# EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

## Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA
## Centers for Disease Control and Prevention (CDC)

For *in vitro* Diagnostic Use

Rx Only

For Use Under Emergency Use Authorization (EUA) Only

***The Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set - EUA will be performed at Centers for Disease Control and Prevention designated laboratories, certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high-complexity tests, as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.***

## INTENDED USE

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA is a real-time PCR test intended for the presumptive qualitative detection of DNA from non-variola *Orthopoxvirus* in human pustular or vesicular rash specimens and viral cell culture lysates submitted to a Centers for Disease Control and Prevention designated laboratory from individuals suspected of monkeypox virus infection by their healthcare provider. Testing is limited to Centers for Disease Control and Prevention designated laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity testing.

This test is also authorized for use with acceptable human pustular or vesicular rash specimens collected using authorized home specimen collection kits that are indicated for use with the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set – EUA when used consistent with their authorization.

Results are for the identification of non-variola *Orthopoxvirus* DNA. This assay does not differentiate vaccinia virus or monkeypox virus from other orthopoxviruses detected by this assay and does not detect variola virus. The non-variola *Orthopoxvirus* DNA is generally detectable in human pustular or vesicular rash specimens and viral cell culture lysates during the acute phase of infection. Refer to the CDC algorithms, Acute, Generalized Vesicular or Pustular Rash Illness Testing Protocol and Evaluating Patients for Smallpox: Acute, Generalized Vesicular or Pustular Rash Illness Protocol in the United States for recommended testing and evaluation algorithms for patients presenting with acute, generalized pustular or vesicular rash illness. Results of this assay are for the identification of non-variola *Orthopoxvirus* DNA. These results must be used in conjunction with other diagnostic assays and clinical observations to diagnose *Orthopoxvirus* infection.

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

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA 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 Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set – EUA is only for use under the Food and Drug Administration's Emergency Use Authorization.

## SPECIAL CONDITIONS OF USE STATEMENTS

- For Emergency Use Authorization (EUA) only.
- For prescription use only.
- For in vitro diagnostic use.

## DEVICE DESCRIPTION AND TEST PRINCIPLE

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA is a real-time polymerase chain reaction (PCR) test that represents certain modifications to the procedure and uses that are not under the indications for use of the FDA cleared Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set.[1] The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA detects most commonly known human pathogenic orthopoxviruses (i.e., vaccinia, cowpox, and monkeypox viruses) but does not detect variola virus, the causative agent of smallpox. The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA can be used to test human pustular or vesicular rash specimens, viral cell culture lysates and human pustular or vesicular rash specimens collected using authorized home specimen collection kits that are indicated for use with the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set – EUA test. With the exception of some reagents and instrumentation, the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set – EUA device description is the same as the description of the cleared device (*K222558*, *K221834*, *K221658*, K181205).

Viral DNA is extracted from patient samples and controls using cleared extraction methods or the authorized KingFisher MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit in combination with the KingFisher Flex Purification System, KingFisher with 96 Deep-well Head. Master mix and amplification reagents are prepared and added to test well plates before addition of the extracted samples, including patient samples and various controls. Real-time PCR amplification is performed using cleared real-time PCR instruments or the authorized QuantStudio 7 Flex Real-Time PCR System. Once the RT-PCR run is complete, results for each sample are analyzed.

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[1] *The Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set was granted De Novo and also received marketing clearances from FDA under section 510(k) of the Act (Product Code: PBK; DEN070001, K181205, K221658, K221834, K222558). The emergency use authorization for the Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set -EUA authorizes certain modifications to the procedure and uses that are not under the cleared indications for use of the product and are an "unapproved use of an approved product" under section 564(a)(2)(B) of the FD&C Act.*

## INSTRUMENTS FOR USE WITH THE TEST

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA test is authorized for use with the following additional extraction instruments and real-time PCR instrumentation:

**Table 1. Instruments and Software for Use with the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA**

| Instrument | Manufacturer | Model | Catalog Number |
|---|---|---|---|
| Automated Extraction Instrument | ThermoFisher Scientific | KingFisher Flex Purification System, KingFisher with 96 Deep-well Head | 5400630 |
| Real Time PCR Instrument | Applied Biosystems | QuantStudio 7 Flex Real-Time PCR System | 4485701 |

## COLLECTION KITS USED WITH THE TEST

- This test can be used with authorized home specimen collection kits that are indicated for use with the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set – EUA when used consistent with their authorization and that collect acceptable human pustular or vesicular rash specimens.

## REAGENTS AND MATERIALS

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA test authorizes the use of the following additional extraction reagents:

**Table 2. Additional Reagents authorized for use with the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA.**

| Reagent Name | Vendor | Catalog# or ID# |
|---|---|---|
| KingFisher MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit | ThermoFisher Scientific | A48383 |

## CONTROLS

Assay controls are run concurrently with all test samples. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set - EUA test uses the same controls described for the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set that was granted De Novo and also received marketing clearances from FDA under section 510(k) of the Act (Product Code: PBK; DEN070001, K181205, K221658, K221834, K222558).

## PERFORMANCE EVALUATION

### 1. *Analytical and Clinical Evaluation*

The analytical and clinical performance was evaluated under the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set that was granted De Novo and also received marketing clearances from FDA under section 510(k) of the Act (Product Code: PBK; DEN070001, K181205, K221658, K221834, K222558).

### 2. *Bridging Studies for Addition of the QuantStudio 7 Flex Real-Time PCR System*

To support addition of the QuantStudio 7 Flex Real-Time PCR System (QS7 Flex) an LoD comparison and clinical evaluation were conducted comparing the QS7 Flex instrument to a cleared real-time PCR instrument using the Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set – EUA test.

The LoD comparison was performed using serial dilutions of *Orthopoxvirus* DNA (ATCC VR-3270SD quantitated genomic control material). The results supported that the QS7 Flex and the cleared real-time PCR instruments have an equivalent performance (Table 3).

**Table 3: Summary of LoD Data Comparing the QS7 Flex instrument and a cleared Real-time PCR instrument.**

| Copies per µL | Average CT<br># Cleared Instrument Positive Replicates | Average CT<br>#QS7 Flex Instrument Positive Replicates | Relationship to LOD |
|---|---|---|---|
| 9 | 33.31<br>20/20 | 33.71<br>20/20 | 3x LOD |
| 3 | 35.37<br>20/20 | 35.48<br>20/20 | LOD |
| 1 | 11/20[a] | 12/20[c] | 0.3x LOD |
| 0.33 | 1/20[b] | 1/20[d] | 0.09x LOD |

[a]The remaining nine replicates all produced equivocal results.
[b]The remaining 19 replicates produced 14 equivocal and five negative results.
[c]The remaining eight replicates all produced equivocal results.
[d]The remaining 19 replicates produced 13 equivocal and six negative results.

In addition to the LoD comparison study, a clinical comparison evaluation was conducted by comparing the performance of the QS7 Flex instrument and cleared real-time PCR instrument using the Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set – EUA test to test163 clinical specimens ("Dry Swab" clinical specimens previously extracted using a cleared method), see Table 4. Of the 163 clinical specimens tested, 147 had a positive or negative result using the cleared real-time PCR instrument, 15 were inconclusive and one was equivocal. Of the 69 positive clinical specimens by the cleared real-time PCR instrument, 69 were also positive by the QS7 Flex, giving a PPA of 100% with a 95% CI of 94.7-100%. Of the 78 negative clinical specimens by the cleared real-time PCR instrument, 67 were also negative by the QS7 Flex and

11 were inconclusive due to failure of the internal specimen adequacy control. Because the inconclusive results were driven by the failure of the internal specimen adequacy control they were not counted against the performance of the QS7 Flex, therefore giving a NPA of 100% with a 95% CI of 94.6-100%.

**Table 4: Summary of Clinical Data Comparing the QS7 Flex instrument and Cleared Real-time PCR instrument.**

| Clinical testing | | Cleared RT-PCR Instrument | | | |
|---|---|---|---|---|---|
| | | **Pos** | **Neg** | **Inc** | **Eqv** |
| **QS7 Flex** | **Pos** | **69** | 0 | 0 | 1 |
| | **Neg** | 0 | **67** | 2 | 0 |
| | **Inc** | 0 | 11* | **12** | 0 |
| | **Eqv** | 0 | 0 | **1** | 0 |

*Of the 11 QS7 Flex Real-Time PCR System inconclusive specimens, ten had a low internal specimen adequacy control signal suggesting that the collection of the clinical specimen may not have been optimal.

3. ***Bridging Studies for Addition of the KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head Used with the Applied Biosciences MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit***

To support addition of the KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head used with the Applied Biosciences MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit, a LoD confirmation and a "Contrived Clinical Sample Equivalency" study were conducted.

For the LoD confirmation study, samples were prepared using NIAID/NIH - NR-1 - Modified Vaccinia Ankara (MVA) cell lysate and supernatant from chicken embryo fibroblast (SL-29) cells infected with VACV, and then extracted with either a cleared method or the King Fisher Flex Magnetic Particle Processor with 96 Deep-Well head with the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit. Extracted samples were then run on a cleared real-time PCR instrument. Results are summarized in Table 5 and are supportive of equivocal performance between the two extraction methods.

**Table 5: Summary of LoD Data Comparing the King Fisher Flex Magnetic Particle Processor with 96 Deep-Well head and a Cleared Extraction Method.**

| **$TCID_{50}$/µL** | **Average CT Cleared Extraction Positive Replicates** | **Average CT KingFisher Flex Extraction System Positive Replicates** | **Relationship to LOD** |
|---|---|---|---|
| 0.15 | 30.95<br>5/5 | 33.80<br>5/5 | 3x LOD |
| 0.05 | 33.40<br>5/5 | 35.36<br>5/5 | LOD |
| 0.015 | 4/5[a] | 3/5[b] | 0.3x LOD |
| PBS | N/A<br>0/5 | N/A<br>0/5 | NEG |

[a]The remaining replicate produced an equivocal result.
[b]The remaining replicates produce one equivocal and one negative result.

In addition to the LoD confirmation evaluation, a "Contrived Clinical Sample Equivalency" study was conducted in which Vaccinia Virus (ATCC, NR-1) diluted in sterile PBS was used to hydrate 30 individual *Orthopoxvirus* negative lesion swabs to create contrived clinical samples comprising of 15 negative samples and 15 samples at 3x LoD (0.15 TCID50/µL). These samples were then extracted with a cleared extraction method and the KingFisher automated extraction, and PCR was performed using a cleared real-time PCR instrument. Results are summarized in Table 6 and demonstrate equivocal performance between the two extraction methods.

**Table 6: Summary of LoD Data Comparing the King Fisher Flex Magnetic Particle Processor with 96 Deep-Well head and a Cleared Extraction Method.**

| | | Cleared Extraction Method | | | |
|---|---|---|---|---|---|
| | | **Pos** | **Neg** | **Inc** | **Eqv** |
| **KingFisher with MagMAX** | **Pos** | **15** | 0 | 0 | 0 |
| | **Neg** | 0 | **15** | 0 | 0 |
| | **Inc** | 0 | 0 | **0** | 0 |
| | **Eqv** | 0 | 0 | **0** | 0 |

## LIMITATIONS

Please refer to the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set that was granted De Novo and also received marketing clearances from FDA under section 510(k) of the Act (Product Code: PBK; DEN070001, K181205, K221658, K221834, K222558).

## WARNINGS

- For in vitro diagnostic use
- Rx Only
- For use under Emergency Use Authorization (EUA) 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 the authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from non-variola orthopoxviruses, including monkeypox virus, 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.

**EUA REVISION HISTORY:**

| Date | Summary of Updates |
|---|---|
| March xx, 2024 | Original authorization |