EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE QUEST DIAGNOSTICS MPOX VIRUS QUALITATIVE REAL-TIME PCR (2-WELL)

For *In vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

(The Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay will be performed at Quest Diagnostics Nichols Institute located at 33608 Ortega Highway, San Juan Capistrano, CA 92675, or other laboratories designated by Quest Diagnostics Nichols Institute that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the requirements to perform high-complexity tests, as described in the laboratory procedures reviewed by the FDA under this EUA).

INTENDED USE

The Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay is a real-time PCR test intended for the qualitative detection of DNA from monkeypox virus (Clade II) and non-variola *Orthopoxvirus* in lesion swab specimens (i.e., swabs of acute pustular or vesicular rash) in universal viral transport media (UTM) from individuals suspected of monkeypox virus infection by their healthcare provider. Testing is limited to laboratories designated by Quest Diagnostics that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the requirements to perform high complexity tests.

Results are for the identification of monkeypox virus (Clade II) and non-variola *Orthopoxvirus* DNA. The monkeypox virus (Clade II) and non-variola *Orthopoxvirus* DNA 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 II) and/or other non-variola *Orthopoxvirus* 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 monkeypox virus (Clade II) or non-variola *Orthopoxvirus* 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 Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of PCR and *in vitro* diagnostic procedures. The

Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

SPECIAL CONDITIONS FOR USE STATEMENTS

For Emergency Use Authorization Only
For Prescription Use Only
For *In vitro* Diagnostic Use Only

DEVICE DESCRIPTION AND TEST PRINCIPLE

Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well)

The Quest Diagnostics Mpox Virus Qualitative Real-time PCR (2-well) assay is a real-time PCR test intended for the qualitative detection of monkeypox[1] virus (Clade II) DNA and non-variola *Orthopoxvirus* DNA using lesion swab specimens collected from patients presenting with an acute pustular or vesicular rash when suspected of monkeypox virus infection by their healthcare provider.

Lesion swab specimens are collected using Copan 330C Universal viral transport media or equivalent. A total of 200 µL of specimen (lesion swab in UTM) is added to 250 µL of MagNA Pure External Lysis Buffer. Specimen lysis and inactivation is conducted prior to loading samples on to the Roche MagNA Pure 96 System for extraction. Onboard the MagNA Pure 96 instrument, the DNA Internal Positive Control (IPC) is added to each specimen, followed by nucleic acid extraction using the MagNA Pure DNA and Viral NA Small Volume Kit. The final elution volume is 50 µL.

Amplification and detection are accomplished using TaqMan chemistry on the ABI 7500 Real-Time PCR or ABI 7500 Fast Real-Time PCR instruments. A total of 10 µL of extracted specimen or control is added to 15 µL of prepared MPX PCR Mix (Mastermix).

The assay detects the following targets with the OPXV, MPXV, and IPC targets multiplexed in the same PCR test well. The RNase P target is evaluated in a separate PCR well.

- **Non-variola *Orthopoxvirus* (OPXV)**: A region of the *Orthopoxvirus* DNA polymerase gene (E9L), which detects several members of the *Orthopoxvirus* genus including monkeypox, vaccinia, and ectromelia viruses.

- **Monkeypox virus (MPXV)**: A region of the monkeypox virus (Clade II) TNF

[1] WHO has updated the name of the disease to "mpox". However, the International Committee on the Taxonomy of Viruses has not updated the name of the virus, and "monkeypox virus" is officially still the name of the virus.

(Tumor Necrosis Factor receptor) gene.

- **Internal Processing Control (IPC)**: An exogenous DNA control added to each specimen to ensure the absence of non-specific PCR inhibition. The Extraction & Amplification Control Primer Mix consists of a Quasar 670 labeled integrated probe and a forward primer and a reverse primer pair specific for a DNA fragment derived from the gene encoding ribulose-1,5-bisphosphate carboxylase oxygenase large unit N-methyltransferase of the plant *Arabidopsis thaliana*.

- **Internal Endogenous Control (RNase P)**: For each specimen, a separate PCR reaction targeting a human RNase P gene sequence is performed to assess specimen adequacy.

INSTRUMENTS USED WITH TEST

The Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay is to be used with the following instrumentation:

- Applied Biosystems 7500 Real-Time PCR System (Software v1.4 or v1.5.1)
- Applied Biosystems 7500 Fast Real-Time PCR System (Software v1.5.1)
- Roche MagNA Pure 96 Instrument

REAGENTS AND MATERIALS

Table 1: Reagents/Materials Used to Perform the Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay

Reagent/Materials	Catalog #	Manufacturer
MagNA Pure 96 DNA and Viral NA Small Volume Kit	06543588001	Roche Diagnostics
MagNA Pure 96 System Fluid	06430112001	Roche Diagnostics
MagNA Pure 96 External Lysis Buffer	06374913001	Roche Diagnostics
Exogenous DNA	MOL9001	Diasorin
TE Buffer, pH 8.0	9859	Ambion
TaqPath qPCR Master Mixes, CG	A15297	Applied Biosystems
DNA Primer Pair (Q670/BHQ1)	MOL9005	Diasorin
DEPC-Treated or Nuclease Free Water	AM9937	Ambion
Primers and Probes: o MPXV-WA Forward Primer(100µM) o MPXV-WA ReversePrimer(100µM) o MPXV-Probe(100µM) o OPXV-Forward(100µM) o OPXV-Reverse(100µM)	Custom	Biosearch

Reagent/Materials	Catalog #	Manufacturer
o OPXV-Probe(100µM) o RNase P-Forward (100 µM) o RNase P-Reverse (100 µM) o RNase P-Probe (100 µM)		

CONTROLS TO BE USED WITH THE QUEST DIAGNOSTICS MPOX VIRUS QUALITATIVE REAL TIME PCR (2-WELL) ASSAY

External Controls:

Two controls (external Positive and Negative controls) are included in each extraction batch, and carried through the full testing process, extraction through amplification and detection:

- The external Positive Control is a 100X intermediate stock that is prepared by diluting 1) vaccinia virus and monkeypox virus genomic DNA) together in TE, or 2) left-over de-identified patient specimen in UTM. The intermediate stock is dispensed into aliquots and the aliquots are stored frozen at–70°C or lower for up to 1 year. The intermediate stock is then diluted to a final stock that yields a MPXV Ct and OPXV Ct value of approximately 30-33.

- The external Negative Control consists of Quest VCM transport media.

Internal Processing Control:

- To ensure the absence of non-specific PCR inhibition of a sample, an internal positive amplification control (IPC) is added to each specimen/control. A sample can be interpreted as negative only if the analysis of the IPC indicates that amplification has occurred in the reaction tube but no signal from OPXV or MPXV target reporter dyes are detected.

Endogenous Internal Control:

- A separate RNase P assay is performed for each clinical specimen. For each batch of specimens evaluated with the RNase P assay, a known RNase P-positive specimen in UTM as well as a clean UTM sample are run as positive and negative controls.

INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the control results are not acceptable, the patient results cannot be interpreted (Refer to Table 2 for a summary of control results).

1) ***Assay Controls – Expected Results***

Controls results (detection cycle or Ct) are generated for the MPXV, OPXV, and IPC assay targets. Acceptable control results are required for the run to be acceptable. Controls must meet the following acceptance criteria to be considered valid (Table 2).

Table 2: Expected Control Results

Control	Acceptance Criteria*		
	MPXV target (FAM channel)	**OPXV target (TAMRA channel)**	**IPC target (Q670 channel)**
Positive Control	POS	POS	Not applicable
Negative Control	NEG	NEG	POS

* For the Positive Control and Negative Control:

- POS means Ct <40.00 and valid amplification curve, and
- NEG means Ct ≥40.00

NOTE: Technically the instrument does not generate a value over 40 Ct. However, Quest uses "Ct ≥40.00" internally as short-hand to indicate that the Ct value was undetermined.

RNase P Controls: The RNase P positive control should generate Ct <45 with a valid amplification curve. The RNase P negative control should be negative (Ct ≥45.00).

2) *Examination and Interpretation of Specimen Results*:

Interpretation of clinical specimen test results can be conducted after the positive and negative controls have been examined and determined to be valid. If the controls are not valid, results for clinical specimens cannot be interpreted.

In the initial run of the specimen:

- The specimen is positive for the MPXV target and/or the OPXV target if the target Ct is <38.00 and the amplification curve is valid.
- The specimen is negative for the MPXV target and/or the OPXV target if the target Ct is ≥40.00.
- The specimen should be re-extracted and re-tested if either of the MPXV target and/or the OPXV target Ct is 38.00 to <40.00. If there is not adequate specimen to re-extract, then the first extraction may be used for the re-test.
- The specimen is positive for the IPC target if the
  - IPC Ct <40.00 and
  - amplification curve is valid and
  - IPC Ct is within ±3 Ct of the Negative Control IPC Ct
- The specimen is negative for the IPC target if the
  - IPC Ct ≥40.00 OR

- amplification curve is NOT valid OR
- The IPC Ct is not within ±3 Ct of the Negative Control IPC Ct

- The specimen is positive for the RNaseP target if the
  - RNaseP Ct <45.00 and
  - Amplification curve is valid

- The specimen is negative for the RnaseP target if the
  - RNaseP Ct ≥45.00 OR
  - amplification curve is NOT valid

In the re-test run of the specimen:

- The specimen is positive if the MPXV target and/or the OPXV target is <40.00 and the amplification curve is valid.

- The specimen is negative for the MPXV target and/or the OPXV target if the target Ct is ≥40.00.

Table 3 includes details on assay cutoffs and interpretation for individual target results. Table 4 includes details on final result interpretation for patient specimens.

Table 3: Results Interpretation, Individual Targets

Run	MPXV target	OPXV target	IPC target	RNase P target
Initial Run	Positive for MPXV target if: • MPXV Ct <38.00 and • Amplification curve is valid Negative for MPXV target if: • MPXV Ct is ≥40.00 Re-test if: • MPXV Ct is 38.00 to <40.00 *	Positive for OPXV target if: • OPXV Ct <38.00 and • Amplification curve is valid Negative for OPXV target if: • OPXV Ct is ≥40.00 Re-test if: • OPXV Ct is 38.00 to <40.00 *	Positive for the IPC target if: • IPC Ct <40.00 and • Amplification curve is valid and • IPC Ct is within ±3 Ct of the Negative Control IPC Ct Negative for the IPC target if • IPC Ct ≥40.00 OR • Amplification curve is NOT valid OR • The IPC Ct is not within ±3 Ct of the Negative Control IPC Ct	Positive for the RNase P target if: • RNase P Ct <40.00 and • Amplification curve is valid Negative for the RNase P target if • RNase P Ct ≥40.00 OR • Amplification curve is NOT valid
Re-test Run**	Positive for MPXV target if: • MPXV Ct <40.00 and • Amplification curve is valid Negative for MPXV target if: • MPXV Ct is ≥40.00	Positive for OPXV target if: • OPXV Ct <40.00 and • Amplification curve is valid Negative for OPXV target if: • OPXV Ct is ≥40.00	Same as above.	Same as above.

* The specimen should be re-extracted and re-tested. If there is not adequate specimen to re-extract, then the first extraction may be used for the re-test.
**Initial result for MPXV is taken as POSITIVE if result is MPXV Ct < 38. Retest result is only for OPXV. Initial result for OPXV is taken as POSITIVE if OPXV Ct < 38. Retest result is only for MPXV.

Table 4: Results Interpretation, Reporting

Target Results				Interpretation/Conditional Comment
MPXV target	OPXV target	IPC target	RNaseP Specimen Adequacy target	
NEG	NEG	POS	POS	Monkeypox virus DNA Not Detected This specimen is negative for monkeypox virus DNA. If clinically indicated, consider collecting another specimen. Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws.

Target Results				Interpretation/Conditional Comment
MPXV target	**OPXV target**	**IPC target**	**RNaseP Specimen Adequacy target**	
POS	POS	Not applicable	Not applicable	Monkeypox virus DNA Detected This specimen is positive for monkeypox virus (Clade II) DNA. Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws.
POS	NEG	Not applicable	Not applicable	Monkeypox virus DNA Detected. This specimen is positive for monkeypox virus (Clade II) DNA. Low levels of DNA in a specimen may result in monkeypox virus DNA Detected and non-variola *Orthopoxvirus* DNA Not Detected. Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws.
NEG	POS	Not applicable	Not applicable	Non-variola *Orthopoxvirus* DNA Detected. This specimen is positive for non-variola *Orthopoxvirus* DNA. Low levels of DNA in a specimen may result in monkeypox virus (Clade II) DNA Not Detected and non-variola *Orthopoxvirus* DNA Detected. This result may occur in the case of low viral DNA concentration or may indicate infection with monkeypox virus (Clade I) or another *Orthopoxvirus,* such as vaccinia. If clinically indicated, consider collecting another specimen. Report to public health authorities, as applicable. Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws.
NEG	NEG	NEG	Not applicable	Invalid. Repeat extraction and PCR. If upon repeat testing the same situation occurs (i.e., all three targets are negative), then the patient result is reported as “Unable to report” due to inhibition (TNP code as appropriate). Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws.
NEG	NEG	Not applicable	NEG	Invalid. Report as “Unable to report” due to failed specimen adequacy control (TNP code as appropriate). Laboratories should notify appropriate Federal, State, or local public health agencies of the test results in accordance with applicable laws.

PERFORMANCE EVALUATION

Analytical and Clinical Performance of the Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay

1) Analytical Sensitivity (Limit of Detection):

The Limit of Detection (LOD) of the assay was evaluated to determine the lowest concentration that is successfully detected with a probability of 95% or greater. Quest Diagnostics initially estimated the LoD using samples spiked with commercially available non-variola *Orthopoxvirus* (live, attenuated vaccinia virus,) and monkeypox virus synthetic DNA quantified by the manufacturer. Samples were prepared using UTM from leftover negative lesion swab samples.

The monkeypox virus synthetic DNA material was diluted to 50, 100, 250, 500, 1000, 5000 and 10000 copies/mL. The non-variola *Orthopoxvirus* material (vaccinia) was quantified using a standard curve of a commercially available quantitated *Orthopoxvirus* synthetic gene fragment and diluted to 100, 250, 500, 1000, 5000 and 10000 copies/mL.

Each sample was extracted 20 separate times and tested. The mean Ct values and percent detected were calculated. The concentration of monkeypox virus synthetic DNA that was successfully detected with at least a 95% detection rate was calculated as 100 copies/mL (Table 5). The concentration of non-variola *Orthopoxvirus* (vaccinia virus) that was successfully detected with at least a 95% detection rate was calculated as 250 copies/mL (Table 6).

Table 5: LoD. Evaluation of samples prepared with monkeypox virus synthetic DNA

Sample		MPXV		OPXV	
Target	Level (copies/mL)	Mean Ct	% Detected	Mean Ct	% Detected
MPXV	10,000	29.83	100% (20/20)	n/a	0/20
	5000	30.69	100% (20/20)	n/a	0/20
	1000	32.82	100% (20/20)	n/a	0/20
	500	33.69	100% (20/20)	n/a	0/20
	250	34.71	100% (20/20)	n/a	0/20
	100	36.00	100% (20/20)	n/a	0/20
	50	36.32	85% (17/20)*	n/a	0/20

Note: the IPC was detected for all samples tested with a Mean Ct of 27.5

Table 6: LoD Evaluation of samples prepared with vaccinia virus

Sample	MPXV		OPXV	
Level (copies/mL)	Mean Ct	% Detected	Mean Ct	% Detected
10,000	n/a	0/20	29.32	100% (20/20)
5000	n/a	0/20	29.00	100% (20/20)
1000	n/a	0/20	33.19	100% (20/20)
500	n/a	0/20	34.46	100% (20/20)
250	n/a	0/20	36.27	100% (20/20)
100	n/a	0/20	36.67	90% (18/20)

Note: IPC target was detected for all samples tested with a mean Ct of 26.5

2) Thermocycler Equivalency Study:

A study was conducted to evaluate the ABI 7500 and ABI 7500 Fast instruments with samples prepared at near LoD concentrations. Positive clinical specimen material was quantitated using a standard curve generated using commercially available synthetic DNA. The positive clinical specimen material was diluted with individual negative lesion specimen (or UTM for the highest concentrations). Testing was conducted with samples prepared at 500, 300, 200, 100, 50, and 25 copies/mL.

Each sample replicate was separately extracted and tested. The performance near the LoD for both the MPXV and OPXV targets was equivalent between the two instruments, with 100% detection (25/25) for sample replicates prepared at 100 copies/mL and <95% detection for samples prepared at lower concentrations (Table 7).

Table 7: Thermocycler Equivalency Study Results

Virus Level (copies/mL)	ABI7500				ABI7500 fast			
	MPXV		OPXV		MPXV		OPXV	
	Mean Ct	% Detected	Mean Ct	% Detected	Mean Ct	% Detected	Mean Ct	% Detected
500	32.56	100% (5/5)	34.11	100% (5/5)	33.08	100% (5/5)	34.51	100% (5/5)
300	33.71	100% (10/10)	35.43	100% (10/10)	34.11	100% (10/10)	35.17	100% (10/10)
200	34.03	100% (5/5)	34.91	100% (5/5)	34.63	100% (5/5)	35.93	100% (5/5)
100	34.87	100% (25/25)	35.89	100% (25/25)	35.15	100% (25/25)	36.32	100% (25/25)
50		60% (3/5)[A]		60% (3/5)[C]		80% (4/5)[E]		40% (2/5)[F]
25		40% (2/5)[B]		20% (1/5)[D]		40% (2/5)		0% (0/5)[G]

[A] Of the two replicates that were not detected, 1/2 had a Ct value between 38.00 and 40.00, and was not repeated.
[B] Of the three replicates that were not detected, 2/3 had a Ct value between 38.00 and 40.00, and was not repeated.
[C] Of the two replicates that were not detected, 2/2 had a Ct value between 38.00 and 40.00, and was not repeated.
[D] Of the four replicates that were not detected, 4/4 had a Ct value between 38.00 and 40.00, and was not repeated.
[E] Of the one replicate that was not detected, 1/1 had a Ct value between 38.00 and 40.00, and was not repeated.
[F] Of the three replicates that were not detected, 3/3 had a Ct value between 38.00 and 40.00, and was not repeated.
[G] Of the five replicates that were not detected, 2/5 had a Ct value between 38.00 and 40.00, and was not repeated.

3) Inclusivity/Exclusivity:

Inclusivity, *in silico* analysis, for MPXV Target:

An *in silico* inclusivity analysis was conducted by aligning the MPXV target primers and probe sequence against available monkeypox virus sequences as of July 7, 2022. The evaluation included a total of 348 monkeypox virus sequences, including 309 sequences of monkeypox virus (Clade II) and 39 sequences of monkeypox virus (Clade I).

Basic local alignment search tool (BLAST) searches were performed using the MPXV primer and probe sets against the NCBI non-redundant nucleotide database. Searches were limited by organism to *Orthopoxvirus* (taxid: 10242) genome sequences longer than 100,000 nucleotides. A match was counted when primer and probe coverage and sequence identity was ≥90% for both primers and the probe. Geneious Prime software was used to confirm sequences that had no BLAST search matches.

Of the 305 sequence entries for monkeypox virus (Clade II) included in the analysis, 305 matched the MPXV primers and probe by at least 90%, with 304/305 sequences showing 100% homology to the MPXV primers/probe. Of the 39 monkeypox virus (Clade I) sequences, none of the sequences evaluated (0/39) matched the MPXV primers or probe by at least 90%. Results are presented in Table 8.

Table 8: Inclusivity of MPXV target

Species	Clade	Number of sequences evaluated	Sequences with ≥90% match to both MPXV primers and probe [B]
Monkeypox virus	(Clade II)	305 [A]	305
Monkeypox virus	(Clade I)	39	0

A One sequence had on mismatch with the MPXV probe; detection is predicted

Inclusivity, *in silico* analysis for OPXV target

In silico inclusivity for OPXV primers and probe sequences were compared against 383 non-variola *Orthopoxvirus* sequences, including:

- 348 monkeypox viruses
- 15 cowpox viruses,
- 11 vaccinia viruses,
- 3 Akhmeta viruses,
- 2 buffalopox viruses,
- 1 horsepox virus
- 1 Alaskapox virus, and
- 1 ectromelia virus

Of the 348 monkeypox virus (Clade II) sequences, 348/348 matched the OPXV primer/probe set by at least 90%, with 346/348 (99.4%) of sequences demonstrating 100% homology to the OPXV target.

For the other non-variola *Orthopoxvirus* sequence entries evaluated, the analysis supports the predicted detection of all viruses, except for one of eleven vaccinia virus sequences and the one Alaskapox virus sequence, which contained multiple mismatches (>3) to the OPXV primers and/or probe.

Exclusivity/Cross-Reactivity (Wet-testing):

The Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay was evaluated for potential cross-reactivity with 13 commercially available microorganisms and viruses at sample concentrations of greater than $1x10^6$ CFU/mL or copies/mL. Additionally, a *T. pallidum* PCR positive remnant specimen was evaluated. The microorganisms or viruses were spiked at high concentrations into pooled UTM from negative lesion swab samples.

No cross-reactivity was observed with any of the fourteen microorganisms and viruses evaluated in the study. Results are presented in Table 9.

Table 9: Cross-reactivity

Organism	Concentration	MPXV Ct	OPXV Ct
Staphylococcus epidermidis, strain PCI 1200	3.8×10^6 cfu/mL	>40	>40
Staphylococcus aureus	1.6×10^6 cfu/mL	>40	>40
Streptococcus pyogenes	3.6×10^6 cfu/mL	>40	>40
Human herpesvirus 7	3.0×10^8 cp/mL	>40	>40
Human herpesvirus 8	1.0×10^7 cp/mL	>40	>40
Coxsackie A16	2.0×10^8 cp/mL	>40	>40
Measles, strain Edmonston	2.5×10^8 cp/mL	>40	>40
Varicella Zoster Virus Culture Fluid, strain Ellen	3.5×10^8 cp/mL	>40	>40
JC polyomavirus, strain MAD-4	1.0×10^8 cp/mL	>40	>40
Epstein Barr Virus Culture Fluid, strain B95-8	3.6×10^6 cp/mL	>40	>40
Human herpesvirus 1, strain MacIntyre	9.2×10^8 cp/mL	>40	>40
Human herpesvirus 2, strain MS	7.3×10^8 cp/mL	>40	>40
Human herpesvirus 5 (CMV), strain AD-169	1.4×10^7 cp/mL	>40	>40
Treponema pallidum	1:100 dilution of specimens with PCR Ct=11	>40	>40

Exclusivity/Cross-Reactivity (*In silico* analysis):

Basic local alignment search tool (BLAST) searches were performed using the MPXV primer and probe sets against the NCBI non-redundant nucleotide database. Cross-reactivity was predicted when primer and probe coverage and sequence identity was ≥85% for both primers and the probe in the two primer and probe sets. Analysis conducted on July 7, 2022, showed that the OPXV primers and probe were 100% exclusive for variola virus and the MPXV primers were 100% exclusive of the Clade I strains.

For the MPXV primer and probe sequences, Quest Diagnostics evaluated sequences from 11 viruses (10 *Orthopoxvirus* and 1 *Molluscipoxvirus*). Of the 388 viral sequences, 323 sequences matched both MPXV primers at least 85% (and possibly could result in amplicon), and 65 sequences lacked forward and/or reverse MPXV primer binding sites. All 388 viral sequences lacked binding sites for the MPXV probe (so even if amplicons were generated, the probe is not predicted to produce a signal). The data supports that cross-reactivity is not predicted for the viruses evaluated. Results are presented in Table 10.

Table 10: Results of In Silico Exclusivity Analysis of MPXV primer and probe vs viral sequences

Species	Taxid	Upper level (genus)	Number of sequences evaluated	Sequences with ≥85% match to both primers	Sequences with ≤80% match to at least one primer	Sequences with ≥80% match to probe
Vaccinia virus	10245	*Orthopoxvirus*	164	161	3	0
Cowpox Virus	10243	*Orthopoxvirus*	96	84	12	0
Variola virus (Smallpox)	10255	*Orthopoxvirus*	58	55	3	0
Camelpox virus	28873	*Orthopoxvirus*	14	14	0	0
Ectromelia virus	12643	*Orthopoxvirus*	13	0	13	0
Tatera virus (gerbilpox)	28871	*Orthopoxvirus*	2	2	0	0
Uasin Gishu disease virus (horsepox)	397342	*Orthopoxvirus*	3	3	0	0
Raccoon poxvirus	10256	*Orthopoxvirus*	3	2	1	0
Volepox virus	28874	*Orthopoxvirus*	2	2	0	0
Skunkpox Virus	160796	*Orthopoxvirus*	2	0	2	0
Molluscum Contagiosum virus	10279	*Molluscipoxvirus*	31	0	31	0

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

Quest Diagnostics conducted an *in silico* analysis for the MPXV primer/probe sequences against non-viral sequences from nine bacterial species and two fungal species that were not evaluated by wet-testing in the cross-reactivity study.

Of the non-viral sequences evaluated, no sequences demonstrated >80% homology with both MPXV primers, and all sequences lacked binding sites for the MPXV probe. Results from the analysis demonstrated that for the 11 microorganisms evaluated, cross-reactivity is not predicted for the MPXV primers/probe included in the Quest Mpox Virus Qualitative Real-Time PCR (2-well) assay. Results are presented in Table 11.

Table 11: Results of *In Silico* Exclusivity Analysis of MPXV primer and probe vs Non-viral sequences

Type	Species	Taxid	Upper level (genus)	Number. of sequences evaluated	Sequences with ≥85% match to Both Primers	Sequences with ≤80% match to at least one Primer	Sequences with ≥80% match to Probe
Bacteria	*Streptococcus agalactiae*	1311	*Streptococcus*	28	0	28	0
Bacteria	*Streptococcus mitis*	28037	*Streptococcus*	1	0	1	0
Bacteria	*Pseudomonas aeruginosa*	287	*Pseudomonas*	56	0	56	0
Bacteria	*Corynebacterium jeikeium*	38289	*Corynebacterium*	4	0	4	0
Bacteria	*Escherichia coli*	562	*Escherichia*	4456	0	4456	0
Bacteria	*Acinetobacter calcoaceticus*	471	*Acinetobacter*	5	0	5	0
Bacteria	*Bacillus fragilis*	817	*Bacteroides*	10	0	10	0
Bacteria	*Enterobacter faecalis*	1351	*Enterococcus*	52	0	52	0
Bacteria	*Lactobacillus species*	1578	*Lactobacillus*	38	0	38	0
Fungi	*Trichophyton rubrum*	5551	*Trichophyton*	967	0	967	0
Fungi	*Candida albicans*	5476	*Candida*	33	0	33	0

4) Interfering Substances:

The assay uses conventional a well-established nucleic acid extraction method; therefore, interference from common endogenous substances is not expected. Interference studies have not been performed for this assay.

5) Specimen Stability:

Quest Diagnostics evaluated specimen stability using samples prepared with diluted clinical specimen material previously determined to be positive for monkeypox virus. The positive specimen material was quantified using a standard curve generated with synthetic DNA containing a monkeypox virus gene fragment. For the specimen stability study, five sample replicates were prepared at each of three concentrations (2x, 5x and 10x LoD); samples were prepared by spiking quantified monkeypox virus material spiked into pooled negative clinical lesion swab matrix in UTM. Five negative sample replicates in clinical matrix were also evaluated for each storage condition.

The storage conditions evaluated included 18-23°C (room temperature), 2-8°C and -20°C as well as samples subjected to three freeze/thaw cycles. For each storage condition and freeze/thaw cycle evaluated, study results demonstrated 100% detection for both MPXV and OPXV targets for all sample replicates spiked with monkeypox virus. In addition, all non-spiked sample replicates generated the expected negative results. In summary, the study results support the recommended storage conditions included in the instructions for

use (up to 24 hours at 18-23°C, up to 7 days at 2-8°C or up to 30 days at -20°C). Study results also support stability for samples subjected to up to three freeze/thaw cycles at -20°C.

6) Clinical Evaluation:

Testing of Consecutive Clinical Specimens (N=492):

Clinical performance of the Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay was evaluated with 492 lesion swab specimens that were collected from individuals suspected of monkeypox virus infection. The study included sequential (i.e., all-comer) specimens, without selection based on prior testing results, patient age, geography, patient sex or for other reasons. Testing included a mixture of VTM and UTM specimens.

An aliquot of each specimen was extracted and tested with the Quest Diagnostics Mpox Virus Qualitative Real-Time PCR (2-well) assay. A separate aliquot was extracted and tested with an FDA-cleared molecular comparator assay that detects non-variola *Orthopoxvirus*.

The 492 lesion swab specimens tested in the study were collected from 383 individuals. A total of 304 specimens were collected from unique individuals. The remaining 188 specimens were collected from 79 individuals for whom more than one specimen was collected and tested. Specimen details are presented in Table 12.

Table 12: Number of Specimens Collected per Individual

	Specimens per Individual	Number of Individuals	Specimens Tested
Specimen Numbers	1	304	304
	2	56	112
	3	17	51
	4	5	20
	5	1	5
Total		383	492

A total of 152 specimens were excluded from the clinical performance resulting in 340 evaluable specimens. Reasons for exclusion included the following:
*Note that some specimens were excluded for more than one reason.

- Additional specimens tested per individual (109 specimens) and/or
- Inconclusive or Equivocal result for the FDA-cleared comparator assay (63 specimens: 26 equivocal and 37 inconclusive) and/or
- Invalid result for the Quest assay (43/492, 8.7%)

The final clinical performance included results for only one specimen per individual. For those individuals with multiple specimens, selection of included/excluded specimens was

conducted in an unbiased manner. The specimens tested included 21.2% (11/52) low positive specimens as determined by the comparator assay (i.e., specimens generated Ct values for the comparator assay that are no lower than 3 Ct below the mean Ct at LoD). The clinical performance for 340 evaluable specimens is presented in Table 13.

Table 13: Clinical Performance

		Cleared Comparator Assay		
		POSITIVE	**NEGATIVE**	**Total**
Quest Mpox Assay	**POSITIVE**	48	2	**50**
	NEGATIVE	4	286	**290**
	TOTAL	**52**	**288**	**340**
Positive Agreement		92.3% (48/52, 95% CI 81.8-97.0%)		
Negative Agreement		99.3% (286/288 95% CI 97.5-99.8%)		

Contrived Positive Specimens:

A clinical evaluation study was conducted to evaluate contrived positive clinical specimens with the Quest Diagnostic Mpox Virus Qualitative Real-Time PCR (2-well) assay. Each contrived clinical specimen was prepared using an individual clinical matrix (i.e., leftover negative lesion swab specimen in UTM). A monkeypox virus-positive specimen, quantified using a standard curve generated with synthetic monkeypox virus DNA, was spiked into each individual clinical specimen matrix. A total of 35 positive contrived specimens were tested, with 20 specimens prepared at 100 copies/mL, the established LoD for the monkeypox virus target, and five specimens each at concentrations of 200, 300 and 500 copies/mL. All 35 contrived positive clinical specimens were detected with both the MPXV and OPXV targets. Positive percent agreement is 100% (95% CI: 90.1-100%). Results are presented in Table 14.

Table 14: Clinical Evaluation, Contrived Positive Clinical Specimens.

MPXV (copies/mL)	**MPXV**		**OPXV**		**IPC**	
	Mean Ct	**% Detected**	**Mean Ct**	**% Detected**	**Mean Ct**	**% Detected**
500	32.56	100% (5/5)	34.11	100% (5/5)	25.4	100% (5/5)
300	33.84	100% (5/5)	35.68	100% (5/5)	25.5	100% (5/5)
200	34.03	100% (5/5)	34.91	100% (5/5)	25.3	100% (5/5)
100	34.75	100% (20/20)	35.79	100% (20/20)	25.5	100% (20/20)

Testing of Negative Clinical Specimens:

A total of 30 presumed negative clinical lesion swab specimens collected from females were tested with the Quest Diagnostic Mpox Virus Qualitative Real-Time PCR (2-well) assay. Each lesion specimen was submitted for HSV testing early in the 2022 monkeypox virus outbreak and therefore expected to be negative for monkeypox virus. Each specimen was evaluated with the multiplexed assay (MPXV, OPXV, IPC targets) and the

separate RNase P assay. Of the 30 presumed negative specimens, the negative percent agreement is 100% (30/30, 95% CI 88.7-100%) with all specimens negative for the MPXV and OPXV targets, and positive for the RNase P target (Table 15).

Table 15: Clinical Evaluation, Negative Specimens

Sample ID	MPXV Ct	OPXV Ct	IPC Ct	RnaseP Ct
Negative 1	>40	>40	26.06	27.26
Negative 2	>40	>40	26.13	27.01
Negative 3	>40	>40	26.04	26.60
Negative 4	>40	>40	26.01	26.12
Negative 5	>40	>40	26.17	19.54
Negative 6	>40	>40	26.10	25.37
Negative 7	>40	>40	26.07	26.10
Negative 8	>40	>40	26.22	27.27
Negative 9	>40	>40	25.70	31.03
Negative 10	>40	>40	25.73	23.28
Negative 11	>40	>40	25.55	23.76
Negative 12	>40	>40	25.78	19.32
Negative 13	>40	>40	25.98	26.12
Negative 14	>40	>40	25.92	28.12
Negative 15	>40	>40	25.75	31.75
Negative 16	>40	>40	25.89	20.25
Negative 17	>40	>40	25.78	24.02
Negative 18	>40	>40	25.55	29.62
Negative 19	>40	>40	25.58	31.09
Negative 20	>40	>40	25.37	25.62
Negative 21	>40	>40	25.83	29.56
Negative 22	>40	>40	25.93	35.63
Negative 23	>40	>40	25.70	27.01
Negative 24	>40	>40	25.83	25.46
Negative 25	>40	>40	25.87	33.30
Negative 26	>40	>40	25.64	34.16
Negative 27	>40	>40	25.69	24.18
Negative 28	>40	>40	25.59	21.80
Negative 29	>40	>40	25.65	22.66
Negative 30	>40	>40	25.73	24.02

Evaluation of Negative Clinical Specimens from Healthy Volunteers

An additional 20 skin swab specimens in UTM were collected from 20 healthy volunteer healthcare workers. All specimens were negative for both the MPXV and OPXV targets, as expected.
Negative Percent Agreement (NPA) = 100% (20/20, 95%CI 83.9-100%)

Evaluation of Clinical Specimens: Assessment of RNase P results, Invalid Rate

A total of 149 sequentially collected lesion swab specimens were tested with the Quest Diagnostics Mpox Virus Qualitative Real-time PCR assay (2-well). All specimens were collected from individuals suspected of monkeypox virus infection.

For 33 of the 149 specimens, both the MPXV and OPXV assay targets were detected (22.1% positivity rate).

Of the remaining 116 specimens, for which the MPXV and OPXV targets were not detected, 97 specimens were positive for RNase P (i.e., final test result is valid) and 19/116 (16.4%) were negative for RNase P (i.e., final test result is invalid). There were no invalid results due to IPC failure.

The invalid rate observed for all specimens tested was 12.8% (19/149).

LIMITATIONS:

- Performance of the assay has been evaluated using contrived clinical lesion swab specimens. Clinical Performance with natural clinical lesion specimens has not been established.
- The assay is indicated for testing of lesion swab specimens. Performance for other specimen types has not been established.
- Performance of the test has only been established in lesion swabs collected in universal viral transport media tubes (UTM Copan 330C) or equivalent media. Copan UTM (cat#330C), Fisher Universal Transport Medium (cat#23001718), Hardy Universal Transport Medium (cat#330CHL), BD UVT (cat# 220220), Quest VCM (cat#220223), and Quidel Universal Transport Medium (cat#330C.DHI) are equivalent. Performance of the test has not been evaluated for dry swabs or for lesion swabs collected in other transport media types.
- Quest Diagnostics did not independently evaluate fresh-frozen testing. Quest Diagnostics adopted standard practices recommended by the Centers for Disease Control and Prevention.
- Quest Diagnostics did not perform an interfering substances study. The assay uses conventional well-established nucleic acid extraction methods and based on our experience with other similar assays, e.g., HSV Real-Time PCR. We do not

anticipate interference from common endogenous substances. Interference studies have not been performed for this assay.

- While monkeypox virus is the only member of the *Orthopoxvirus* genus known to be circulating among humans in the US at this time, a positive result for the non-variola *Orthopoxvirus* target most likely represents the presence of monkeypox virus clade II. However, there is a small possibility that this result could represent the presence of monkeypox virus clade I or a different non-variola *Orthopoxvirus* such as vaccinia virus. If clinical concern for such an infection exists, healthcare providers should contact the CDC and their local public health authorities for guidance.

WARNINGS:

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by laboratories designated by Quest Diagnostics that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests;
- This product has been authorized only for the detection of nucleic acid from monkeypox virus or other non-variola orthopoxviruses, not for any other viruses or pathogens; and,
- 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 infection with the 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.

REVISION HISTORY:

Date	**Summary of Updates**
September 7, 2022	Original authorized
September 15, 2022	Minor edits to the description of the control materials
December 9, 2022	Update to validated specimen stability in UTM parameters to address Condition of Authorization O. in the September 7, 2022 Letter of Authorization and add use of leftover deidentified positive patient specimens as an acceptable positive control material
May 22, 2023	Update for evaluating the clinical performance of the assay using natural clinical lesion swab specimens to address Condition of Authorization N in the September 7, 2022 Letter of Authorization

November 22, 2024	Update the labeling to indicate that monkeypox virus is the only orthopoxvirus currently circulating in North America (no longer limited to clade II).