# InfectionDetect™

## Mpox qPCR Assay

Assay for Detection of Monkeypox

# INSTRUCTIONS FOR USE

REFERENCE NUMBERS

**Mpox qPCR Assay**
Dispense-Ready
6803 (200 reactions)
Ready-to-Use
6802 (96 reactions)

**Mpox qPCR Assay with Extraction Control**
Dispense-Ready
6805 (200 reactions)
Ready-to-Use
6806 (96 reactions)

**Mpox qPCR Assay with Extraction Control**
**For LightCycler® 480**
Dispense-Ready
6807 (200 reactions)
Ready-to-Use
6808 (96 reactions)

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# 1 Intended purpose

InfectionDetect™ Mpox qPCR Assay is a semi-quantitative real-time Polymerase Chain Reaction (PCR) assay intended for the detection of nucleic acids from the Monkeypox virus, for research purposes. The assay is used with real-time PCR systems. Monkeypox DNA can be found in the upper respiratory tracts, rectal tracs and skin lesions of infected individuals. Samples can be obtained either by oropharyngeal swabs, rectal swabs and/or skin lesion swabs. Samples can be purified on automated platforms or in manual workflows. The assay is provided in a multiplex format analysing two monkeypox viral targets and one human target sampling control in the same PCR reaction tube. The assay is not intended for *in vitro* diagnostic purposes.

## 1.1 Intended user

InfectionDetect™ Mpox qPCR Assay is intended for use by healthcare professionals, or qualified laboratory personnel, instructed and trained in the techniques of real-time PCR as well as proficient in handling biological samples, for research purposes.

# 2 Test principle

## 2.1 Explanation of the assay

The InfectionDetect™ Mpox qPCR Assay combines real-time qPCR with PentaBase's novel and selective technologies comprising both standard synthetic oligonucleotides as well as proprietary modified synthetic oligonucleotides, such as HydrolEasy® probes and SuPrimers™, for specific and sensitive amplification. The technology applies to several common real-time PCR instruments, as well as PentaBase's own portfolio of instruments using standard procedures. Pentabase-modified oligonucleotides contain at least one synthetic DNA analogue comprising a flat heteroaromatic, hydrophobic molecule and a linker. These modifications are inserted into the oligonucleotides at fixed positions during synthesis. Using the InfectionDetect™ Mpox qPCR Assay, the presence of virus DNA in a sample can be detected quickly, sensitively, and selectively by qPCR analysis.

### 2.1.1 HydrolEasy® probe

A HydrolEasy® probe is similar to a standard hydrolysis probe (also referred to as a TaqMan® probe[1]) labelled with a fluorophore at the 5' end and a quencher at the 3' end, but with the addition of pentabases. HydrolEasy® probes are based on oligonucleotides modified with pentabases, giving the probe a significantly improved signal-to-noise ratio, higher specificity, and higher sensitivity compared to conventional hydrolysis probes. HydrolEasy® probes in the InfectionDetect™ Mpox qPCR Assay are labelled with either FAM, HEX, or Cy5.

### 2.1.2 SuPrimers™

SuPrimers™ are standard DNA primers modified with one or more pentabases. Pentabases in primers may provide increased specificity, sensitivity, and reduce primer-dimer formation.

## 2.2 Product variants

InfectionDetect™ Mpox qPCR Assay is supplied as either Dispense Ready (DR) (ref. no 6803) or Ready-to-Use (RTU) (ref. no 6802). The DR version includes Primer-Probe Mix and Master Mix in separate tubes, which need to be dispensed into suitable plasticware before the addition of template. The RTU version is pre-dispensed and only need the addition of DNA before qPCR.
InfectionDetect™ Mpox qPCR Assay can be supplied with an extraction control to ensure each PCR wells performance, supplied as either Dispense Ready (DR) (ref. no 6805) or Ready-to-Use (RTU) (ref. no 6806) and for the LightCycler480 edition it is supplied as either Dispense Ready (DR) (ref. no 6807) or Ready-to-Use (RTU) (ref. no 6808).

## 2.3 Principle of the procedure

The InfectionDetect™ Mpox qPCR Assay is designed for use with real-time PCR instruments for nucleic acid amplification and detection of the target sequence in biological samples.

The InfectionDetect™ Mpox qPCR Assay targets two viral sequences of the monkeypox virus, amplifying the OPG002 Crm-B secreted TNF-alpha-receptor-like protein J2R, and the OPG191 Ankyrin-like protein B7R (**Table 1**). Selective amplification of J2R and B7R sequences are achieved by using sequence-specific forward and reverse primers together with sequence specific HydrolEasy® probes labelled with FAM or HEX, respectively. Selective amplification of a region within the human Ribonuclease P gene (*RNase P*) is used as sampling control and is achieved by combining non-competitive sequence-specific forward and reverse primers with a sequence specific Cy5-labelled HydrolEasy® probe, which has no homology with the monkeypox genome. The amplified target is detected by measuring the increased fluorescence generated by release of the fluorophore from the fluorescently labelled oligonucleotide probe, specifically targeting either the monkeypox sequences or the human sequence of interest. A thermostable DNA polymerase enzyme is used for amplification.

InfectionDetect™ Mpox qPCR assay with Extraction Control amplifies a region of the Solanum tuberosum. Selective amplification of the Solanum tuberosum phyB gene sequence is achieved by using sequence-specific forward and reverse primers together with sequence specific HydrolEasy® probes labelled with CAL fluor® red 610.

[1]Taqman is a registered tradename of Roche Molecular Systems, Inc



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**Table 1.** Amplified genomic regions by the InfectionDetect™ Mpox qPCR Assay.

| Targeted Regions | Gene | Fluorophore |
|---|---|---|
| B7R | OPG191 Ankyrin-like protein | FAM |
| J2R | OPG002 Crm-B secreted TNF-alpha-receptor-like protein | HEX |
| RNase P | Human RNase P | Cy5 |
| PhyB Gene | Solanum tuberosum | CalFluorRed610* |

*CalFluorRed610 is replaced with ATTO425 in the LightCycler® 480 product variants (ref. no. 6807-6808).

# 3 Reagents and materials

The materials provided with the InfectionDetect™ Mpox qPCR Assay can be found in **Table 2**. Materials and instruments required, but not provided can be found in **Table 3**.

## 3.1 Storage and stability

Refer to the label for expiry date. This assay should be stored at -20°C. Repeated thawing and freezing should be kept to a minimum.

### 3.1.1 In-use stability

When in use, the assay components should be returned to the freezer promptly after use to minimise the time at room temperature and exposure to light.
Used Ready-to-Use PCR tubes and dispensed Primer-Probe and Master Mix should be disposed following your local guidelines on disposal of biological waste. The reagents included are not for reuse.

## 3.2 Materials provided

**Table 2.** List of materials provided with the InfectionDetect™ Mpox qPCR Assay as either Dispense Ready (DR) or Ready-to-Use (RTU).

| **InfectionDetect™ Mpox qPCR Assay** | |
|---|---|
| **Dispense-Ready (DR)** | |
| **Kit components** | **Content** |
| Mpox qPCR Assay Primer-Probe Mix | Synthetic DNA. |
| AmpliQueen™ qPCR Master Mix | Enzymes and buffer for reverse transcription and qPCR. |
| Mpox qPCR Positive Control | Buffer solution including inactivated monkeypox DNA and human DNA. |
| Mpox qPCR Negative Control | Buffer solution free of monkeypox DNA. |
| **Ready-to-Use (RTU)** | |
| **Kit components** | **Content** |
| Mpox qPCR Assay | Synthetic DNA.<br>Enzymes and buffer for reverse transcription and qPCR. |
| Mpox qPCR Positive Control | Buffer solution including inactivated monkeypox DNA and human DNA. |
| Mpox qPCR Negative Control | Buffer solution free of monkeypox DNA. |
| **InfectionDetect™ Mpox qPCR Assay with Extraction Control** | |
| **Dispense-Ready (DR)** | |
| **Kit components** | **Content** |
| Mpox qPCR Assay Primer-Probe Mix | Synthetic DNA. |
| AmpliQueen™ qPCR Master Mix | Enzymes and buffer for reverse transcription and qPCR. |
| Mpox qPCR Positive Control | Buffer solution including inactivated monkeypox DNA and human DNA. |
| Mpox qPCR Negative Control | Buffer solution free of monkeypox DNA. |
| Mpox qPCR Extraction Control* | Buffer solution including Solanum tuberosum DNA. |
| **Ready-to-Use (RTU)** | |
| **Kit components** | **Content** |
| Mpox qPCR Assay | Synthetic DNA.<br>Enzymes and buffer for reverse transcription and qPCR. |
| Mpox qPCR Positive Control | Buffer solution including inactivated monkeypox DNA and human DNA. |
| Mpox qPCR Negative Control | Buffer solution free of monkeypox DNA. |
| Mpox qPCR Extraction Control* | Buffer solution including Solanum tuberosum DNA. |

*Only included in the product variants with ref. no. 6805-6808.



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## 3.3 Materials and instruments required but not provided

Materials and instruments required but not provided are listed in **Table 3**. InfectionDetect™ Mpox qPCR Assay is designed to run on open platforms and has been validated using samples purified with the BasePurifier™ 32 Nucleic Acid Extraction System (BasePurifier™, PentaBase ref, no. 715) and analysed with the BaseTyper™ 48.4, BaseTyper™ 96.4 and BaseTyper™ 96.6 (PentaBase, ref. no. 754, 758, 759), CFX384 (Bio-Rad, ref. no. 1855484), LightCycler® 480 II (Roche, ref. no. 05 015 278 001), or QuantStudio™ 5 (Applied Biosystems™ ref. no. A28574) Real-Time PCR instruments. There is currently no evidence available to PentaBase suggesting that there are certain relevant commercially available nucleotide purification methods and instruments or three-channel real-time qPCR instruments that are not compatible with the InfectionDetect™ Mpox qPCR Assay. However, when running InfectionDetect™ Mpox qPCR Assay on instruments not validated by PentaBase, it is highly recommended that a specific validation is performed using clinical samples and reference controls to verify cycle thresholds and cut-offs. Please contact PentaBase A/S or your local distributor for support.

**Table 3.** Materials and instruments required but not provided.

| **Materials** |
|---|
| Plasticware compatible with the used real-time PCR instrument[2] |
| Pipettes (1-10 µL, 10-100 µL) |
| Pipette Tips |
| Centrifuge for spinning PCR tubes, strips or plates |
| **Collection Kits (one of the following)** |
| Rectal swab |
| Oropharyngeal swab |
| Skin lesion swab |
| **DNA extraction method or instrument** |
| DNA Extraction kit (e.g., Viral DNA and RNA Extraction Kit, PentaBase A/S) |
| Extraction instrument (e.g., BasePurifier™, PentaBase A/S) |
| **Real-time qPCR** |
| Real-time PCR instrument including: BaseTyper™ (PentaBase A/S), QuantStudio 5 (Applied Biosystems), LightCycler® 480 (Roche) or CFX Opus 96 (Bio-Rad) |

# 4 Warnings and precautions

- For research use only. Not for *in vitro* diagnostics.
- Treat all biological specimens as if capable of transmitting infectious agents. All biological specimens should be treated with universal precautions, as it is often impossible to know which specimens might be infectious.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Good laboratory practices and careful adherence to the procedures specified in these Instructions for Use are necessary. Wear laboratory coats, laboratory gloves and eye protection when handling biological samples and reagents. Laboratory gloves must be changed between handling different biological samples to avoid contamination of reagents.
- Remove gloves and wash hands thoroughly after handling samples and reagents.
- Do not use reagents that have expired.
- The components included in this assay should only be used for the purposes stated in these instructions for use.
- Do not use damaged InfectionDetect™ Mpox qPCR Assay tubes.
- Do not use a InfectionDetect™ Mpox qPCR Assay pre-dispensed in a Ready-to-Use PCR tube that has been dropped while open.
- Do not open the tubes or unseal wells during or after amplification following completing the PCR program.
- Be aware of the placement and orientation of the PCR tubes in the PCR machine in relation to how the samples are named in the PCR software.
- Baseline drift, a slowly rising signal in the amplification plot with no or late exponential phase, may lead to false positive results if not corrected. Refer to Section 7.1 for more information.
- Consult relevant nucleic acid extraction and real-time qPCR Instrument User Guides for additional warnings, precautions, and procedures to reduce the risk of contamination.
- Dispose of used InfectionDetect™ Mpox qPCR tubes, pipette tips and specimen tubes according to local, state and federal regulations for biological material.
- Due to the high sensitivity of the assays, contamination of the work area with previous positive samples might cause false-positive results. Therefore, use extreme caution not to contaminate reagents and handle samples according to good laboratory practice.
- Minimise the exposure of InfectionDetect™ Mpox qPCR Assay to light due to the presence of light sensitive HydrolEasy® probes.
- The reagents should not be diluted to a lower concentration than stated in this Instructions for Use. This may affect the performance of the assay.
- Do not substitute the reagents with others, as it may affect the performance of the assay.

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[2] Only when using Dispense Ready version

- Specimen collection must be performed using appropriate swab types. Inadequate or inappropriate sample collection, storage, and transport may yield incorrect or invalid results. **NOTE:** Cotton or calcium alginate swabs, or swabs with wood shafts have not been thoroughly tested with InfectionDetect™ Mpox qPCR Assay.
- Oropharyngeal specimen collection should be performed at least 30 minutes after tooth brushing, eating, or drinking, as it may influence the performance of the assay.
- Ensure there is no sign of leakage from the collection tube before running the analysis.

# 5 Sample handling

Handle all biological samples and controls as if they are capable of transmitting infectious agents. Please follow your local guidelines for handling and disposal of sample material.

## 5.1 Sample collection

The specimens should either be rectal, oropharyngeal, or from skin lesions. Ineffective or inappropriate sample collection can result in false test results. Training in specimen collection is therefore recommended to ensure the best quality.

## 5.2 Sample transport and storage

Transportation of collected specimens must comply with all applicable regulations for the transport of biological agents. Specimens should be stored in suitable buffers, such as viral transport media. Please follow the specific instructions for use of the transport vial.

## 5.3 Sample purification

Specimens should be subjected to DNA purification prior to analysis by InfectionDetect™ Mpox qPCR Assay, using suitable DNA purification methods such as the BasePurifier™ 32 Nucleic Acid Extraction System (PentaBase A/S, ref. no. 715) and the Viral DNA and RNA Extraction Kit (PentaBase A/S, ref. no. 727) according to the manufacturer’s instructions. Be aware that the outcome from the purification method may influence the results of the InfectionDetect™ Mpox qPCR Assay.
InfectionDetect™ Mpox qPCR product variants ref. no. 6805-6808 includes an extraction control sample. There is enough extraction control to include into each extraction well.

**NOTE:** The extraction control must be added directly to a nucleotide extraction procedure. Use 10 µL extraction control directly in each extraction well.

## 5.4 Positive and Negative Control

At least one Positive and one Negative Control should be included in each qPCR run. There are enough Positive and Negative Control samples included in the kit to an average of four samples per qPCR run. If less than four samples are analysed on average per qPCR run, additional controls can be ordered from PentaBase or your local distributor.

The Positive control contains 100 B7R and J2R copies and 1 ng human genomic DNA per microliter.

**NOTE:** The Controls have to be added directly to the InfectionDetect™ Mpox qPCR Assay and not to a nucleotide extraction procedure. Use 5 µL of Positive and Negative Control directly in separate qPCR tubes.

# 6 Procedure

## 6.1 Dispense Ready

1. Add 10 µL AmpliQueen™ qPCR Master Mix to each PCR tube (vial, strip or plate).
2. Add 5 µL Primer-Probe Multiplex Mix to the PCR tubes.
3. Add 5 µL of template (sample, positive, or negative control) to the needed PCR tubes.
4. Seal all tubes.
   ‘Optional step: Briefly vortex PCR tubes (2-3 sec.) to enhance elimination of air bubbles’
5. Spin down the PCR tubes (1-2 minutes at 4000-5000 rpm) to ensure that all reagents are collected at the bottom of the tubes and to eliminate air bubbles.
6. Place the PCR tubes in the real-time PCR instrument and run the RT-qPCR program (**Table 4-9**).

## 6.2 Ready-to-Use

1. Spin down the PCR strips (1-2 minutes at 4000-5000 rpm) to ensure that all reagents are collected at the bottom of the strips to eliminate air bubbles.
2. Open PCR tubes and add 5 µL of template (sample, positive or negative control). Continue with different templates and tubes until all templates are added to individual tubes.
3. Seal all tubes.
   ‘Optional step: Briefly vortex PCR strips (2-3 sec.) to enhance elimination of air bubbles’
4. Spin down the PCR strips (1-2 minutes at 4000-5000 rpm) to ensure that all reagents are collected at the bottom of the strips and to eliminate air bubbles.
5. Place the PCR tubes in the real-time PCR instrument and run the RT-qPCR program (**Table 4-9**).

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## 6.3 Real-time qPCR program

The assay can be run with different PCR programs depending on the PCR machine. Please refer to the relevant table (**Table 4**-**Table 8**).

**Table 4.** Real-time qPCR program for running InfectionDetect™ Mpox qPCR Assay on BaseTyper 48™.

| Protocol | Temperature [°C] | Time [sec] | Cycles | Ramping [°C/sec] | Channel |
|---|---|---|---|---|---|
| **Stage 1** | | | | | |
| Hold | 95 | 120 | 1 | 8.0 | |
| **Stage 2 (Cycle 1-45)** | | | | | |
| 2-step amplification | 94 | 5 | 45 | 8.0 | FAM (green)<br>HEX (yellow)<br>Cy5 (red)<br>*Acquisition at 60°C |
| | 60* | 30 | | 8.0 | |

**Table 5.** Real-time qPCR program for running InfectionDetect™ Mpox qPCR Assay on BaseTyper 96™.

| Protocol | Temperature [°C] | Time [sec] | Cycles | Ramping [°C/sec] | Channel |
|---|---|---|---|---|---|
| **Stage 1** | | | | | |
| Hold | 95 | 120 | 1 | 6.1 | |
| **Stage 2 (Cycle 1-45)** | | | | | |
| 2-step amplification | 94 | 5 | 45 | 6.1 | FAM (green)<br>HEX (yellow)<br>Cy5 (red)<br>*Acquisition at 60°C |
| | 60* | 30 | | 6.1 | |

**Table 6.** Real-time qPCR program for running InfectionDetect™ Mpox qPCR Assay on QuantStudio 5.

| Protocol | Temperature [°C] | Time [sec] | Cycles | Ramping [°C/sec] | Channel |
|---|---|---|---|---|---|
| **Stage 1** | | | | | |
| Hold | 95 | 120 | 1 | 1.6 | |
| **Stage 2 (Cycle 1-45)** | | | | | |
| 2-step amplification | 94 | 5 | 45 | 1.6 | FAM (green)<br>VIC (yellow)<br>Cy5 (red)<br>*Acquisition at 60°C |
| | 60* | 30 | | 1.6 | |

**Table 7.** Real-time qPCR program for running InfectionDetect™ Mpox qPCR Assay on CFX Opus 96.

| Protocol | Temperature [°C] | Time [sec] | Cycles | Ramping [°C/sec] | Channel |
|---|---|---|---|---|---|
| **Stage 1** | | | | | |
| Hold | 95 | 120 | 1 | - | |
| **Stage 2 (Cycle 1-45)** | | | | | |
| 2-step amplification | 94 | 5 | 45 | - | FAM (green)<br>HEX (yellow)<br>Cy5 (red)<br>*Acquisition at 60°C |
| | 60* | 30 | | - | |

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**Table 8.** Real-time qPCR program for running InfectionDetect™ Mpox qPCR Assay on LightCycler 480.

| Protocol | Temperature [°C] | Time [sec] | Cycles | Ramping [°C/sec] | Channel |
|---|---|---|---|---|---|
| **Stage 1** | | | | | |
| Hold | 95 | 120 | 1 | 4.4 | |
| **Stage 2 (Cycle 1-45)** | | | | | |
| 3-step amplification | 94 | 5 | 45 | 2.2 | |
| | 63 | 1 | | 2.2 | |
| | 58* | 25 | | 2.0 | 3 color hydrolysis probe:<br>FAM/FAM-dT (green)<br>HEX/sima-dT (yellow)<br>Cy5 (red)<br>*Acquisition at 58°C<br><br>4 color hydrolysis probe:<br>LC Cyan 500 (Cyan)<br>FAM/FAM-dT (green)<br>LC Red610 (orange)<br>Cy5 (Red)<br>*Acquisition at 58°C |

# 7 Data Analysis

For the InfectionDetect™ Mpox qPCR Assay, determining the cycle threshold (Ct) is a central part of the data analysis procedure. The Ct is defined as the cycle in which the fluorescence signal of a given assay exceeds the threshold value, which is set as part of the analysis procedure. The Ct values of the PCR program (**Table 4-Table 8**, stage 2) are compared to predefined cut-off values to determine if the individual samples are positive or negative for monkeypox (Section 7.2).

## 7.1 Baseline and threshold settings

Results from InfectionDetect™ Mpox qPCR Assay can be analysed using both automatic and manual baseline and threshold settings. If automatic baseline and threshold settings are used, it is recommended to also perform a visual inspection of the amplification curves since some cases might need manual adjustment of baseline and/or threshold due to baseline drift and/or incorrect baselining. When setting the baseline manually, it is recommended to use 5 cycle intervals such as from cycle 10 to cycle 15 depending on the amplification curve of the sample. When setting the threshold manually, the threshold should be set to cross at the beginning of the exponential PCR phase and above any background or baseline fluorescence. If there is significant background or baseline fluorescence, adjust the baseline interval.

## 7.2 Channel Acquisition

An overview of the channel settings for the different machines and product variants. The PhyB is only included in ref. no. 6805-6808 and is excluded in the analysis of ref. no 6802-6803 for the BaseTyper 48™, BaseTyper 96™, CFX Opus 96 and QuantStudio 5.

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**Table 9.** Channel settings for the different machines and product variants.

| Machine | Target | Channel acquisition | Assay ref. no. |
|---|---|---|---|
| **BaseTyper 48™, BaseTyper 96™, CFX Opus 96** | B7R | FAM | 6802-6803<br>6805-6806* |
| | J2R | HEX | |
| | RNase P | Cy5 | |
| | PhyB gene* | Texas Red | |
| **QuantStudio 5** | B7R | FAM | 6802-6803<br>6805-6806* |
| | J2R | VIC | |
| | RNase P | Cy5 | |
| | PhyB gene* | ROX | |
| **LightCycler480 4 color hydrolysis probe** | B7R | FAM | 6807-6808 |
| | J2R | LightCycler Red 610** | |
| | RNase P | Cy5 | |
| | PhyB gene | LightCycler Cyan 500 | |
| **LightCycler480 3 color hydrolysis probe** | B7R | FAM | 6802-6803 |
| | J2R | HEX | |
| | RNase P | Cy5 | |
| | J2R | Channel 3 | |
| | RNase P | Channel 4 | |

*phyB gene is only included in product variants ref. no. 6805-6808 and not in ref. no. 6802-6803.
**J2R crosstalks into the FAM channel.

## 7.3 Interpretation of results

An overview of the possible outcomes of the analysis is shown in **Table 11**. The results are only valid if the included Positive Control Ct values are below 33 for B7R and J2R, and below 32 for the RNase P sample control. No template control (NTC) should produce no Ct values for the B7R and J2R monkeypox-specific targets. NTC Ct values above cycle 39 for the RNase P sample control are acceptable.
For product variants ref. no. 6805-6808, the included extraction control, PhyB, should have a Ct value between 27 and 30 for the results to be valid.

**Table 10.** Analysis outcomes and Ct threshold based on target amplification curves for InfectionDetect™ Mpox qPCR Assay. Conclusions are based on target Ct values compared to the cut-off values.

| Sample status | Target | Outcome | Ct | Conclusion | Comments |
|---|---|---|---|---|---|
| **Case 1** | B7R | + | <42 | Monkeypox detected | When both B7R and J2R Ct values are below 42, and the Ct value of RNase P is below 35, monkeypox has been detected in the sample. |
| | J2R | + | <42 | | |
| | RNase P | + | <35 | | |
| | PhyB gene* | +/- | 27-30 | | |
| **Case 2** | B7R | - | ≥42 | | Monkeypox has been detected in the sample, if J2R is positive, in two separate runs. |
| | J2R | + | <42 | | |
| | RNase P | +/- | <35 | | |
| | PhyB gene* | +/- | 27-30 | | |
| **Case 3** | B7R | + | <42 | | The RNase P signal may be suppressed when B7R and J2R are positive, especially in samples with high amounts of viral RNA. |
| | J2R | + | <42 | | |
| | RNase P | - | ≥35 | | |
| | PhyB gene* | +/- | 27-30 | | |
| **Case 4** | B7R | + | <42 | Possibly Vaccinia Virus detected | The B7R gene has a small cross-reactivity with Vaccinia virus. Due to this cross-reactivity, the B7R signal alone cannot result in detection of Monkeypox. |
| | J2R | - | ≥42 | | |
| | RNase P | +/- | <35 | | |
| | PhyB gene* | +/- | 27-30 | | |
| **Case 5** | B7R | - | ≥42 | Monkeypox not detected | A RNase P signal is required for a sample to be considered valid. |
| | J2R | - | ≥42 | | |
| | RNase P | + | <35 | | |
| | PhyB gene* | + | 27-30 | | |
| **Case 6** | B7R | - | ≥42 | Invalid | The sample does not contain enough material for the analysis. Take a new specimen if possible. |
| | J2R | - | ≥42 | | |
| | RNase P | - | ≥35 | | |
| | PhyB gene* | - | <27-30< | | |

*phyB gene is only included in product variants ref. no. 6805-6808. Exclude it from the data analysis if using product variants with ref. no. 6802-6804.

### 7.3.1 Monkeypox detected

Monkeypox is detected if a sample has Ct values for both viral B7R and J2R assays below 42 (**Table 11**, case 1). Please notice that the RNase P signal may be suppressed in some samples, and particularly when a sample contains large

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amounts of viral DNA. These samples are considered valid if the Ct values of both B7R and J2R are below 42, even when RNase P is negative (**Table 11**, case 3).

If a sample only has signal in one of the two viral genes, the sample should be rerun before drawing a conclusion (**Table 11**, case 2). If the sample again has signal for J2R, monkeypox is detected in the sample. If the sample is positive for only B7R in two consecutive runs, it can be a sign of Vaccinia virus, and cannot be concluded as monkeypox detected (**Table 11**, case 4). If the second run does not show signal for any of the viral genes, but positive for the RNP sample control, monkeypox has not been detected in the sample.

**NOTE**: A lack of signal in either B7R or J2R may be due to mutations present in the target regions of the assay.

### 7.3.2 Monkeypox not detected

Monkeypox has not been detected if the sample is positive for RNase P but does not show signal for B7R and J2R (**Table 11**, case 5).

### 7.3.3 Invalid samples

#### 7.3.3.1 No sample signals

In the case of no or late amplification of RNase P (Ct ≥ 35), the test is invalid unless either B7R or J2R are present (Ct < 42) (**Table 11**, case 6). The concentration or the quality of the DNA in the sample is too low. Add more sample, if possible, or collect a new specimen. Repeat the extraction and run the test again. If there are no target signals after repeating the test, no conclusions can be drawn.

#### 7.3.3.2 No positive control signals

If no signal occurs from the positive control sample(s), make sure that the qPCR program has been defined correctly and that the instrument is acquiring on the correct channels (**See Table 10**) in Step 2 or 3 (depending on the program) of Stage 2 (**Table 4-9**). Results from a run where the control signals are not present should not be used. The control reagents might be degraded, or the InfectionDetect™ Mpox qPCR Assay is not functional. If you cannot locate the root cause of the problem, please contact PentaBase A/S or your local distributor for support.

#### 7.3.3.3 Signal in NTC

NTC Ct values above cycle 39 for the RNase P sample control are acceptable. If NTC signals are at or below cycle 39, make sure that the threshold has been set correctly above any background fluorescence. If this is the case, the reagents may be contaminated. Find the cause of contamination by checking or replacing all potential sources of the contamination, such as pipettes and instruments. If the contamination cannot be located, contact PentaBase A/S or your local distributor.

#### 7.3.3.4 No extraction control signal

If no signal occurs from the extraction control, make sure that the qPCR program has been defined correctly and that the instrument is acquiring in Step 2 or 3 (depending on the program) of Stage 2 (**Table 4-9**). Results from a sample where the control signal is not present should not be used. If the control signal is absent in multiple samples reagents might be degraded or the InfectionDetect™ Mpox qPCR Assay is not functional. If you cannot locate the root cause of the problem, please contact PentaBase A/S or your local distributor for support.

**NOTE:** This section only applies to product variants with ref. no. 6805-6808.

# 8 Limitations

- Performance of the InfectionDetect™ Mpox qPCR Assay has only been tested on specimens from oropharyngeal swabs, rectal swabs, and skin lesion swabs.
- Please note that an estimate of 1 out of 40,000 samples carry a mutation in the RNase P gene that make them test negative for the internal control. For such cases, use a different assay.
- Incorrect collection, transportation or handling of the sample could cause false-negative test results. Also, a very low amount of virus DNA in the specimen or amplification inhibitors could give false-negative test results.
- If mutations occur in the targeted region of the virus (B7R and J2R markers) it may affect the sensitivity of the test and may result in false-negative results.
- The B7R gene has a small cross-reactivity with Vaccinia virus. Due to this cross-reactivity, the B7R signal alone cannot result in detection of Monkeypox.

Research Use Only

# 9 Symbols

The following symbols are used in labelling of InfectionDetect™ Mpox qPCR Assay.

Date of manufacture

For Research Use Only, not for *in vitro* diagnostics

Use-by date

Do not reuse

Contains sufficient for $<n>$

Manufacturer

Temperature limit

Consult electronic available instructions for use

# 10 Manufacturer

For technical assistance please contact your local distributor or PentaBase A/S. A complete list of distributors is available at www.pentabase.com.

PentaBase A/S
Petersmindevej 1A
DK-5000 Odense C

Telephone: +45 36 96 94 96
Email: info@pentabase.com
Webpage: www.pentabase.com

NOTICE TO USERS: Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is established.

Reseach Use Only