

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K181205

B. Purpose for Submission:

Modification of device

C. Measurand:

Non-variola Orthopoxvirus DNA target sequence

D. Type of Test:

In vitro molecular diagnostic test

E. Applicant:

Centers for Disease Control and Prevention

F. Proprietary and Established Names:

Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3315: Nucleic acid based reagents for detection of non-variola orthopoxviruses

2. Classification:

Class II (Special Controls)

3. Product code:

PBK

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set is intended for the *in vitro* qualitative presumptive detection of non-variola *Orthopoxvirus* DNA extracted from human pustular or vesicular rash specimens and viral cell culture lysates submitted to a Laboratory Response Network (LRN) reference laboratory. The assay detects non-variola *Orthopoxvirus* DNA, including *Vaccinia*, *Cowpox*, *Monkeypox* and *Ectromelia* *viruses* at varying concentrations. This assay does not differentiate *Vaccinia virus* or *Monkeypox virus* from other *Orthopoxviruses* detected by this assay and does not detect *Variola virus*. Refer to the CDC algorithm, Acute, Generalized Vesicular or Pustular Rash Illness Testing 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 presumptive identification of non-variola *Orthopoxvirus* DNA. These results must be used in conjunction with other diagnostic assays and clinical observations to diagnose *Orthopoxvirus* infection. The assay should only be used to test specimens with low/moderate risk of smallpox. If a high risk of smallpox exists, viral culture should **not** be attempted. Negative results obtained with this device do not preclude *Variola virus* infection and should not be used as the sole basis for treatment or other patient management decisions.

Use is limited to Laboratory Response Network (LRN) designated laboratories.

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

Distribution of device is limited to designated laboratories in the Laboratory Response Network.

4. Special instrument requirements:

Real-time PCR instrumentation and software.

I. Device Description:

Variola virus, a member of the *Orthopoxvirus* genus, is the causative agent of smallpox and was certified eradicated in 1980 by the World Health Organization. At that time, smallpox vaccinations were ceased worldwide as a result. However, in recent years, concerns over the potential use of *Variola virus* as a biological weapon led the United States to resume smallpox vaccinations on a limited basis. Since the smallpox vaccine contains live

Vaccinia virus, it is possible for vaccine recipients and/or their close contacts to develop adverse reactions to the vaccine including the emergence of pustules on the skin.

The Laboratory Response Network (LRN) is part of a national bioterrorism preparedness initiative created to ensure an effective laboratory response to biological threats by helping to improve the nation's public health laboratory infrastructure. Member laboratories must meet specific membership requirements and pass rigorous proficiency tests demonstrating their ability to accurately identify agents of concern. One of the major goals is the development and validation of rapid and specific assays for detection of biothreat agents and emerging infectious diseases. Accordingly, scientists at the Centers for Disease Control and Prevention have developed several real-time PCR based assays to detect non-variola *Orthopoxvirus* and other potential biothreat agents in an effort to meet the need for rapid detection.

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set was developed for use in conjunction with clinical observations and other tests as described in the CDC algorithm, Acute, Generalized Vesicular or Pustular Rash Illness Testing Protocol in the United States. The assay is designed to aid in the identification of the causative agent of a pustular or vesicular rash illness and to help rule out the presence of *Variola virus* in patients presenting with pustular rash illness.

This assay detects most commonly known human pathogenic *Orthopoxviruses* (e.g. *Vaccinia*, *Cowpox*, and *Monkeypox viruses*) but does not detect *Variola virus*, the causative agent of smallpox. *Vaccinia virus* infection in the United States usually occurs in conjunction with smallpox vaccination or contact with a smallpox vaccine recipient. *Monkeypox* and *Cowpox viruses* are endemic to locations outside the United States, with the exception of the 2003 monkeypox outbreak associated with prairie dogs, which became infected due to imported African rodents.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set

2. Predicate 510(k) number(s):

DEN070001/K053469

3. Comparison with predicate:

Item	Device (K181205)	Predicate (DEN070001/K053469)
Intended Use	The Non-variola <i>Orthopoxvirus</i> Real-time PCR Primer and Probe Set is intended for the <i>in vitro</i> qualitative presumptive detection of non-variola	The Non-variola Orthopoxvirus Real-time PCR Primer and Probe Set is intended for the <i>in vitro</i> qualitative presumptive detection of non-variola

Item	Device (K181205)	Predicate (DEN070001/K053469)
	<p><i>Orthopoxvirus</i> DNA extracted from human pustular or vesicular rash specimens and viral cell culture lysates submitted to a Laboratory Response Network (LRN) reference laboratory. The assay detects non-variola <i>Orthopoxvirus</i> DNA, including <i>Vaccinia</i>, <i>Cowpox</i>, <i>Monkeypox</i> and <i>Ectromelia</i> viruses at varying concentrations. This assay does not differentiate <i>Vaccinia virus</i> or <i>Monkeypox virus</i> from other <i>Orthopoxviruses</i> detected by this assay and does not detect <i>Variola virus</i>. Refer to the CDC algorithm, <u>Acute, Generalized Vesicular or Pustular Rash Illness Testing Protocol</u> in the United States for recommended testing and evaluation algorithms for patients presenting with acute, generalized pustular or vesicular rash illness.</p> <p>Results of this assay are for the presumptive identification of non-variola <i>Orthopoxvirus</i> DNA. These results must be used in conjunction with other diagnostic assays and clinical observations to diagnose <i>Orthopoxvirus</i> infection. The assay should only be used to test specimens with low/moderate risk of smallpox. If a high risk of smallpox exists, viral culture should not be attempted. Negative results obtained with this device do not preclude <i>Variola virus</i> infection and should not be used as the</p>	<p><i>Orthopoxvirus</i> DNA extracted from human pustular or vesicular rash specimens and viral cell culture lysates submitted to a Laboratory Response Network (LRN) reference laboratory. The assay detects non-variola <i>Orthopoxvirus</i> DNA, including <i>vaccinia</i>, <i>cowpox</i>, <i>monkeypox</i> and <i>ectromelia</i> viruses at varying concentrations. This assay does not differentiate <i>vaccinia virus</i> or <i>monkeypox virus</i> from other <i>Orthopoxviruses</i> detected by this assay and does not detect <i>variola virus</i>. Refer to the CDC algorithm, <u>Acute, Generalized Vesicular or Pustular Rash Illness Testing Protocol in the United States</u> for recommended testing and evaluation algorithms for patients presenting with acute, generalized pustular or vesicular rash illness.</p> <p>This assay should be used in conjunction with other diagnostic assays and clinical observations for the following indications:</p> <p>(1) to serve as an aid in determining whether <i>vaccinia virus</i> is the causative agent of a vaccination adverse event in recipients of the smallpox vaccine (which uses live <i>vaccinia virus</i>)</p> <p>(2) to serve as an aid in determining <i>vaccinia virus</i> infection in smallpox vaccinee contacts presenting with unclear</p>

Item	Device (K181205)	Predicate (DEN070001/K053469)
	<p>sole basis for treatment or other patient management decisions.</p> <div> Use of this assay is limited to Laboratory Response Network (LRN) designated laboratories. </div>	<p>etiology and pustules resembling <i>vaccinia virus</i> infection</p> <p>(3) to determine infection with <i>vaccinia</i> or other non-variola <i>Orthopoxviruses</i> in individuals presenting with pustular or vesicular rash illness</p> <p>(4) to aid in the differential diagnosis of smallpox</p> <p>(5) to aid in the identification of viral cell cultures from patients with low/moderate risk of smallpox.</p> <p>NOTE: If a high risk of smallpox exists, viral culture should not be attempted.</p> <div> Use of this assay is limited to Laboratory Response Network (LRN) designated laboratories. </div>
Principle of Operation	Unchanged	Nucleic acid amplification and fluorescent probe detection
Sample Types	Unchanged	<ul style="list-style-type: none"> • Vesicle fluid, skin, crust, “roof” • Dry or wet swab of lesion (dry swab is preferred) • Touch prep (slide) of lesion • Fresh biopsy of pustule or vesicle (no formalin) • Viral cell culture lysates
Instrumentation and Software	Unchanged	Real-time PCR instrumentation and software

K. Standard/Guidance Document Referenced (if applicable):

Not applicable.

L. Test Principle:

With the exception of some reagents, the Non-variola *Orthopoxvirus* Real-time PCR Primer

and Probe Set device description remains unchanged from the original submission (K053469).

The Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set Assay uses a fluorogenic probe, consisting of an oligonucleotide with a reporter dye (FAM) attached to the 5' end and a quencher dye (BHQ1) attached at or near the 3' end. The probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of the *Taq* polymerase degrades the probe causing the reporter dye to separate from the quencher dye, thereby generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes and the fluorescence intensity is monitored during the PCR in real-time. The *Taq* polymerase used in this assay is inactive at room temperature and is activated by incubation at 95°C, thus minimizing the production of nonspecific amplification products.

Each extracted DNA sample is tested using the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe set along with an internal control primer and probe set(s) to demonstrate adequate DNA extraction and isolation, proper function of common reagents and equipment, and the absence of inhibitory substances.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

N/A

b. Linearity/assay reportable range:

N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

N/A

d. Detection limit:

The limit of detection for the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set was determined through an analytical sensitivity study.

e. Analytical specificity:

Inquiries regarding performance characteristics for the Non-variola *Orthopoxvirus* Real-time PCR Primer and Probe Set should be directed to the Centers for Disease Control and Prevention.

f. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

N/A

b. Clinical specificity:

N/A

c. Other clinical supportive data (when a. and b. are not applicable):

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

N/A

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.