase P. The RNase P target is an internal control which can be evaluated for successful DNA extraction and PCR reaction.

PART 3. COMPONENTS AND STORAGE

3.1. Kit Components

QuantiVirus™ MPXV Test Kit includes the following components:

- 5x PCR Master mix
- One set of Primers/Probe Mix specific to J2L and B6R genes in the Monkeypox genomic region as well as human RNase P (RPP30) gene.
- Positive Control (PC), Extraction Control (EC) and No Template Control (NTC)

The QuantiVirus™ MPXV Test Kit is available in 3 pack sizes: 24-reactions, 48-reactions, and 480-reactions. Individual components and their descriptions are listed in **Tables 2a** to **2c** below.

Table 2a. Kit Components (Pack-Size: 24 Reactions)

Name of Component	Part #	Description	Pack Size: 24 Reactions kit	Label Volume for Each Vial	Storage Temp
Primer/Probe Mix	1012241	Primer/probe Mix (J2L, B6R & Human RPP30 gene primers and probes)	1 vial	48 µL	-25°C to -15°C
Master Mix	1012251	Meridian Inhibitor-Tolerant Master mix	1 vial	48 µL	-25°C to -15°C
Positive Control (PC)	1012261	Synthetic DNA templates for J2L & B6R	1 vial	10 µL	-25°C to -15°C
Extraction Control (EC)	1012271	Human Specimen Extraction Control	1 vial	40 µL	-25°C to -15°C
No Template Control	1012281	Nuclease-Free Water	1 vial	50 µL	-25°C to -15°C

Table 2b. Kit Components (Pack-Size: 48 Reactions)

Name of Component	Part #	Description	Pack Size: 48 Reactions kit	Label Volume for Each Vial	Storage Temp
Primer/Probe Mix	1012242	Primer/probe Mix (J2L, B6R & Human RPP30 gene primers and probes)	1 vial	96 µL	-25°C to -15°C
Master Mix	1012252	Meridian Inhibitor-Tolerant Master mix	1 vial	96 µL	-25°C to -15°C
Positive Control (PC)	1012262	Synthetic DNA templates for J2L & B6R	1 vial	40 µL	-25°C to -15°C
Extraction Control (EC)	1012272	Human Specimen Extraction Control	1 vial	60 µL	-25°C to -15°C
No Template Control	1012282	Nuclease-Free Water	1 vial	100 µL	-25°C to -15°C

Table 2c. Kit Components (Pack-Size: 480 Reactions)

Name of Component	Part #	Description	Pack Size: 480 Reactions Kit	Label Volume for Each Vial	Storage Temp
Primer/Probe Mix	1012243	Primer/probe Mix (J2L, B6R & Human RPP30 gene primers and probes)	1 vial	960 µL	-25°C to -15°C
Master Mix	1012253	Meridian Inhibitor-Tolerant Master mix	1 vial	960 µL	-25°C to -15°C
Positive Control (PC)	1012263	Synthetic DNA templates for J2L & B6R	1 vial	100 µL	-25°C to -15°C
Extraction Control (EC)	1012273	Human Specimen Extraction Control	1 vial	100 µL	-25°C to -15°C
No Template Control	1012283	Nuclease-Free Water	1 vial	500 µL	-25°C to -15°C

3.2. Shelf-Life

Storage of kits is proposed to be at -25°C to -15°C. Based on individual component shelf life, the shelf life of the kit is 13 months. Do not use expired reagents from the kit.

PART 4. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

4.1. Reagents for Viral DNA Isolation

Several methods exist for DNA isolation. For consistency, please use the MGIEasy® Nucleic Acid Extraction Kit (cat# 1000020261)

DNA quality and quantity are critical for the test accuracy. Follow manufacturer's Instructions for Use (IFU).

4.2. Consumables

- White 0.2 mL nuclease-free PCR tubes or plates (96-well) recommended by the instrument manufacturer
- Nuclease-free, low-binding microcentrifuge tubes
- Nuclease-free pipet tips with aerosol barriers

4.3. Other Reagents

- Molecular grade nuclease-free water.

4.4. Equipment

- Applied Biosystems™ QuantStudio 5 Real-Time PCR Instrument (QuantStudio™ Design and Analysis Software v1.4), ABI 7500 Fast DX (SDS Software v1.4), Bio-Rad CXF 384 Real-Time PCR Instrument (CFX Maestro Software 2.0) and Roche LightCycler 480 II (LightCycler480 SW 1.5.1.61).
- Dedicated pipettes* (adjustable, 10–100 µL, 100–200 µL, 1000 µL) for sample preparation
- Dedicated pipettes* (adjustable, 1–20 µL, 10–100 µL, 100–200 µL, 1000 µL) for PCR Master Mix preparation
- Dedicated pipettes* (adjustable, 1–20 µL, 10–100 µL) for dispensing of template RNA/DNA
- 12-channel multichannel pipettor (P-10) for transferring reactions to PCR plates.
- Microcentrifuge
- Benchtop centrifuge* with rotor for 1.5 mL tubes
- Benchtop mini centrifuge with rotor for PCR strips
- Benchtop plate centrifuge
- Vortex instrument
- Compatible 96-well PCR plate
- Compatible clear PCR plate sealer
- Reagent reservoir (holding 25 mL liquid or more)
- Spectrophotometer

Note: *Prior to use, ensure that instruments and equipment have been maintained and calibrated according to the manufacturer's recommendations.

PART 5. WARNING AND PRECAUTIONS

5.1. Warnings and Precautions

- For *in vitro* diagnostic use.
- For prescription use only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use by laboratories certified under CLIA that meet the requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from monkeypox virus, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of monkeypox virus, including in vitro diagnostics that detect and/or diagnose infection with non-variola *Orthopoxvirus*, under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

- Handle all specimens as if infectious using safe laboratory procedures. Refer to CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Monkeypox: https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html
- Use extreme caution to prevent contamination of PCR reactions with the positive and negative controls provided.
- Minimize exposure of the 5x PCR Master Mix to room temperature for optimal amplification.
- Avoid over exposure of the primer-probe mixes to light for optimal fluorescent signal.
- Use of non-recommended reagent volumes may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended volumes and concentrations of the RNA/DNA sample may result in a loss of performance and may also decrease the reliability of the test results.
- Use of non-recommended consumables with instruments may adversely affect test results.
- Do not re-use any remaining reagents after PCR amplification is completed.
- Additional validation testing by user may be necessary when using non-recommended instruments.
- Perform all experiments under proper sterile conditions using aseptic techniques.
- Perform all procedures using universal precautions.
- Wear personal protective apparel, including disposable gloves, throughout the assay procedure.
- Do not eat, drink, smoke, or apply cosmetics in areas where reagents or specimens are handled.
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Discard all materials in a safe and acceptable manner, in compliance with all legal requirements.
- Dissolve reagents completely, then mix thoroughly by pipetting up and down several times or vertexing if needed.
- If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately.
- Do not use components beyond the expiration the date printed on the kit boxes.
- Do not mix reagents from different lots.
- Return all components to the appropriate storage condition after preparing the working reagents.
- Do not interchange vial or bottle caps, as cross-contamination may occur.
- Keep all the materials on ice when in use.
- Do not leave components out at room temperature for more than 2 hours.
- Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data.

The product contains no substances which at their given concentration are considered to be hazardous to health or environment (**Table 3**).

Table 3. Summary of Hazardous Materials Identification System (HMIS)

Health	0
Flammability	0
Reactivity	0

5.2. Handling and Storage

This kit is shipped on dry ice. If any component of the kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packaging note or the reagents, please contact DiaCarta or the local distributors as soon as possible.

The kit should be stored immediately upon receipt at -15°C to -25°C in a constant-temperature freezer and must be protected from light. When stored under the specified storage conditions, the kit is stable until the stated expiration date. It is recommended to store the PCR reagents in a pre-amplification area and the controls in a postamplification (DNA template-handling) area. The kit can undergo up to 6 freeze-thaw cycles without affecting performance.

All reagents must be thawed at ambient temperature for a minimum of 30 minutes before use. Do not exceed 2 hours at ambient temperature. The primer and probe mixes contain fluorophore labeled probes and should be protected from light. It is recommended that all reagents should be kept on ice when setting up the assay mixes.

Attention should be paid to expiration dates and storage conditions printed in the box and labels of all components. Do not use expired or incorrectly stored components.

5.3. General Considerations

Effective use of real-time PCR tests requires good laboratory practices, including maintenance of equipment that is dedicated to molecular biology. Use nuclease-free lab ware (pipettes, pipette tips, reaction vials) and wear gloves when performing the assay. Use aerosol-resistant pipette tips for all pipetting steps to avoid cross-contamination of the samples and reagents.

Prepare the assay mixes in designated pre-amplification areas using only equipment dedicated to this application. Add template DNA in a separate area (preferably a separate room). Use extreme precautions to prevent DNase contamination that could result in degradation of the template DNA, or PCR carryover contamination, which could result in a false positive signal.

Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation times and temperatures may result in erroneous or discordant data.

PART 6. SAMPLES & CONTROLS

6.1. Samples and Controls

Patient samples must be collected according to appropriate CDC guidelines. Positive, Extraction Control and No Template Controls must be included in every run to accurately interpret patient test results.

Table 4. Assay Controls

Control	Used to Monitor	Assays
Positive Control (Synthetic DNA)	PCR reaction	Target gene assay
No Template Control (nuclease-free water)	Cross-contamination for assay procedure	Target gene assay
Extraction Control (Human specimen DNA)	DNA extraction and PCR	RPP30 gene assay

a. Positive Control (PC)

A positive control is a mix of synthetic DNA templates for the target sequences for J2L and B6R genes of the MPXV genome. Positive controls must show the appropriate values in FAM and HEX channel for the run to be valid. Positive control monitors the function of each assay component.

b. Extraction Control (EC)

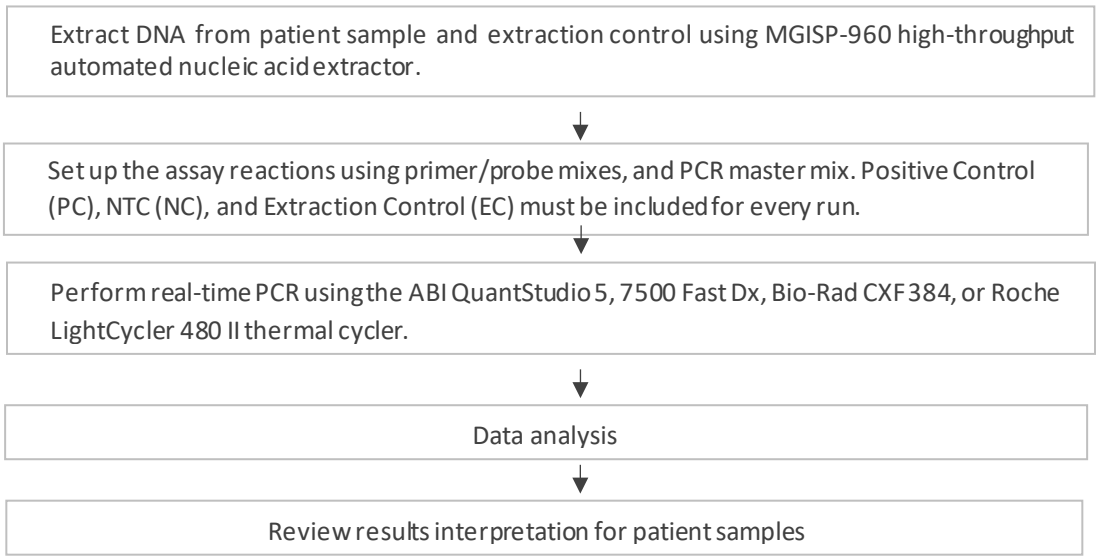
Extraction Control is template material with target sequences for the human RNase P (RPP30) gene DNA. The extraction control RP DNA undergoes the full extraction procedure. As the Extraction Control, there should be amplification for RP gene, but no amplification for the viral gene (J2L or B6R). This control should be run with every batch of extraction.

c. No Template Control (NTC)

Nuclease-free water is used in place of template. No amplification should be observed in all channels, assuring the absence of contamination during assay set-up.

PART 7. WORKFLOW

The brief procedure for performing the assay include the following steps:



The workflow begins with DNA extraction from lesion swab specimens. DNA is isolated and purified from the specimens using the appropriately chosen viral DNA extraction method, please refer to the above list in **PART 4.A**. The purified DNA is amplified using QuantiVirus™ MPXV Test Kit on either ABI QuantStudio 5, ABI 7500 Fast Dx, Bio-Rad CXF 384, or Roche LightCycler 480 II Real-Time PCR instrument. In the process, the probes anneal to the specific target sequences located between one pair of unique forward and reverse primers for the J2L and B6R genes in the MPXV genome. The RPP30's primers and probe target the human RNase P gene in each specimen to monitor successful DNA extraction. During the extension phase of the PCR cycle, the 5' exonuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the PCR instrument. For details, please refer to manufacturer's Instructions for Use (IFU).

7.1. Sample Collection and Handling

Sample collection device is not a part of the assay kit. All testing for MPXV should be conducted in consultation with a healthcare provider. We recommend using CDC guidelines for sample collection and storage available at link below:
https://www.cdc.gov/poxvirus/monkeypox/clinicians/prep-collection-specimens.html

Sample Collection

Once the swabs have been collected as per the CDC guidelines above, it is recommended to use Viral Transport Medium (VTM) System (for transportation/ temporary storage of lesion swabs. Specimens collected in the VTM should be processed within 24 hours from collection and stored at 2 – 25°C during that time as per the manufacturer's instructions.

7.2. Viral DNA Isolation

7.2.1. Automated High-throughput Extraction

- The QuantiVirus™ MPXV Test Kit uses MGISP-960 automated high-throughput nucleic acid extractor and MGIEasy® Nucleic Acid Extraction Kit (cat# 1000020261)

  - Aliquot MPXV samples into 96-well deep well plates*.
  - While aliquoting samples, prepare the extraction reagents (MLB MIX, MW1, MW2, and Elution water) with MGISP-960.
  - Load the samples and extraction reagents into MGISP-960 as follows:

Reagents	Position
Samples	POS 17 and POS 15
Buffer MLB Mixture	POS 21 and POS 22
Buffer MW1	POS 22
Buffer MW2	POS 14 and POS 16
Nuclease-Free Water	POS 13

  - Each machine can extract two 96-well plates of samples, with full load, we can extract 384 samples in one run with two MGISP-960.
  - Load the Script of "Nucleic Acid Extraction" and run the extraction.
  - The whole run will take approximate 60 minutes.

*__Note__: For each batch of clinical samples to be tested, spike 20µL Extraction Control (EC) from QuantiVirus™ MPXV Test Kit into 160µL sterile DNase-free water prior to DNA extraction. Treat the spiked EC as a sample and process the spiked EC and clinical samples for viral DNA isolation.

7.3. Preparation of Reagents and Assay Mixes

7.3.1. Preparation of Reagents

1) Thaw the primer and probe mix, Positive Control, Nuclease-Free Water and 5x PCR Master Mix provided.
2) Thaw all reaction mixes at room temperature for a minimum of 30 minutes.
3) Keep all thawed reagents on ice.
4) Vortex all components except the PCR Master Mix and 5x Primer and Probe Mix for 5 seconds and perform a quick spin.
5) The PCR Master Mix and Primer/probe mix should be mixed gently by inverting the tube a few times.

Prior to use, ensure that any precipitate in the PCR Master Mix is re-suspended by pipetting up and down multiple times. Do not leave kit components at room temperature for more than 2 hours. The PCR reactions are set up in a total volume of 10 µL/reaction. **Table 2** shows the component volumes for each 10 µL reaction.

Table 5. Assay Components and Reaction Volume

Components	Volume/Reaction
5x PCR Master Mix	2 µL
5x Primer and Probe Mix	2 µL
DNA sample or Controls	Sample: 6 µL Controls: add 2 µL of controls and add 4 µL of nuclease-free water to make 6 µL volume
Total Volume	10 µL

For accuracy, 5x PCR Master Mix, 5x primers and probes should be pre-mixed into assay mixes as described in **Table 6** below.

7.3.2. Preparation of Assay Mixes

Assay mixes should be prepared just prior to use. Label a microcentrifuge tube (not provided) for each reaction mix, as shown in Table 6. For each control and virus detection reaction, prepare sufficient working assay mixes for the DNA samples, one Positive Control, one extraction control and one nuclease-free water for No Template Control (NTC), according to the volumes in **Table 6**. Include reagents for 1 extra sample to allow sufficient overage for the PCR set-up. The assay mixes contain all of the components needed for PCR except the templates (sample or controls).

Table 6. Preparation of Assay Mixes

	Volume of 5x PCR Master Mix	Volume of 5x Primer and probe Mix
Assay Mix	2 µL x (n+ 3+ 1)	2 µL x (n+ 3+ 1)

n = number of reactions (DNA samples), +3 is for 3 controls. Prepare enough for 1 extra sample (+1) to allow for sufficient coverage for the PCR set-up.

A reaction mix containing all reagents, except for the DNA sample or control templates, should be prepared for the total number of samples and controls to be tested in one run. The Positive Control (PC), Extraction Control (EC) and No Template Control (NTC) should be included in each run.

7.4. Suggested Run Layout

For each reaction, add 4 µL of the appropriate assay mix to the plate or tubes. Add up to 6 µL of template.

The assay has been validated on the following PCR instruments:

Table 7. Validated PCR Instruments

Company	Model
Bio-Rad	CFX384
Thermo Fisher (ABI)	QuantStudio 5
Thermo Fisher (ABI)	7500 Fast Dx
Roche	LightCycler 480 II

Table 8a. Plate Layout for 384-Well Plate

	1	2	3	4		21	22	23	24
A	NTC	EC	S1	S2		S19	S20	S21	PC
B	S22	S23	S24	S25		S42	S43	S44	S45
C	S46	S47	S48	S49	→	S66	S67	S68	S69
D	S70	S71	S72	S73		S90	S91	S92	S93
E	S94	S95	S96	S97		S114	S115	S116	S117
F	S118	S119	S120	S121		S138	S139	S140	S141
G	S142	S143	S144	S145		S162	S163	S164	S165
H	S166	S167	S168	S169	→	S186	S187	S188	S189
I	S190	S191	S192	S193		S210	S211	S212	S213
J	S214	S215	S216	S217		S234	S235	S236	S237
K	S238	S239	S240	S241		S258	S259	S260	S261
L	S262	S263	S264	S265		S282	S283	S284	S285
M	S286	S287	S288	S289	→	S306	S307	S308	S309
N	S310	S311	S312	S313		S330	S331	S332	S333
O	S334	S335	S336	S337		S354	S355	S356	S357
P	S358	S359	S360	S361		S378	S379	S380	S381

*Column 5 to 20 are omitted

A single experiment can analyze up to 381 unknown samples. PC, Positive Control; EC, Extraction Control; NTC, No Template Control (water); S1 – S381, Samples 1 – 381 (up to 381 unknown samples can be loaded).

After all reagents have been added to the plate, tightly seal the plate to prevent evaporation. Spin at 1,000 rpm for 1 minute to mix and collect all the reagents at the bottom of the plate wells. Place in the real-time PCR instrument immediately.

Table 8b. Plate Layout for 96-well Plate

		1	2	3	4	5	6	7	8	9	10	11	12
A	Assay Mix	NTC	EC	S1	S2	S3	S4	S5	S6	S7	S8	S9	PC
B	Assay Mix	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
C	Assay Mix	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33
D	Assay Mix	S34	S35	S36	S37	S38	S39	S40	S44	S42	S43	S44	S45
E	Assay Mix	S46	S47	S48	S49	S50	S55	S52	S53	S54	S55	S56	S57
F	Assay Mix	S58	S59	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69
G	Assay Mix	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81
H	Assay Mix	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93

A single experiment can analyze up to 93 unknown samples. PC, Positive Control; EC, Extraction Control; NTC, No-Template Control (water); S1 – S93, Samples 1 – 93 (up to 93 unknown samples can be loaded).

After all reagents have been added to the plate, tightly seal the plate to prevent evaporation. Spin at 1000 rpm for 1 minute to mix and collect all the reagents at the bottom of plate wells. Place in the real-time PCR instrument immediately.

7.5. Instrument Set-Up

Set up the PCR reaction thermocycling conditions on ABI QuantStudio 5, ABI 7500 Fast Dx, Bio-Rad CXF 384 Real-Time PCR Instrument, or Roche LightCycler 480 II as follows.

7.5.1. Selection of Detectors

a. For ABI QuantStudio 5 and ABI 7500 Fast Dx, assign the target J2L as “FAM”, the target B6R as “VIC/HEX” and the RNase P (Internal control) as “Cy5”, respectively.
b. For Bio-Rad CFX 384/96, select all channel.
c. For Roche LightCycler 480 II, in detection format select “FAM”, “HEX” and “Cy5.”

7.5.2. Setup the thermocycling parameters for QuantStudio 5 (QS5) Real-Time PCR Instrument, ABI 7500 Fast Dx, Bio-Rad CFX384, and Roche LightCycler 480 II as shown in **Table 9a** and **Table 9b**.

Table 9a. PCR Cycling Parameters on ABI QS5 and ABI 7500 Fast Dx

Step	Temperature (°C)	Time (Seconds)	Ramp Rate (°C/s)	Cycles	Data Collection
Polymerase Activation	95	120	1.6	1	OFF
Denaturation	95	3	1	´45	OFF
Annealing and Extension	60	30	1		FAM, HEX and Cy5

Table 9b. PCR Cycling Parameters on Bio-Rad CFX 384 and LightCycler 480 II

Step	Temperature (°C)	Time (Seconds)	Cycles	Data Collection
Polymerase activation	95	120	1	OFF
Denaturation	95	3	´45	OFF
Annealing and Extension	60	30		FAM, HEX and Cy5

7.5.3. Start the Run

PART 8. DATA ANALYSIS

8.1. Assessment of real-time PCR Results

Save and analyze the data following the instrument manufacturer's instruction.

Adjust the threshold above any background signal to around the middle of the exponential phase of the amplification curve in the log view (e.g., **Figure 2**). The procedure chosen for setting the threshold should be used consistently. Exact threshold setting may be different for individual instruments and can be adjusted based on the amplification curves if needed.

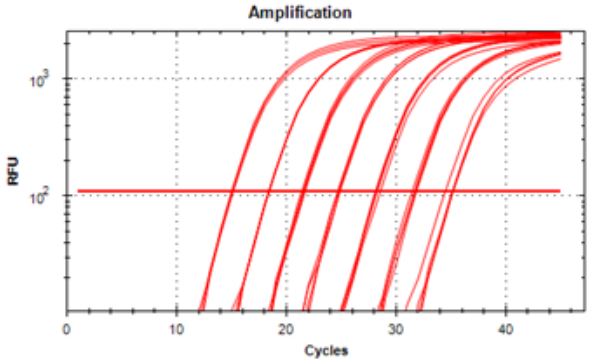


Figure 2. Amplification Curve of 10 -fold Serial Dilution of Templates Showing the Threshold Setting

8.2. Assessment of the Assay Run

8.2.1. ABI QuantStudio 5

A. Cq Values for Controls

The QuantiVirus™ MPXV Test Kit protocol dictates that the controls should be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Cq values must meet the acceptance criteria in **Table 10a** below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 10a. Acceptable Cq Values for Positive Control, Extraction Controls and No Template Control

Control		Acceptable Cq	Test valid/invalid
Extraction control	RPP30 gene	< 38	Valid
Positive control	J2L gene	< 30	Valid
	B6R gene	< 30	Valid
Non-template control		≥ 45	Valid

B. Cq Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in **Table 10b** below.

Table 10b. Individual Assay Results

Target	Cut-Off	Result
J2L Virus Gene (MPXV-specific)	Cq < 40	POS
J2L Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
B6R Virus Gene (MPXV-specific)	Cq < 40	POS
B6R Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
Human RPP30 Gene	Cq < 38	DNA input OK
Human RPP30 Gene	Cq ≥ 38	DNA input fail

8.2.2. ABI 7500 FAST Dx

A. Cq Values for Controls

The QuantiVirus™ MPXV Test Kit protocol dictates that the controls be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Cq values must meet the acceptance criteria in **Table 11a** below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 11a. Acceptable Cq Values for Positive Control, Extraction Control and No Template Control

Control		Acceptable Cq	Test valid/invalid
Extraction Control	RPP30 gene	< 38	Valid
Positive Control	J2L gene	< 30	Valid
	B6R gene	< 30	Valid
No Template Control		≥ 45	Valid

B. Cq Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in **Table 11b** below.

Table 11b. Individual Assay Results

Target	Cut-Off	Result
J2L Virus Gene (MPXV-specific)	Cq < 40	POS
J2L Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
B6R Virus Gene (MPXV-specific)	Cq < 40	POS
B6R Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
Human RPP30 Gene	Cq < 38	DNA input OK
Human RPP30 Gene	Cq ≥ 38	DNA input fail

8.2.3. Bio-Rad CFX384

A. Cq Values for Control

The QuantiVirus™ MPXV Test Kit protocol dictates that the controls be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Cq values must meet the acceptance criteria in **Table 12a** below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 12a. Acceptable Cq Values for Positive Control, Extraction Control and No Template Control

Control		Acceptable Cq	Test valid/invalid
Extraction control	RPP30 gene	< 38	Valid
Positive control	J2L gene	< 30	Valid
	B6R gene	< 30	Valid
Non-template control		≥ 45	Valid

B. Cq Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in Table 12b below:

Table 12b. Individual Assay Results

Target	Cut-Off	Result
J2L Virus Gene (MPXV-specific)	Cq < 40	POS
J2L Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
B6R Virus Gene (MPXV-specific)	Cq < 40	POS
B6R Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
Human RPP30 Gene	Cq < 38	DNA input OK
Human RPP30 Gene	Cq ≥ 38	DNA input fail

8.2.4. Roche LightCycler 480 II

A. Cq Values for Control

The QuantiVirus™ MPXV Test Kit protocol dictates that the controls be analyzed before the analysis of patient samples. The kit positive, extraction and no template control Cq values must meet the acceptance criteria in **Table 13a** below for the assay to be valid. If kit control(s) fail, the test is invalid and needs to be repeated. Patient sample data is analyzed and interpreted only after all the kit controls pass.

Table 13a. Acceptable Cq Values for Positive Control, Extraction Control and No Template Control

Control		Acceptable Cq	Test valid/invalid
Extraction Control	RNase P gene	< 38	Valid
Positive Control	J2L gene	< 30	Valid
	B6R gene	< 30	Valid
No Template Control		≥ 45	Valid

B. Cq Values for Samples

Assessment of the results for each individual assay should be based on the Cq values, according to the criteria outlined in **Table 13b** below.

Table 13b. Individual Assay Results

Target	Cut-Off	Result
J2L Virus Gene (MPXV-specific)	Cq < 40	POS
J2L Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
B6R Virus Gene (MPXV-specific)	Cq < 40	POS
B6R Virus Gene (MPXV-specific)	Cq ≥ 40	NEG
Human RPP30 Gene	Cq < 38	DNA input OK
Human RPP30 Gene	Cq ≥ 38	DNA input fail

PART 9. INTERPRETATION OF RESULTS

Positive Control, Extraction Control, and No Template Control in the kit must function as outlined in **Tables 10a, 11a, 12a** and **13a** above. If the controls do not function as required, the test is invalid. All the samples need to be retested.

When MPXV J2L, BR6 and human RPP30 genes or one of MPXV gene (J2L or BR6) and RPP30 gene are detectable, the patient sample is positive. When both MPXV genes (J2L and BR6) or one of MPXV gene (J2L or BR6) are detectable and RPP30 gene is not detectable, the patient sample is positive. When MPXV J2L and BR6 are not detectable, but human RPP30 gene is detectable, the patient sample is negative. When neither of MPXV genes nor human RPP30 gene is detectable, the result is invalid and repeat the test is needed (**Table 14**)

Table 14. Interpretation of the Results

J2L Gene	B6R Gene	RPP30 Gene	Status	Result	Action
Undetected	Undetected	Undetected	Invalid	Inconclusive	Repeat test one more time. If the repeat result remains invalid, consider collecting new specimen.
Undetected	Undetected	Detected	Valid	MPXV-Negative	Report results to healthcare provider. Consider testing for other pathogens.
Detected	Detected	Detected	Valid	MPXV-Positive	Report results to healthcare provider and CDC.
Undetected	Detected	Detected			
Detected	Undetected	Detected			
Detected	Detected	Undetected			
Undetected	Detected	Undetected			
Detected	Undetected	Undetected			

PART 10. ASSAY LIMITATIONS

a) **For use under an Emergency Use Authorization only.** This assay is for in vitro diagnostic use under FDA Emergency Use Authorization only.
b) Use of the QuantiVirus™ MPXV Test Kit is limited to personnel who have been trained in the procedures of a molecular diagnostic assay.
c) Laboratories are required to report results to the appropriate public health authorities.
d) The performance of QuantiVirus™ MPXV Test was established using lesion swab samples.
e) Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
f) Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction kits have not been evaluated.
g) If the virus mutates in the PCR target region, MPXV may not be detected or may be detected less predictably.

h) False Positive results may arise from the contamination during specimen handling or preparation, or between patient samples.
i) Negative results do not preclude MPXV infection and should not be used as the sole basis for treatment or other patient management decisions.
j) False Negative results may arise from:
   - Improper sample collection
   - Degradation of the viral DNA during shipping/storage
   - The presence of PCR inhibitors
   - Mutation(s) in the sequence of Monkeypox virus
k) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
l) This assay does not detect variola virus (smallpox virus).
m) While monkeypox virus clade II is the only member of the *Orthopoxvirus* genus known to be circulating among humans in the US at this time, a positive result most likely represents the presence of monkeypox virus clade II, although there is a small possibility that this result could represent the presence of monkeypox virus clade I. If clinical concern for such an infection exists, healthcare providers should contact the CDC and their local public health authorities for guidance.
n) The assay is indicated for testing of lesion swab specimens. Performance for other specimen types has not been established.
o) Performance of the test has only been established in lesion swabs collected in VTM. Performance of the test has not been evaluated for dry swabs or for lesion swabs collected in other transport media types.
p) A specimen with a result of "MPXV Negative" does not preclude monkeypox virus infection and should not be used as the sole basis for treatment or other patient management decisions. Collection of multiple specimens (and specimens collected at different time points) from the same patient may be necessary to detect the virus.
q) Detection of monkeypox virus DNA may be affected by sample collection methods (e.g., if a specimen is improperly collected, transported, or handled), patient factors (e.g., presence, type, and duration of symptoms), and/or stage of infection (e.g., if collected too early or too late in the course of illness).
r) False-negative results may arise from degradation of the viral DNA during storage and transport of the specimens.
s) The impacts of specific vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs on the performance of this test have not been evaluated.
t) The clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of monkeypox virus and their prevalence, which may change over time.

PART 11. CONDITIONS OF AUTHORIZATION FOR LABORATORIES

The QuantiVirus™ MPXV Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:
https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-emergency-use-authorizations-medical-devices.
However, to assist clinical laboratories using the QuantiVirus™ MPXV Test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[a] using your product must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories[a] using your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories[a] that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories[a] using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories[a] must have a process in place to track adverse events and report to you (via email: information@diacarta.com; 1-800-246-8878) and to FDA pursuant to 21 CFR Part 803.

F. All laboratory personnel using your product must be appropriately trained in real time PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

G. DiaCarta, Inc, authorized distributor(s) and authorized laboratories[a] must collect information on the performance of your product and must report any significant deviations from the established performance characteristics of your product of which they become aware to DMD/ OHT7/OPEQ/CDRH (via email: CDRH-EUAReporting@fda.hhs.gov). In addition, authorized distributor(s) and authorized laboratories report to DiaCarta, Inc. (via email: information@diacarta.com; 1-800-246-8878).

H. DiaCarta, Inc., authorized distributor(s) and authorized laboratories[a] must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

[a] The letter of authorization refers to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests" as "authorized laboratories."

PART 12. PERFORMANCE EVALUATION

12.1. Analytical Sensitivity (LOD, Limit of Detection)

To determine the Limit of Detection (LoD) and analytical sensitivity of the kit, studies were performed using serial dilutions of analyte and the LoD was determined to be the lowest concentration of template that could reliably be detected with 95% of all tested positive.

The LoD of each target assay was determined using contrived specimens using inactivated MPXV (USA/MA001/2022) from ZeptoMetrix LLC (Cat# 0810657CFHI, NY 14201). Its titration is about $1.23x10^8$ $TCID_{50}$/mL. Lesion swab specimens (MPXV negative samples in VTM) were acquired from San Francisco Department of Public Health (SF DPH lab) and pooled as background diluent in preparation of the contrived samples for the preliminary LoD and LoD studies.

The stock solution was first diluted with 10 mM Tris Buffer (pH 8.0) to reach $1x10^6$ $TCID_{50}$/mL for all the following tests. All further dilutions were done with a pool of MPXV negative lesion swab clinical background prepared fresh for each test. Extraction was performed on the MGISP-960 automated extractor with a sample input volume of 200 µL and elution volume of 30 µL. All PCR measurements were performed on the Bio-Rad CFX384 instrument.

The LoD was confirmed by testing dilutions that cover 80, 100 and 200 $TCID_{50}$/mL and 24 replicates at each concentration. Final LoD was confirmed as the lowest concentration that reached positive detection rate of 95% and above, and the LoD of the Monkeypox Detection Assay was determined to be 100 $TCID_{50}$/mL (**Table 15**).

Table 15. LoD Summary for QuantiVirus™ MPXV Test

Virus Concentration ($TCID_{50}$/mL)	Hit Rate (%) #detected/#tested			Mean C_q(SD*)
	Each Target		Overall**	
200	J2L	100% (24/24)	100% (24/24)	32.93 (0.89)
	B6R	91.67% (22/24)		35.37 (0.90)
	RP	100% (24/24)		26.13 (0.44)
100	J2L	91.67% (22/24)	100% (24/24)	33.81 (0.89)
	B6R	70.83% (17/24)		37.17 (2.45)
	RP	100% (24/24)		26.46 (0.31)
80	J2L	83.33% (20/24)	87.5%(21/24)	34.10 (0.73)
	B6R	62.50% (15/24)		38.02 (2.93)
	RP	100% (24/24)		25.99 (0.55)

*SD: Standard Deviation **Overall Hit Rate refers to hit at least one MPXV gene

Additionally, equivalent performance near the LoD was demonstrated on four different RT-PCR instruments (Bio-Rad CFX384, ABI QuantStudio 5, Roche LightCycler 480 II and ABI 7500 Fast DX) using inactivated NATtrol™ Monkeypox Virus External Run Control (USA/MA001/2022, Lineage B.1, Clade IIb) from ZeptoMetrix LLC (Cat# NATMPXV-ERC). The virus was originally obtained from BEI Resources, and its titer is about 15,000 copies/mL. Lesion swab specimens (MPXV negative sample in VTM) were acquired from San Francisco Department of Public Health (SF DPH lab) and pooled as background diluent in preparation of the contrived samples for these studies.

The stock solution was first diluted with 10 mM Tris Buffer (pH 8.0) to reach 10,000 copies/mL for all the following tests. All further dilutions were done with a pool of lesion swab clinical background prepared fresh for each test. Extraction was performed on the MGISP-960 automated extractor with a sample input volume of 200 µL and elution volume of 30 µL. The final comparison of sensitivity was done with dilutions that cover 20, 25, 30, 35, 40, 50, 60 and 80 copies/mL and 24 replicates at each concentration. The data demonstrated equivalent performance near the LoD for each MPXV gene target for the Bio-Rad CXF 384 Real-Time PCR Instrument, the ABI QuantStudio 5, the Roche LightCycler 480 II and the ABI 7500 Fast Dx. (**Tables 16a, 16b, 16c and 16d**)

Table 16a. Near LoD Performance for QuantiVirus™ MPXV Test in Bio-Rad CFX384

Virus Concentration (copies/mL)	Hit Rate (%) #detected/#tested Each Target		Mean C_q (SD)
50	J2L	100% (24/24)	33.01 (0.46)
	B6R	95.8% (23/24)	35.56 (0.87)
	RP	100% (24/24)	28.16 (0.23)
40	J2L	95.8% (23/24)	33.72 (0.73)
	B6R	91.7%(22/24)	35.75 (0.80)
	RP	100% (24/24)	28.06 (0.19)

Table 16b. Near LoD Performance for QuantiVirus™ MPXV Test in ABI QuantStudio 5

Virus Concentration (copies/mL)	Hit Rate (%) #detected/#tested Each Target		Mean C_q (SD)
50	J2L	100% (24/24)	33.85 (0.53)
	B6R	95.8% (23/24)	37.07 (0.82)
	RP	100% (24/24)	28.11 (0.40)
40	J2L	100% (24/24)	34.23 (0.63)
	B6R	91.7%(22/24)	37.36 (0.76)
	RP	100% (24/24)	28.03 (0.36)

Table 16c. Near LoD Performance for QuantiVirus™ MPXV Test in Roche LightCycler 480 II

Virus Concentration (copies/mL)	Hit Rate (%) #detected/#tested Each Target		Mean C_q (SD)
80	J2L	100% (24/24)	32.82 (0.66)
	B6R	100% (24/24)	34.04 (1.09)
	RP	100% (24/24)	29.15 (0.21)
60	J2L	100% (24/24)	33.08 (0.68)
	B6R	91.7% (22/24)	34.22 (1.00)
	RP	100% (24/24)	29.12 (0.57)

Table 16d. Near LoD Performance for QuantiVirus™ MPXV Test in ABI 7500 Fast Dx

Virus Concentration (copies/mL)	Hit Rate (%) #detected/#tested		Mean Cq(SD)
	Each Target		
25	J2L	100% (24/24)	34.71 (1.07)
	B6R	100% (24/24)	36.61 (1.50)
	RP	100% (24/24)	25.07 (0.46)
20	J2L	95.8% (23/24)	34.80 (1.22)
	B6R	91.7%(22/24)	36.91 (1.28)
	RP	100% (24/24)	25.09 (0.28)

12.2. Inclusivity/Exclusivity

12.2.1. Inclusivity: In silico analysis for MPXV targets

An *in silico* inclusivity analysis was conducted by aligning the MPXV target primer and probe sequences against available monkeypox virus sequences from GenBank at NCBI database as of Sept 12th, 2022. A total of 1099 clade I/II Monkeypox virus isolated sequences were analyzed and the sequence identify was 92% – 100% for both primer and probe (**Table 17**).

Table 17. Inclusivity of MPXV Target

Species	Number of Sequence evaluated	Sequence with >90% match to both MPXV primers and probe
Monkeypox virus	1099	1099

12.2.2. Exclusivity: *In silico* analysis for cross-reactivity

In Silico Exclusivity Analysis of MPXV primers/probe against virus sequences

For the MPXV primer and probe sequences, DiaCarta evaluated sequences from 10 viruses (8 *Orthopoxvirus*, 1 *Papillomavirus* and 1 *Molluscipoxvirus*). The data supports that cross-reactivity is not predicted for the viruses evaluated. Results are presented in **Table 18**.

Table 18. Organisms used for *In Silico* exclusive analysis of MPXV primer and probe vs viral sequences

Species	Taxid	Upper Level (Genus)	Sequence ID
Vaccinia virus	10245	*Orthopoxvirus*	NC_006998
Cowpox Virus	10243	*Orthopoxvirus*	NC_003663
Variola Virus (Smallpox)	10255	*Orthopoxvirus*	NC_001611
Camelpox Virus	28873	*Orthopoxvirus*	NC_003391
Ectromelia Virus	12643	*Orthopoxvirus*	NC_004105
Raccoon Poxvirus	10256	*Orthopoxvirus*	NC_027213
Volepox Virus	28874	*Orthopoxvirus*	NC_031033
Skunkpox Virus	160796	*Orthopoxvirus*	NC_031038
Molluscum Contagiosum virus	10279	*Molluscipoxvirus*	NC_001731
Human papilloma virus	173087	*Papillomavirus*	NC_027779.1

In Silico Exclusivity Analysis of MPXV primers/probe against Bacterial and Fungal sequences

An *in silico* analysis for the MPXV primer/probe sequences was conducted against non-viral sequences from bacterial species, fungal and protozoan species.

Of the non-viral sequences evaluated, no sequences demonstrated >80% homology with both MPXV primers, however, some sequences did have binding sites for the MPXV probe. It is not expected that there is any cross reactivity for these organisms due to absence of >80% homology in the assay's primers although some have probe binding sites. Results from the analysis demonstrated that for the microorganisms evaluated, cross-reactivity is not predicted for the MPXV primers/probe included in the QuantiVirus™ MPXV Test assay. Results are presented in **Table 19**.

Table 19. Organisms used for *In Silico* exclusive analysis of MPXV primer and probe vs Non-viral sequences

Type	Species	Taxid	Upper level (Genus)	Sequence ID
Bacteria	*Acinetobacter calcoaceticus*	471	*Acinetobacter*	NZ_CP020000.1
Bacteria	*Bacillus fragilis*	817	*Bacillus*	NZ_CP069563.1
Fungi	*Candida albicans*	5476	*Candida*	NC_032089.1
Bacteria	*Chlamydia trachomatis*	813	*Chlamydia*	NC_000117.1
Bacteria	*Corynebacterium jeikeium*	38289	*Corynebacterium*	NC_007164.1
Bacteria	*Enterobacter faecalis*	1351	*Enterobacter*	NZ_CP091884.1
Bacteria	*Escherichia coli*	562	*Escherichia*	NC_000913.3
Bacteria	*Lactobacillus species*	1578	*Lactobacillus*	NZ_CP018218.1
Bacteria	*Mycoplasma genitalium*	2097	*Mycoplasma*	NC_000908.2
Bacteria	*Mycoplasma pneumoniae*	2104	*Mycoplasma*	NZ_LR214945.1
Bacteria	*Neisseria gonorrhoeae*	485	*Neisseria*	NZ_AP023069.1
Bacteria	*Pseudomonas aeruginosa*	287	*Pseudomonas*	NC_002516.2
Bacteria	*Streptococcus agalactiae*	1311	*Streptococcus*	NZ_CP012480.1
Bacteria	*Streptococcus canis*	1329	*Streptococcus*	NZ_CP053790.1
Bacteria	*Streptococcus dysgalactiae sub. equisimilis*	119602	*Streptococcus*	NZ_AP023394.1
Bacteria	*Streptococcus intestinalis*	29389	*Streptococcus*	GCF_009695625.1
Bacteria	*Streptococcus milleri*	33040	*Streptococcus*	NZ_LR134288.1
Bacteria	*Streptococcus mitis*	28037	*Streptococcus*	CP047883.1
protozoan	*Trichomonas vaginalis*	5722	*Trichomonas*	GCA_002891335.1
Fungi	*Trichophyton rubrum*	5551	*Trichophyton*	GCF_000151425.1

12.2.3. Exclusivity/Cross-Reactivity (Wet Lab analysis)

The QuantiVirus™ MPXV Test Kit was evaluated for potential cross-reactivity with the following commercially available microorganisms at sample concentrations of greater than $1x10^5$ CFU/mL, copies/mL or $1x10^6$ $TCID_{50}$/mL. The test was done in Bio-Rad CFX384. The results showed that no cross-reactivity was observed with any of the microorganisms (**Table 20**).

Table 20. Cross-Reactivity

Organism	Concentration	J2L (Cq)	B6R (Cq)	RPP30 (Cq)
Staphylococcus epidermidis	1.28 x 10^8 cfu/mL	45.0	45.0	24.5
Staphylococcus aureus	1.32 x 10^9 cfu/mL	45.0	45.0	24.2
Streptococcus pyogenes	1.28 x 10^8 cfu/mL	45.0	45.0	24.4
Human herpesvirus 7	1.0 x 10^5 cfu/mL	45.0	45.0	25.0
Human herpesvirus 8	6.0 x 10^7 cp/mL	45.0	45.0	24.3
Coxsackie A16	1.0 x 10^5 cp/mL	45.0	45.0	24.4
Measles	2.3 x 10^{10} cp/mL	45.0	45.0	25.0
Varicella Zoster Virus	1.0 x 10^5 cp/mL	45.0	45.0	24.9
JC polyomavirus	1.3 x 10^8 cp/mL	45.0	45.0	25.3
Epstein Barr Virus	1.0 x 10^5 cp/mL	45.0	45.0	24.5
Human herpesvirus 1	9.4 x 10^{10} cp/mL	45.0	45.0	24.6
Human herpesvirus 2	2.8 x 10^6 $TCID_{50}$/mL	45.0	45.0	24.5
Human herpesvirus 5	1.7 x 10^8 cp/mL	45.0	45.0	25.0
Treponema pallidum	4.0 x 10^5 cfu/mL	45.0	45.0	24.2
Vaccinia virus	10^5 copies/mL	45.0	45.0	n.a.
Cowpox virus	10^5 copies/mL	45.0	45.0	n.a.
MPXV (ATCC)	10^5 copies/mL	21.8	22.0	n.a.

*cp/mL=copies/mL

12.3. Interfering Substances

A study was performed to evaluate the impact of potentially interfering substances on the performance of the QuantiVirus™ MPXV Test Kit. Before DNA extraction, the following substances were spiked in MPXV-negative clinical samples in VTM with either the presence or the absence of contrived MPXV reference material. The interfering substances study demonstrated that these interferents did not have a significant impact on the performance of the QuantiVirus™ MPXV Test (**Table 21**). The test was done in the Bio-Rad CFX384

Table 21. Interfering Substances

Interference Substance	Without MPXV reference material			With MPXV reference material (3x LoD)		
	J2L (Cq)	B6R(Cq)	RP (Cq)	J2L (Cq)	B6R (Cq)	RP (Cq)
Abreva (7%)			24.67	33.61	35.81	27.31
Acyclovir (7 mg/mL)			25.74	33.88	35.99	27.89
Albumin (2.2 mg/mL)			24.52	35.06	36.55	26.68
Mucin (60 µg/mL)			24.46	33.74	35.36	26.85
Hydrocortisone Cream (7%)			25.19	33.26	35.44	28.20
Benadryl Cream (7%)			24.80	30.01	31.94	27.25
Carmex (7%)			24.80	32.63	35.06	27.77
Casein (7 mg/mL)			23.96	33.52		26.55
Lanacane (3.5%)			24.29	33.53	35.19	27.06
KY Jelly (7%)			25.41	32.43	33.40	28.07
Douche (7%)			24.74	34.36	34.91	27.28
Neosporin (3%)			24.23	34.12	36.11	27.72
Urine (7%)			25.24	34.15		28.20
Zine Oxide Ointment (7%)			24.71	34.17	35.56	28.17
Vagisil Cream (1%)			24.50	33.15	33.51	27.28
Cornstarch (2.5 mg/mL)			24.58	34.31	35.68	27.80
Blood/EDTA (5%)			25.66	34.49	35.75	28.17

Female urine (7%)			27.20	34.79	37.57	28.63
Male urine (7%)			27.53	35.73	36.76	28.38
Feces (0.22%)			26.49	34.51	36.11	28.26
Seminal fluid (7%)			27.71	34.56	35.56	28.98

12.4. Sample Stability

Heat-inactivated monkeypox virus (hMPXV/USA/MA001/2022) was purchased from ZeptoMetrix LLC (Cat# 0810657CFHI). The virus was originally isolated from a human in Massachusetts, USA in May of 2022 and obtained through BEI Resources. The titer of the stock solution was determined by endpoint dilution assay and confirmed to be $1.23x10^8$ TCID50/mL by ZeptoMetrix.

Negative clinical matrix was created through a pool of MPXV negative lesion swab specimens in VTM and used as background diluent in preparation of the contrived samples for the sample stability study. Contrived samples at both 2x and 5x LoD level were prepared, representing weak positive and moderate positive clinical samples, respectively. Both dilutions, as well as aliquots of the negative clinical matrix, were sealed with parafilm, kept at room temperature and away from direct light. At each pre-defined time point of 0, 6 and 24 hours, 10 replicates of 5x LoD dilution, 30 replicates of 2x LoD dilution and 10 replicates of negative clinical matrix were aliquoted, extracted and tested with the QuantiVirus™ MPXV Test Kit. The confirmed sample stability is up to 24 hours at room temperature without observable degradation (**Table 22**).

Table 22. Lesion Swab Samples Stability in VTM

Sample Stability		0 hours			6 hours			24 hours		
		Avg	Std	CV	Avg	Std	CV	Avg	Std	CV
5x LoD	J2L	33.57	0.426	1.27%	33.31	0.726	2.18%	32.85	0.683	2.08%
	B6R	35.01	0.308	0.88%	34.68	0.835	2.41%	33.81	0.648	1.92%
	RNase P	29.00	0.396	1.37%	28.75	0.311	1.08%	28.69	0.220	0.77%
2x LoD	J2L	35.55	2.656	7.47%	34.32	1.170	3.41%	34.97	2.843	8.13%
	B6R	36.89	2.229	6.04%	36.67	3.478	9.48%	35.69	2.010	5.63%
	RNase P	28.91	0.309	1.07%	28.50	0.343	1.20%	28.41	0.201	0.71%
Negative	J2L	45	N/A	N/A	45	N/A	N/A	45	N/A	N/A
	B6R	45	N/A	N/A	45	N/A	N/A	45	N/A	N/A
	RNase P	27.89	0.242	0.87%	27.43	0.187	0.68%	27.62	0.677	2.45%

12.5. Clinical Performance

Clinical evaluation was performed using 30 monkeypox virus contrived positive samples and 30 negative clinical lesion samples, using QuantiVirus™ MPXV Test Kit. Contrived samples targeted 2x and 5x LoD for monkeypox virus. Each contrived clinical specimen was prepared using an individual clinical matrix (i.e., leftover negative lesion swab specimen in VTM) for a total 30 samples spiked with Heat-inactivated monkeypox virus (hMPXV/USA/MA001/2022), purchased from ZeptoMetrix LLC (Cat# 0810657CFHI, about $1.23x10^8$ $TCID_{50}$/mL).

All 30 contrived positive clinical specimens (15 samples at 2x LoD and 15 samples at 5xLoD) were detected by both J2L and B6R targets with QuantiVirus™ MPXV Test Kit (Table 23). Positive percent agreement (PPA) is 100% (95%CI: 0.858-1.00). The negative percent agreement (NPA) is 100% (95%CI: 0.858-1.00) (Table 24), with all 30 specimens negative for the J2L and B6R targets and positive for the RNase P target

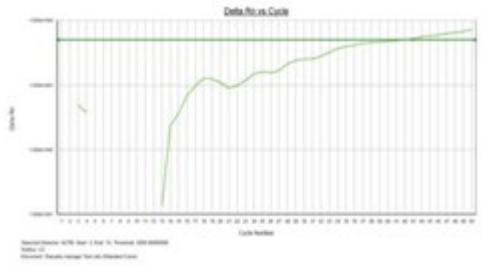
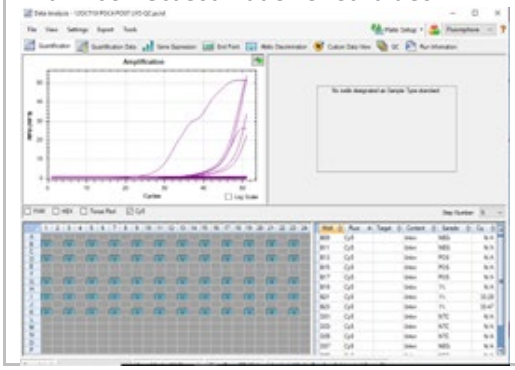
Table 23. Summary of Contrived Clinical Study Results Generated on Bio-Rad CFX384

Virus Concentration	Total (N)	J2L		B6R		RP	
		Mean Cq (SD)	Hit Rate	Mean Cq (SD)	Hit Rate	Mean Cq (SD)	Hit Rate
Negative	30	N/A	0/30	N/A	0/30	29.76 (1.15)	30/30
2x LoD	15	33.03 (0.55)	15/15	35.19 (0.67)	15/15	32.36 (1.78)	15/15
5x LoD	15	31.38 (0.28)	15/15	33.44 (0.26)	15/15	31.83 (1.41)	15/15

Table 24. Summary of Contrived Clinical Study with PPA and NPA

QuantiVirus™ MPXV Test	Contrived Clinical Samples		PPA (%)	NPA (%)
	Positive	Negative		
Positive	30	0	100% (95%CI: 0.858-1.00)	100% (95%CI: 0.858-1.00)
Negative	0	30		
Total	30	30		

PART 13. ASSAY TROUBLESHOOTING

Problem	Cause	Solution
Fluorescence signals in No Template Control (e.g., Cq <= 40) 	➢ The positive signal may be caused by contamination during setting-up of the PCR. ➢ The signal is not true target amplification, but background curves generated by the software of the PCR instrument.	Repeat the PCR with new reagents. Follow the general rules of GLP in a PCR lab. It is recommended to set up the PCR reactions in a separate area, where no DNA is handled and with equipment designated for pre-PCR activities. Make sure the workspace and instruments are decontaminated regularly. Ignore the Ct value of NTC if the amplification curve looks not real but background noise.
The Positive Control did not meet the criteria set for acceptable values of the virus RNA detection kit. The assay is invalid.	➢ Kit was not stored at the recommended conditions. ➢ Its shelf-life expired.	Check the kit label for storage conditions and expiration date and use a new kit if necessary.
The edge wells have abnormal amplification curves, resulting in high baseline threshold with incorrect estimation of Ct values. 	➢ Edge wells show high background fluorescence which prevents software from calling Ct values for sample wells.	All wells showing high background fluorescence must be deselected, threshold reset to a lower value and then reanalyzed using user defined threshold setting.

PART 14. CUSTOMER AND TECHNICAL SUPPORT

Visit diacarta.com/support for the latest service and support information.

- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

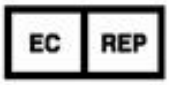
Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Contact:
Email: support@diacarta.com
Phone: +1 510-878-6662, option 4 (tech support)

1. Thermo Fisher Scientific® QuantStudio™ 5 System
2. Applied Biosystems™ 7500 Fast Real-Time PCR Systems
3. Bio-Rad CFX 384 System
4. Roche LightCycler® 480 II
5. MGISP-960 High-throughput Automated Sample Preparation System

PART 15. SYMBOLS USED IN PACKAGING

Symbols Used in Packaging

Symbol	Definition
	In vitro Diagnostics
	Authorized Representative in the European Community
	Catalog Number
	Manufactured By
	Temperature Limitation
	Batch Code
	Expiration Date
	Contains sufficient for <n> tests
1011-11-17	Date Format (year-month-day)
1011-11	Date Format (year-month)

HMIS

Health	0
Flammability	0
Reactivity	0

The product contains no substances which at their given concentration, are considered to be hazardous to health.

PART 16. REFERENCES

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