VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX™ System

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English

REF **445313**

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For Emergency Use Authorization (EUA) only
For use with the BD MAX™ System

INTENDED USE

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

Results are for the identification of monkeypox virus (MPXV, clade I/II) DNA which is generally detectable in human pustular or vesicular lesion specimens during the acute phase of infection. Positive results are indicative of the presence of monkeypox virus (MPXV, clade I/II) DNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results obtained with this device do not preclude monkeypox virus (MPXV, clade I/II) infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

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

The VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX™ System is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR, in vitro diagnostic procedures, and use of the BD MAX System.

The VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX™ System is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE PROCEDURE

Deoxyribonucleic acid (DNA) is isolated and purified using BD MAX™ ExK™ TNA-3 kit from lesion swabs collected in BD Universal Viral Transport System (UVT), Copan Universal Transport Media System (UTM), or Healthlink Inc Transport Medium (UTM). Patient samples are transferred to the Sample Buffer Tube (SBT) provided with the BD MAX™ ExK™ TNA-3 kit and placed in the BD MAX™ instrument. The BD MAX™ ExK™ TNA-3 reagent strip contains a combination of lytic and extraction reagents designed to perform cell lysis and DNA extraction. Following cell lysis, the released nucleic acids are captured by magnetic affinity beads. The beads with the bound nucleic acids are washed and then the DNA is eluted. Rehydration buffer is used to rehydrate the Monkeypox reaction tube. Eluted DNA is added to the rehydrated reaction tube. This final rehydrated reaction tube is transferred to a PCR cartridge for real-time PCR.

The VIASURE Monkeypox virus Real-time PCR Reagents for BD MAX™ System utilizes multiplexed primers and probes targeting DNA from the tumor-necrosis factor gene (G2R) and an open reading frame (F3L) of the monkeypox genome, and the human beta globin gene (HBB). The primer and probe sets are based on literature for specific detection of monkeypox virus (clade I/II) by amplifying two unique regions of the monkeypox virus genome (i.e., G2R and F3L). An internal control targeting the human beta-globin gene will be co-amplified along with monkeypox gene targets (if present) and will serve as an endogenous nucleic acid extraction control present in all properly collected patient samples. This control serves as both an extraction control and an internal amplification control.

PRINCIPLES OF THE PROCEDURE

A combination of lytic and extraction reagents is used to perform cell lysis and DNA extraction. Nucleic acids released from the target organisms are captured on magnetic affinity beads. The beads, together with the bound nucleic acids, are washed and the nucleic acids are eluted by a combination of heat and pH variation. Rehydration buffer is used to rehydrate the Monkeypox virus reaction tube. Eluted DNA is added to the rehydrated monkeypox virus reaction tube and mixed. After reconstitution, the BD MAX™ System dispenses a fixed volume of RT-PCR-ready solution containing extracted nucleic acids into the PCR Cartridge.

Microvalves on the cartridge are sealed by the system prior to initiating PCR in order to contain the amplification mixture and thus prevent evaporation and contamination. The amplified DNA targets are detected using hydrolysis (TaqMan®) probes, labeled at one end with a fluorescent reporter dye (fluorophore), and at the other end, with a quencher moiety. Probes labeled with different fluorophores are used to detect the target analytes in different optical channels of the BD MAX™ System. When the probes are in their native state, the fluorescence of the fluorophore is quenched due to its proximity to the quencher. However, in the presence of target DNA, the probes hybridize to their complementary sequences and are hydrolyzed by the 5'–3' exonuclease activity of the DNA polymerase as it synthesizes the nascent strand along the DNA template. As a result, the fluorophores are separated from the quencher molecules and fluorescence is emitted. The amount of fluorescence detected in the optical channels is directly proportional to the quantity of the corresponding probe that is hydrolyzed. The BD MAX™ System monitors these signals at each cycle of the PCR and interprets the data at the end of the reaction to provide qualitative test results for each analyte.

REAGENTS AND MATERIALS

Materials Provided

Catalog Number	Contents	Quantity
445313	**Monkeypox virus reaction tube (1H foil)** A mix of enzymes, primer-probes, buffer, dNTPs, stabilizers, and endogenous internal control in a stabilized format.	24 (2 x 12 tubes)
	Rehydration Buffer tube (11 foil) Solution to reconstitute the stabilized product.	24 (1 x 24 tubes)

Equipment and Materials Required but Not Provided

- BD MAX™ System (BD Catalog Number 441916)
- BD MAX™ Sample Rack (BD Catalog Number 441935, 443550, 443551, 444807, or 444808)
- BD PCR Cartridges (BD Catalog Number 437519)
- BD MAX™ ExK™ TNA-3 (BD Catalog Number 442821)
- Copan Universal Transport Medium (UTM®) (Copan Catalog Number 305C, 306C)
- BD Universal Viral Transport System (BD Catalog Number 220528, 220531)
- Healthlink Inc. Transport Medium (UTM®) System (Healthlink Catalog Number 3C036N.HL, 3C038N.HL)
- ATCC Quantitative Synthetic Monkeypox Virus DNA (Cat #: VR-3270SD)
- Microbiologics Negative Cellularity Control (NCC) (Swab - Cat #: HE0067NS, pellet - Cat #: HE0058N)
- Vortex Genie 2 (VWR Catalog Number 58815-235 or equivalent)
- Multi-Tube Vortex Mixer (VWR Catalog Number 58816-115 or equivalent)
- Rack compatible with a multi-tube vortexer (e.g., Cryogenic Vial Holder or equivalent)
- Variable Volume Calibrated Pipettor (400 µL volume capable)
- Aerosol resistant micropipette tips
- Disposable gloves, powderless

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WARNINGS AND PRECAUTIONS

- In accordance with Regulation (EC) No 1907/2006 (REACH), VIASURE Real Time PCR Detection Kits do not require Material Safety Data Sheets on account of their classification as non-hazardous to health and the environment, because they do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) No 1272/2008 (CLP), or which are in concentrations higher than the value established in the mentioned regulation for their declaration.
- For in vitro diagnostic use
- For use under Emergency Use Authorization only.
- For use by trained laboratory personnel.
- For Prescription Use only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories; use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform moderate or high complexity tests.
- This product has been authorized only for the detection of nucleic acid from monkeypox virus, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of 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.
- Positive results are indicative of the presence of monkeypox virus DNA.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in the CLSI Document M29-A4[1] and in Biosafety in Microbiological and Biomedical Laboratories.[2] Only personnel proficient in handling infectious materials and the use of VIASURE Monkeypox virus Real Time Reagents for BD MAX™ System and the BD MAX™ System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, follow appropriate site procedures.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Protect reagents against heat and humidity. Prolonged exposure to humidity may affect product performance.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Do not interchange or re-use caps, as contamination may occur and compromise test results.
- Check Unitized Reagent Strips for proper liquid fills (ensure that the liquids are at the bottom of the tubes).
- Check Unitized Reagent Strips to ensure that all pipette tips are present.
- Proceed with caution when using chemical solutions, as Extraction Tube barcode readability may be altered.
- Good laboratory technique is essential to the proper performance of this assay. Extreme care should be taken to preserve the purity of all materials and reagents.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX System, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents at all times. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges.
- To avoid contamination of the environment by amplicons, do not break apart the BD PCR Cartridge after use. The seals of the BD PCR Cartridges are designed to prevent contamination.
- The laboratory should routinely perform environmental monitoring to minimize the risk of cross-contamination.
- Wear protective clothing and disposable gloves while handling all reagents.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth.
- Do not smoke, drink, chew or eat in areas where specimens or kit reagents are being handled.
- Collect and dispose of all used and unused reagents and any other contaminated disposable materials following procedures for biohazardous or potentially biohazardous waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to adequately treat and dispose of them (or have them treated and

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disposed of) in accordance with any applicable regulations. Do not discharge liquid waste down the drain where prohibited.

- Consult the BD MAX™ System User's Manual[3] and BD MAX™ ExK™ TNA-3 package insert for additional warnings, precautions and procedures.

STORAGE AND STABILITY

- The VIASURE Monkeypox Real-Time PCR Reagents kit ships at ambient temperature and is stable at 2–25 °C through the stated expiration date. Do not use if expired.
  NOTE: The reagents are considered unusable by the BD MAX™ System on the expiration date printed on the product label.
- The VIASURE Monkeypox Real-Time PCR Reagents are provided in sealed pouches. To protect from humidity, immediately re-seal after opening shelf.

INSTRUCTIONS FOR USE

NOTE: Wear gloves when handling specimens. If gloves come in contact with the specimen, immediately change them to prevent contamination of other specimens.

Swab Specimen Collection/Transport in Universal Viral Transport (UVT) or Universal Transport Medium (UTM)

1. Lesion swab specimens should be collected and expressed directly into the Universal Viral Transport System or the Universal Transport Media System* according to their respective package insert instructions.
2. After collection, specimens can be stored for up to 24 hours at 2-25°C or 6 days at 2-8°C.
3. If delivery and processing of samples exceeds the specified time period, specimens should be transported in dry ice and once in laboratory stored frozen at -70°C or colder for up to 30 days.

* Performance of this test has only been established in human lesion swabs collected in Universal Viral Transport Media System and Universal Transport Medium System manufactured by Copan (Copan UTM/UVT). Copan UTM/UVT, BD™ Universal Viral Transport System (UVT) and Healthlink Inc. Transport Medium System are equivalent.

BD MAX™ Sample Buffer Tube Preparation for use with lesion swab specimens in Universal Viral Transport (UVT) or Universal Transport Media (UTM)

NOTE: If frozen, allow Universal Viral Transport (UVT) or Universal Transport Media (UTM) specimen to come to room temperature before proceeding.

1. Remove a BD MAX™ ExK™ TNA-3 Sample Buffer Tube from the BD MAX™ ExK™ TNA-3 kit.
2. Uncap the Sample Buffer Tube and transfer (using a calibrated, variable pipette) 400 µL from the UVT/UTM directly into the Sample Buffer Tube.
3. Recap the Sample Buffer Tube with a blue septum cap and vortex or mix by inversion 5 times.
4. Label the Sample Buffer Tube with patient information.
   NOTE: Do not obscure the barcodes on the tube. Obscuring the barcode may result in BD MAX™ System catalog failure and inability to test the sample.
5. Repeat Steps 1 to 4 for each UVT/UTM that will be tested on the BD MAX™ System.
6. Proceed directly with the BD MAX™ System Operation.

BD MAX™ System Operation

NOTE: Refer to the BD MAX™ System User's Manual[3] for detailed instructions (Operation section).

1. Power on the BD MAX™ System (if not already done) and log in by entering **<user name>** and **<password>**.
2. Gloves must be changed before manipulating reagents and cartridges.
3. Remove the required number of BD MAX™ ExK™ TNA-3 Unitized Reagent Strips from the BD MAX™ ExK™ TNA-3 kit. Gently tap each Unitized Reagent Strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes. Remove the required number of BD MAX™ ExK™ TNA-3 Extraction Tube(s) from the protective pouch. Remove excess air, and close pouch with the zip seal.
4. From the VIASURE Monkeypox Real-Time PCR Reagent kit, remove the required number of Monkeypox Reaction and Rehydration Tube(s) from their protective pouches. Remove excess air and close each pouch with the zip seal.
5. For each specimen to be tested, place one (1) Unitized Reagent Strip on the BD MAX™ System Rack. Assemble the strip as in Figure 1.

Figure 1: Snap Extraction Tube, Master Mix Tube, and Primer/Probe Tube into Unitized Reagent Strip

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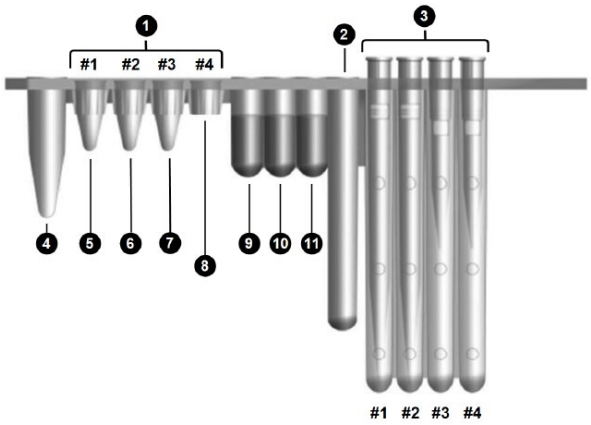


Figure 1 Key: (1) Snap-in Tubes **(2)** Waste Reservoir **(3)** Pipette Tips **(4)** Lysis Tube **(5)** Extraction Tube **(6)** VIASURE Monkeypox virus Reaction Tube **(7)** VIASURE Rehydration Buffer Tube **(8)** Empty **(9)** Wash Buffer **(10)** Elution Buffer **(11)** Neutralization Buffer

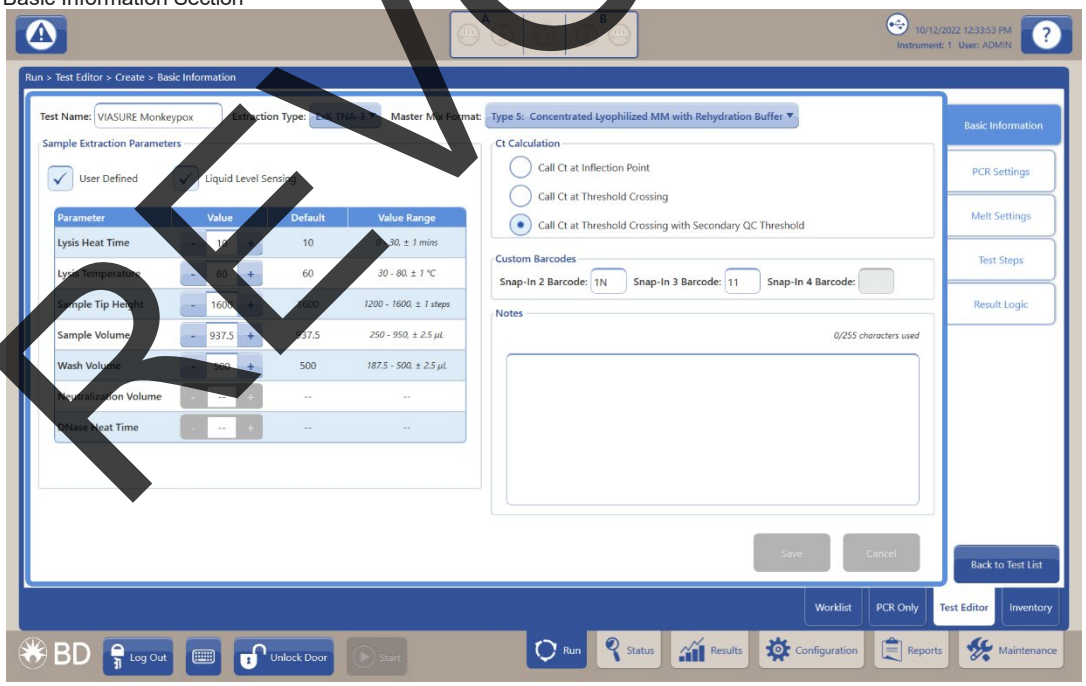
Note: Failure to add extraction tube, reaction tube, or rehydration tube may result in instrument contamination.

Note: A conical snap-in tube is fully seated in the strip when a 'click' is heard. Refer to above for reagent placement in the Unitized Reagent Strip.

- Position 1= Snap the BD MAX™ ExK™ TNA-3 Extraction Tube into Position 1.
- Position 2= Snap the VIASURE Monkeypox virus Reaction Tube into Position 2.
- Position 3= Snap the VIASURE Rehydration Buffer tube into Position 3.
- Position 4= Leave Position 4 empty (no conical snap-in tube).

6. Create the User Defined Protocol (UDP) as follows:
   - Navigate to Run > Test Editor tab.
   - Click "Create".
   - Complete each section of the user protocol as outlined in the screen shots below.

Basic Information Section



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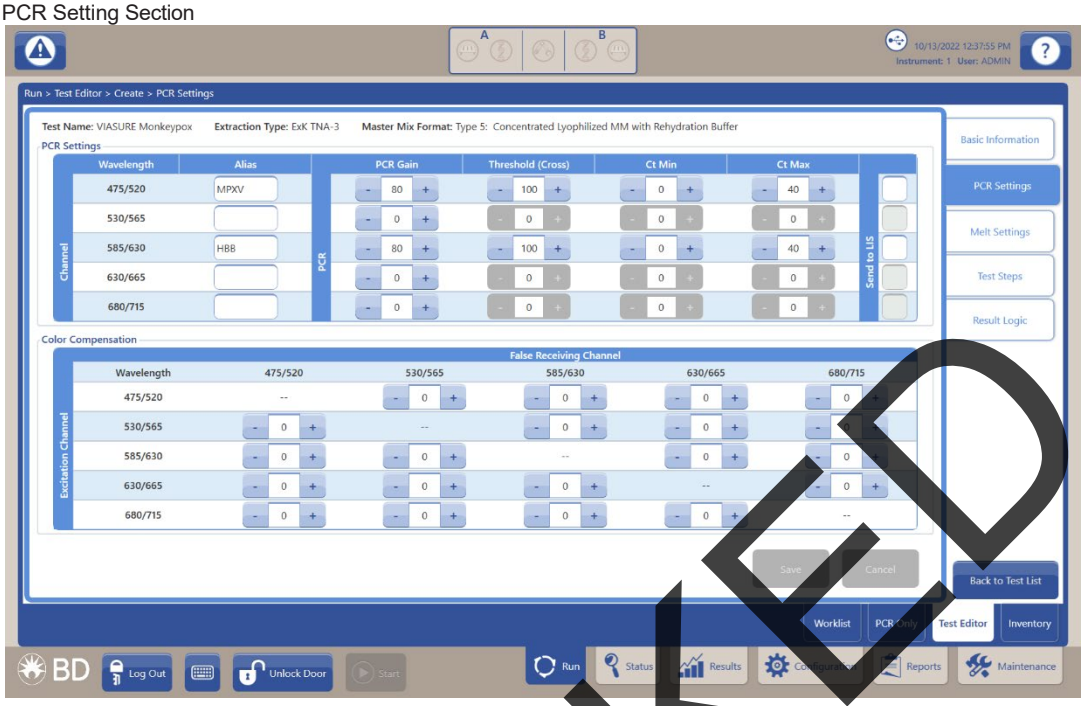
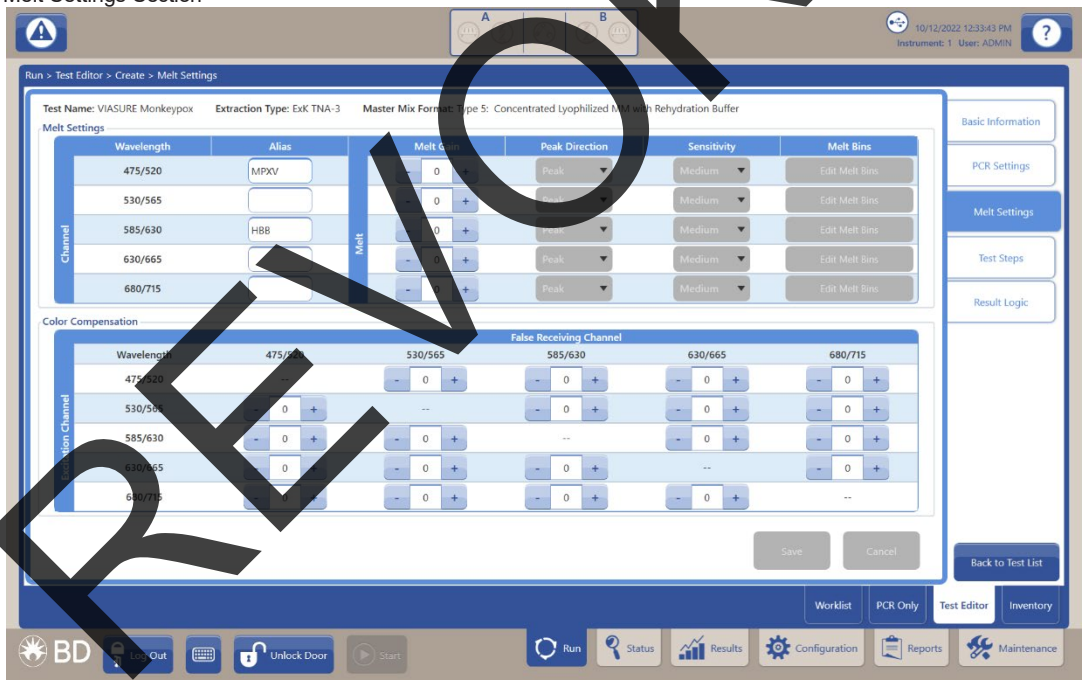
PCR Setting Section



Melt Settings Section



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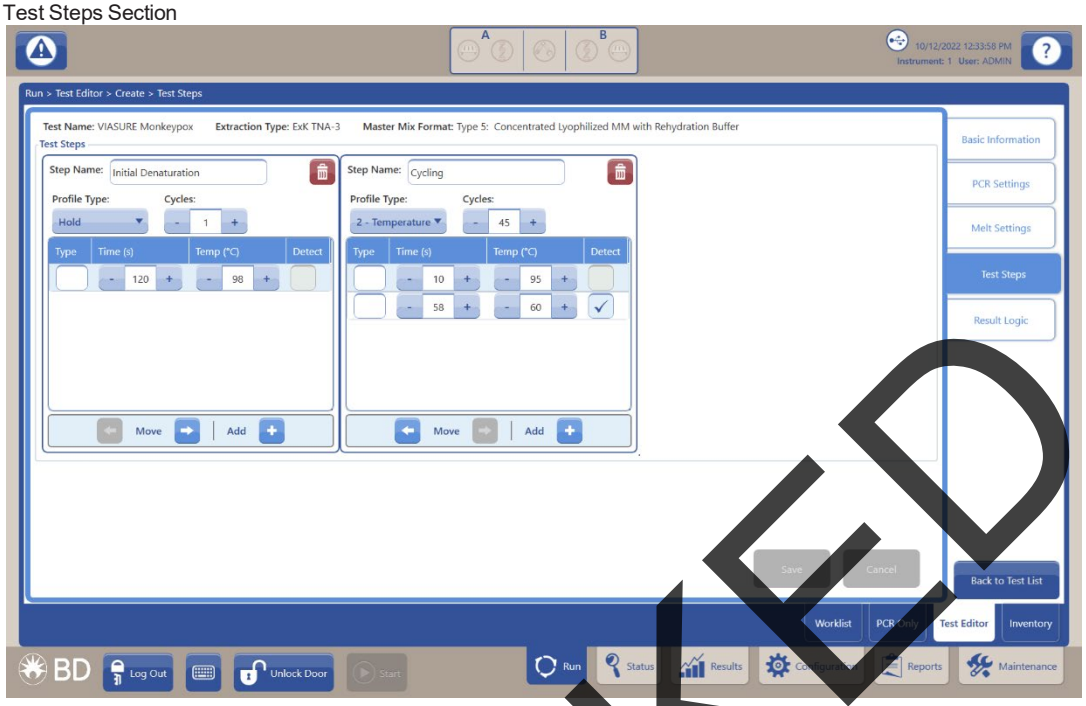
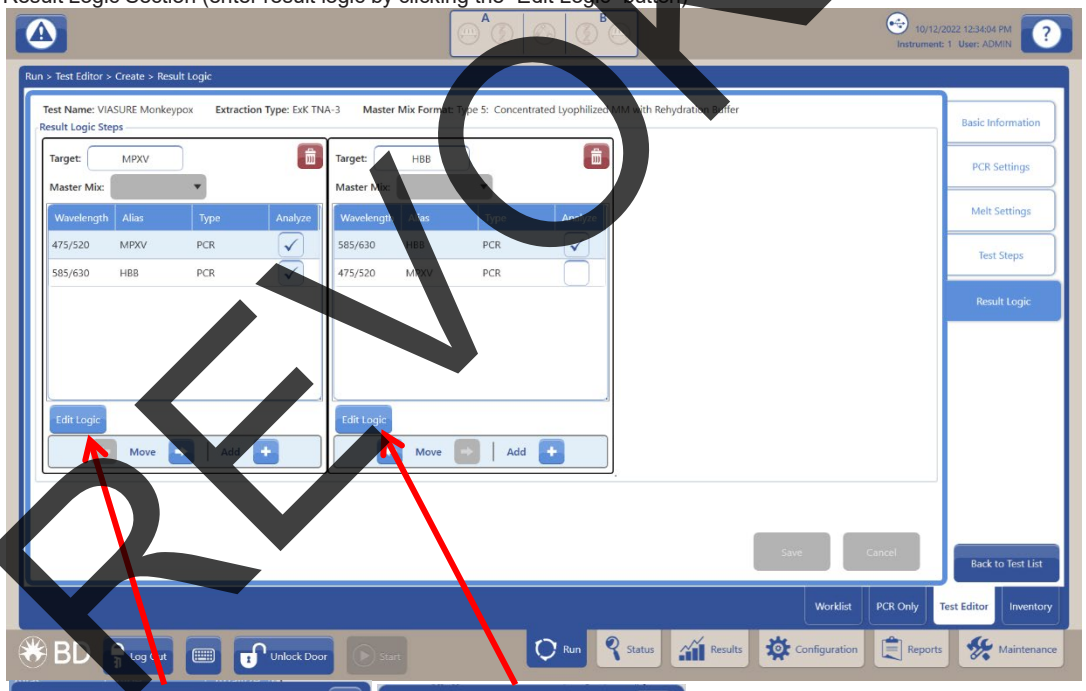
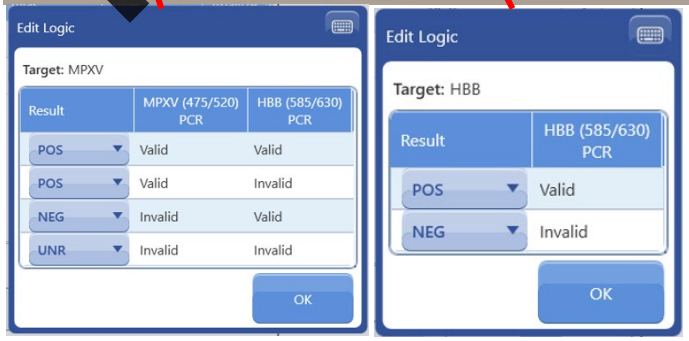
Test Steps Section



Result Logic Section (enter result logic by clicking the “Edit Logic” button)





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7. Click **<SAVE>** after all information has been entered into the Test Editor. The UDP only needs to be created once, and steps 6 and 7 do not need to be repeated for subsequent runs.
8. Click on the Run tab, then Inventory. Enter the kit lot number for the VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX System kit in the barcode field (for lot traceability) by either scanning the barcode with the scanner or by manual entry and then save.

   Note: Repeat step 8 each time a new kit lot is used.
9. Navigate to the Worklist (RUN > WORKLIST). Using the pull down menu select the UDP previously created in Step 6 (example: BD Monkeypox DNA3).
10. Enter the Sample Buffer Tube ID, Patient ID and Accession Number (if applicable) into the Worklist, either by scanning the barcode with the scanner or by manual entry.
11. Select the appropriate kit lot number (found on the outer box) from the pull down menu.
12. Repeat Steps 9 to 11 for all remaining Sample Buffer Tubes.
13. Place the Sample Buffer Tubes into the BD MAX™ System Rack(s) corresponding to the Unitized Reagent Strips previously assembled.
14. Place the required number of BD PCR Cartridge(s) into the BD MAX™ System (refer to Figure 2).

Figure 2: Load BD PCR Cartridges

15. Load rack(s) onto the BD MAX™ System (refer to Figure 3).

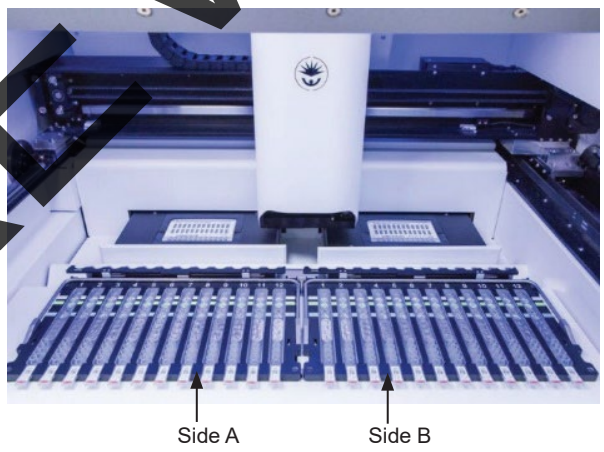

Figure 3: Load Rack(s) onto the BD MAX™ System

16. Close the BD MAX™ System lid and click <Start> to begin the processing.

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QUALITY CONTROL

1. Quality control procedures monitor the performance of the assay. Laboratories must establish the number, type, and frequency of testing of control materials according to guidelines or requirements of local, provincial, state, and federal and/or country regulations or accreditation organizations in order to monitor the effectiveness of the entire analytical process. For general Quality Control guidance, the user may wish to refer to CLSI MM3 and EP12.[1,2]
2. External Control materials are not provided by BD. External Positive and Negative Controls are not used by the BD MAX™ System software for the purpose of sample test result interpretation. External Controls are treated as if they were patient samples. (Refer to the table in the Results Interpretation section for the interpretation of External Control assay results.)
3. It is recommended that one (1) External Positive Control and one (1) External Negative Control be run at least daily until adequate process validation is achieved on the BD MAX™ System in each laboratory setting. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Reduced frequency of control testing should be in accordance with applicable regulations.
4. The External Positive Control is intended to monitor for substantial reagent failure. The External Negative Control is intended to detect reagent or environmental contamination (or carry-over) by target nucleic acids.
5. Various types of External Controls are recommended to allow the user to select the most appropriate for their laboratory quality control program.
    a. External Negative Control (ENC): Commercially available control material, such as Microbiologics® Helix Elite™ Inactivated Standard Negative Cellularity Control (refer to **Table 1**). BD recommends that the External Negative Control be prepared prior to the External Positive Control in order to reduce the potential for contamination as a result of control preparation.
    b. External Positive Control (EPC): Commercially available control material, such as from ATCC® listed below (refer to Table 1).

Table 1. Commercially Available Standards for External Controls

Commercially Available Standards	Part Number
ATCC Quantitative Synthetic Monkeypox virus DNA	VR-3270SD
Microbiologics® Helix Elite™ Inactivated Standard Negative Cellularity Control (Pellet)	HE0058N
Microbiologics® Helix Elite™ Inactivated Standard Negative Cellularity Control (Swab)	HE0067NS

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6. Preparation of External Negative Control Standards including:

    Microbiologics® Helix Elite™ Inactivated Standard Negative Cellularity Control (Pellet) (HE0058N)
    a. Add 400 µL of nuclease-free water into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube.
    b. Rehydrate the Microbiologics® Negative Cellularity Control Standard with 100 µL of nuclease free water.
    c. Dilute the rehydrated standard 1:10 in nuclease free water (10 µL standard to 90 µL nuclease free water).
    d. Spike 75 µL of the diluted standard into the Sample Buffer Tube.
    e. Cap the External Negative Control Sample Buffer Tube and vortex for 10–30 seconds or invert 8–10 times. Process on the BD MAX™ System.

    Microbiologics Helix Elite™ Inactivated Standard Negative Cellularity Control (Swab) (HE0067NS)
    a. Add 400µL of nuclease free water into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube.
    b. Place a Microbiologics Helix Elite™ Inactivated Standard Negative Cellularity Control (Swab) directly into the BD MAX™ ExK™ TNA-3 Sample Buffer Tube.
    c. Express the swab thoroughly.
    d. Discard the expressed swab.
    e. Cap the External Negative Control sample buffer tube and vortex for 10-30 seconds or invert 8-10 times. Process on the BD MAX™ instrument.
7. Preparation of External Positive Control Standards including:

    ATCC® Quantitative Synthetic Monkeypox virus DNA (VR-3270SD) and Microbiologics® Helix Elite™ Inactivated Standard Negative Cellularity Control (Pellet) (HE0058N)
    a. Add 400 µL of nuclease-free water into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube.
    b. Rehydrate the Microbiologics® Negative Cellularity Control Standard with 100 µL of nuclease free water.
    c. Dilute the rehydrated Microbiologics® Negative Cellularity Control Standard 1:10 in nuclease free water (10 µL standard to 90 µL nuclease free water).
    d. Spike 75uL of the diluted Microbiologics® Negative Cellularity Control Standard into the Sample Buffer Tube.
    e. Serially dilute the ATCC® Quantitative Synthetic Monkeypox virus DNA (VR-3270SD) 1:10,000 in nuclease free water

(i.e. 10 µL standard to 990 µL nuclease free water followed by a subsequent 10 µL diluted standard to 990 µL nuclease free water).

f. Spike 50 µL of the 1:10,000 diluted standard into the Sample Buffer Tube.

g. Cap the External Negative Control Sample Buffer Tube and vortex for 10–30 seconds or invert 8–10 times. Process on the BD MAX™ System.

ATCC® Quantitative Synthetic Monkeypox virus DNA (VR-3270SD) and Microbiologics Helix Elite™ Inactivated Standard Negative Cellularity Control (Swab) (HE0067NS)

a. Add 400 µL of nuclease-free water into a BD MAX™ ExK™ TNA-3 Sample Buffer Tube.

b. Place a Microbiologics Helix Elite™ Inactivated Standard Negative Cellularity Control (Swab) directly into the BD MAX™ ExK™ TNA-3 Sample Buffer Tube.

c. Express the swab thoroughly.

d. Discard the expressed swab.

e. Serially dilute the ATCC® Quantitative Synthetic Monkeypox virus DNA (VR-3270SD) 1:10,000 in nuclease free water (i.e. 10 µL standard to 990 µL nuclease free water followed by a subsequent 10 µL diluted standard to 990 µL nuclease free water).

f. Spike 50 µL of the 1:10,000 diluted standard into the Sample Buffer Tube.

g. Cap the External Negative Control Sample Buffer Tube and vortex for 10–30 seconds or invert 8–10 times. Process on the BD MAX™ System.

8. All External Controls should yield the expected results (Table 2) with no failed external controls (Unresolved, Indeterminate, Incomplete Results).

Table 2. VIASURE Monkeypox virus Real Time PCR Reagents External Control Expected Results

Control Type	Control	Used to Monitor	Expected Result	
			MPX	HBB
Negative External Control	Known Negative Specimen	Reagent and/or environmental contamination	NEG	POS
	Microbiologics® Negative Cellularity Control			
Positive External Control	Known Positive Specimen	Substantial reagent failure including primer and probe integrity.	POS	POS
	ATCC® Synthetic Monkeypox virus			

9. An External Negative Control that yields a positive test result is indicative of a specimen handling and/or contamination event. Review the specimen handling technique to avoid mix-up and/or contamination. An External Positive Control that yields a negative result is indicative of a specimen handling/preparation problem. Review the specimen handling/preparation technique.

10. An External Control that yields an Unresolved, Indeterminate, or Incomplete test result is indicative of a reagent or a BD MAX™ System failure. Check the BD MAX™ System monitor for any error messages. Refer to the Troubleshooting section of the BD MAX™ System User's Manual[3] for interpretation of warning and error codes. If the problem persists, use reagents from an unopened pouch or use a new VIASURE Monkeypox virus Real Time PCR Reagent for BD MAX™ System kit.

11. The human beta globin gene serves as both an Extraction and Internal Amplification Control. In the event that MPX results are negative, a HBB result must be positive for the MPX result to be valid negative results. An Unresolved (UNR) result is indicative of specimen-associated inhibition or reagent failure. Repeat any sample reported as Unresolved according to the Repeat Test Procedure section below.

RESULTS INTERPRETATION

Results are available on the results tab in the Results window on the BD MAX™ System monitor. The BD MAX™ System software, version 5.14A or later, automatically interprets the test result when the Monkeypox User Defined Protocol (UDP) is used. Results are based on the following decision algorithm:

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Table 3. VIASURE Monkeypox virus Real Time PCR Reagents Assay Result Interpretation

Assay Result Reported	Interpretation of Result
MPXV POS	Monkeypox virus DNA Detected
MPXV NEG	No Monkeypox virus DNA detected and endogenous control (HBB) Detected
UNR	No Monkeypox virus DNA detected and no endogenous control (HBB) Detected (Indicative of an inadequate specimen or reagent failure)
IND	Indeterminate due to a BD MAX™ System failure (with Warning or Error Codes)[a]
INC	Incomplete Run (with Warning or Error Codes)[a]

[a] Refer to the Troubleshooting section of the BD MAX™ System User's Manual for interpretation of warning and error codes

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the external positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The table below lists the expected results. If results are obtained that do not follow these guidelines, prepare a new specimen. If repeat testing yields similar results, collect a fresh sample from the patient for testing.

Table 4. Interpretation of Patient Specimen Results

Endogenous Control (HBB)	MPX	Results Interpretation[a,b]	Actions
POS/NEG	POS	Positive	Report as Detected
POS	NEG	Negative	Report as Not Detected
NEG	UNR	UNR, IND, INC	Repeat Test[c]

[a] UNR = Unresolved

[b] Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system

[c] Repeat Test by preparing a fresh Sample Buffer Tube from the original primary sample

Repeat Test Procedure

When an Indeterminate (IND), Unresolved (UNR), or Incomplete (INC) result is obtained a repeat test from the primary sample must be performed. If an External Control fails, repeat testing of all specimens conducted on the same day using freshly prepared External Controls (see Quality Control).

Unresolved Result

Unresolved results (UNR) may be obtained in the event that specimen-associated inhibition or reagent failure prevents proper target or HBB amplification. Sample(s) can be repeated from the primary sample. Uncap a new BD MAX™ TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 400 µL from the UVT/UTM specimen directly into the BD MAX™ TNA-3 Sample Buffer Tube. Restart from the BD MAX™ System Operation section.

Indeterminate Result

Indeterminate results (IND) may be obtained in the event that a System failure occurs. Sample(s) can be repeated from the primary sample. Uncap a new BD MAX™ TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 400 µL from the UVT/UTM specimen directly into the BD MAX™ TNA-3 Sample Buffer Tube. Restart from the BD MAX™ System Operation section.

Incomplete Result

Incomplete results (INC) may be obtained in the event that Specimen Preparation or the PCR did not reach its expected time points. Sample(s) can be repeated from the primary sample. Uncap a new BD MAX™ TNA-3 Sample Buffer Tube and transfer (using a calibrated, variable pipette) 400 µL from the UVT/UTM specimen directly into the BD MAX™ TNA-3 Sample Buffer Tube. Restart from the BD MAX™ System Operation section.

External Control Failure

External Controls should yield expected results when tested. If samples have to be repeated due to an incorrect External Control result, the samples should be repeated from the primary sample along with freshly prepared External Controls. Restart from the BD MAX™ System Operation section.

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LIMITATIONS OF THE PROCEDURE

- VIASURE Monkeypox virus Real Time PCR Reagents have been evaluated only for use on the BD MAX™ System with BD MAX ExK TNA-3 kit and the external quality controls listed above.
- Reliable results depend on proper sample collection, storage and handling procedures.
- The performance of this test was established using lesion swab specimens collected in Universal Viral Transport System (UVT) or Universal Transport Media System (UTM) manufactured by Copan, BD or Healthlink Inc. only. Assay performance has not been validated for use with other collection media and/or specimen types. Use of other collection media and/or specimen types may lead to false positive, false negative or invalid result.
- While monkeypox virus clade II is the only member of the *Orthopoxvirus* genus known to be circulating among humans in the US at this time, a positive result most likely represents the presence of monkeypox virus clade II, although there is a small possibility that this result could represent the presence of monkeypox virus clade I. If clinical concern for such an infection exists, healthcare providers should contact the CDC and their local public health authorities for guidance
- The performance of this test was evaluated using contrived clinical lesion swab specimens. Clinical Performance with natural clinical lesion swab specimens has not been established
- The clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of monkeypox virus and their prevalence, which may change over time.
- As with any molecular test, mutations within the target regions of the VIASURE Monkeypox virus Real Time PCR Reagents could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Detection of MPXV DNA is dependent on the number of copies present in the specimen. Detection of monkeypox virus DNA may be affected by sample collection methods (e.g., if a specimen is improperly collected, transported, or handled), patient factors (e.g., presence, type, and duration of symptoms), stage of infection (e.g., if collected too early or too late in the course of illness) and/or presence of interfering substances.
- A specimen with a result of “MPXV Negative” does not preclude monkeypox virus infection and should not be used as the sole basis for treatment or other patient management decisions. Collection of multiple specimens (and specimens collected at different time points) from the same patient may be necessary to detect the virus.
- False negative or invalid results may occur due to interference. The HBB endogenous control is included to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification. Interfering substances studies have not been performed for this assay. The assay uses conventional well-established nucleic acid extraction methods used for other similar assays. Interference from common endogenous substances is not anticipated.
- Detection of HBB indicates that human nucleic acid is present and implies that human biological material was collected, and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of monkeypox virus DNA. All MPXV-negative specimens must have a positive HBB result to be identified as valid negatives.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- Good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The VIASURE Monkeypox virus Real Time PCR Reagents Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-emergency-use-authorizations-medical-devices

To assist clinical laboratories running the VIASURE Monkeypox virus Real Time PCR Reagents, the relevant Conditions of Authorization are listed below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories* using the VIASURE Monkeypox virus Real Time PCR Reagents must include with result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using the VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX™ System must use the VIASURE Monkeypox virus Real Time PCR Reagents as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized ancillary reagents, and authorized material required to perform the VIASURE Monkeypox virus Real Time PCR Reagents test are not permitted.
- Authorized laboratories must have a process in place to track adverse events and report to Becton, Dickinson and Company Customer Technical Support 1.800.638.8663 and to FDA pursuant to 21 CFR Part 803.
- Authorized laboratories that receive the VIASURE Monkeypox virus Real Time PCR Reagents test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
- All laboratory personnel using the VIASURE Monkeypox virus Real Time PCR Reagents must be appropriately trained in real-time PCR techniques, use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

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- Authorized laboratories using the VIASURE Monkeypox virus Real Time PCR Reagents must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Becton, Dickinson and Company, authorized distributors, and authorized laboratories using VIASURE Monkeypox virus Real Time PCR Reagents must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.
- Authorized laboratories will collect information on the performance of the VIASURE Monkeypox virus Real Time PCR Reagents and report to DMD/OHT7 /OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to Becton, Dickinson and Company Customer Technical Support 1.800.638.8663 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the VIASURE Monkeypox virus Real Time PCR Reagents test of which they become aware.

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

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

LoD studies determine the lowest detectable concentration of the Monkeypox virus at which approximately 95% of all (true positive) replicates test positive. To determine the LoD, quantified monkeypox virus synthetic DNA material, obtained from BEI resources, NIAID, NIH (Catalog Number NR-58627) or heat inactivated monkeypox virus strain USA/MA001/2022 was serially diluted in TE buffer and spiked into the BD MAX ExK TNA-3 Sample Buffer Tube containing pooled negative lesion swab matrix, a total of 5 concentrations levels, with 3-fold serial dilutions between each level.

Confirmation of the estimated LoD was performed with one reagent lot and 20 replicates prepared using pooled lesion swab clinical matrix. The LoD is the lowest concentration (reported as copies/mL or $TCID_{50}$/mL) of monkeypox that can be reproducibly distinguished from negative samples ≥95%. The LoD for the assay is indicated below.

Table 5: LoD Determination of Monkeypox virus

Material	Concentration	Percent Positivity	Mean Ct	
			MPX	HBB
Synthetic DNA NR-58627	288 copies/mL	100% (20/20)	33.5	22.4
Heat inactivated Monkeypox virus USA/MA001/2022	90 $TCID_{50}$/mL	100% (20/20)	34.1	24.6

Reactivity/Inclusivity

An *in silico* comparison of the G2R and F3L primer sets was performed using all available high quality Monkeypox virus sequences submitted to the comprehensive INSDC (International Sequence Database Collaboration) nucleotide sequence collection for DNA as of October 5, 2022 (N=1415). Alignments against the G2R and F3L genes showed that both G2R and F3L primer/probe sets are a perfect match to 3.13% of sequences in the database, 3.2% of the sequences were a perfect match to the G2R primer set region, and 99% were a perfect match to the F3L primer set region. In total, 99.1% are a perfect match to either the G2R or F3L region primer set.

Cross-Reactivity and Microbial Interference

An *in silico* comparison of the G2R and F3L primer sets were screened against all available high quality DNA sequences submitted to the comprehensive INSDC (International Sequence Database Collaboration) nucleotide sequence collection for DNA as of October, 5, 2022. The analysis was limited to a maximum of 100,000 sequences. Any sequence that contained that contained ≥80% similarity to any individual primer or probe. Additional emphasis was placed on the organisms outlined in Table 6.

G2R Assay:

The primer and probe sequence of G2R assay showed high sequence homology human genomic DNA, and Cowpox virus. One identified human genome sequence matched the MPX1-Forward primer in two locations with 5 mismatches. In the unlikely event that these primers could extend, the resulting amplicon would be 1,525bp and no probe matches sequence between the binding sites. Therefore, it is not considered to be a cross-reactivity risk. Musa schizocarpa (wild banana), and Ophonus ardosiacus (ground beetle) are not considered clinically relevant. Akhmeta virus is a related zoonotic orthopoxvirus isolated from rodents in the country of Georgia. It is not considered to be a human pathogen. Orthopoxvirus Abatino is another closely related, likely zoonotic, Orthopoxvirus isolated from a macaque, and not known to be a human pathogen.

F3L Assay:

The forward primer, reverse primer and probe sequences showed no significant homology with the human genome, other orthopoxvirus or human microflora. As a result, there is no prediction of potential false positive results.

Table 6: Organisms Evaluated in-silico for Cross-reactivity.

Organism	Organism
Acinetobacter calcoaceticus	*Mycoplasma genitalium*

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Organism	Organism
Bacteroides fragilis	*Mycoplasma pneumoniae*
Camelpox virus	*Neisseria gonorrhoeae*
Candida albicans	*Pseudomonas aeruginosa*
Chlamydia trachomatis	*Staphylococcus aureus*
Corynebacterium diptheriae	*Staphylococcus epidermidis*
Corynebacterium jeikeium	*Streptococcus agalactia*
Cowpox virus	*Streptococcus* Group C
Ectromelia virus (mousepox)	*Streptococcus* Group G
Enterococcus faecalis	*Streptococcus mitis*
Escherichia coli	*Streptococcus pyogenes*
Herpes simplex virus 1	*Treponema pallidum*
Herpes simplex virus 2	*Trichomonas vaginalis*
Human genomic DNA	*Trichophyton rubrum*
Human papilloma virus (HPV)	Vaccinia virus
Lactobacillius species	Varicella-zoster virus (chickenpox)
Molluscum contagiosum virus	Variola virus (smallpox)

Interfering Substances

The VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX™ System uses a conventional, well-established nucleic acid extraction method; therefore, interference from common endogenous substances is not expected. Interference studies have not been performed for this assay.

Clinical Evaluation

The performance of VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX™ System was further evaluated using 30 contrived positive clinical samples prepared in negative lesion swab matrix and 30 negative lesion swab clinical samples. Low positive contrived clinical samples were prepared by spiking quantified synthetic monkeypox virus DNA (NR-58627) into a BD MAX ExK TNA-3 Sample Buffer Tube that contained individual negative clinical matrix to approximately 1-2x LoD and 3-5x LoD. The low positive samples showed 95% agreement with the expected results, moderate positive samples showed 100% agreement with the expected results, and negative samples showed 100% agreement with the expected results.

Table 7: Clinical Evaluation with Contrived Lesion Swab Samples

Concentration	Total Valid Results	MPXV Positive	Mean Ct[a]	
			MPXV	HBB
Moderate Positive ~3-5x LoD	10	100% (10/10)	31.7	25.7
Low Positive ~1-2x LoD	20	95% (19/240)	33.2	26.5
Negative	30	0% (0/30)	n/a	27.2

[a] – only positive replicates were used for mean Ct calculations.

Percent Agreement	Result (%)	95% Confidence Interval
PPA	96.7% (29/30)	83.3% - 99.4%
NPA	100.0% (30/30)	88.6% - 100.0%

AVAILABILITY

Catalog Number	Description
437519	BD PCR Cartridges
442821	BD MAX™ ExK™ DNA-3
445313	VIASURE Monkeypox virus Real Time PCR Reagents for BD MAX™ System

REFERENCES

REVOKED

1. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections; Approved Guideline. Document M29 (refer to the latest edition).
2. Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in microbiological and biomedical laboratories. Chosewood L.C. and Wilson D.E. (eds) (2009). HHS Publication No. (CDC) 21–1112.
3. BD MAX™ System User's Manual (refer to the latest revision) BD Life Sciences, Sparks, MD 21152 USA.

SYMBOLS FOR COMPONENTS AND REAGENTS

***In vitro* diagnostic Device**

Contains <n> test

Catalogue number

Keep dry

Manufacture

Consult instructions for use

Batch Code (MPX106L-xxx MPX112L-xxx)

Use by (yyyy-mm: year and month)

Temperature limitation

For Use under Emergency Use Authorization Only

For prescription Use Only

Do not use if package is damaged.

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Change History

Revision	Date	Change Summary
(01)	2022-12	Initial release.

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BD

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