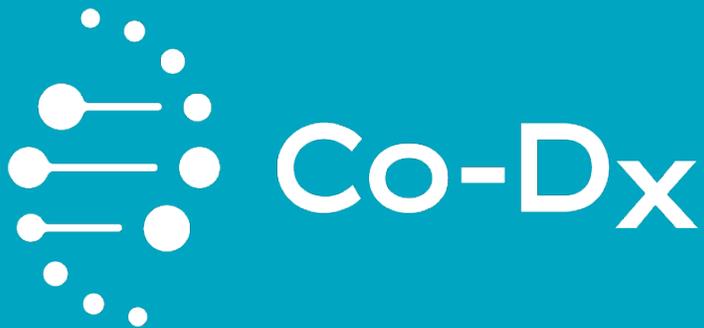


Oct  
2022



# Instructions for Use for Co-Dx Logix Smart™ Monkeypox

**REF** MPXA-R-001

Co-Dx Logix Smart™ Monkeypox RUO  
CO-DIAGNOSTICS, INC.

**RUO**

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## 1 MANUFACTURER



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Email: [info@co-dx.com](mailto:info@co-dx.com)  
Website: [www.co-dx.com](http://www.co-dx.com)

## 2 INTENDED USE

The **Co-Dx Logix Smart™ Monkeypox RUO** is for the simultaneous qualitative detection of Monkeypox and other related Orthopoxviruses.

For research use only. Not for use in diagnostics procedures.

## 3 PRODUCT DESCRIPTION

The **Co-Dx Logix Smart™ Monkeypox RUO** is a research-use-only test, based on real-time polymerase chain reaction technology (PCR). It tests for the simultaneous qualitative detection of Monkeypox and other related Orthopoxviruses.

The **Co-Dx Logix Smart™ Monkeypox RUO** includes an internal control to identify possible inhibition, confirm the integrity of the reagents, and verify the quality of the sample extraction. The **Co-Dx Logix Smart™ Monkeypox RUO** also includes a positive control (PC) which includes synthetic DNA molecules carrying sequences that are homologous to monkeypox viruses targeted by this assay. PCs represent a source of cross contamination. Take precautions to prevent and minimize the risk of cross contamination.

CoPrimers™ in the **Co-Dx Logix Smart™ Monkeypox RUO** include the following:

- CoPrimers™ that target monkeypox (MPX) F8L are labelled with the Quasar® 670 fluorophore.
- CoPrimers™ that target the Human RNaseP DNA, which acts as the Internal Positive Control (IPC), are labelled with CAL Fluor® Red 610 fluorophore.

## 4 RUO COMPONENTS

See Table 1 for components included in the **Co-Dx Logix Smart™ Monkeypox RUO**.

**Table 1**

*RUO Component Information*

Lid Color	Component	Symbol	Catalog Number	Description	Amount
<b>Brown</b>	Co-Dx Logix Smart™ Monkeypox RUO Master Mix	MM	MPXA-MM-001	Proprietary blend of CoPrimers™ and PCR reagents	1×500 µL (100 reactions)
<b>Red</b>	Co-Dx Logix Smart™ Monkeypox RUO Positive Control	PC	MPXA-PC-001	Proprietary blend of target templates	1×500 µL (100 reactions)
<b>Clear</b>	No Template Control	NC	MPXA-NC-001	DNase/RNase-free water	1×500 µL (100 reactions)

The product code for this product is MPXA-R-001. Contact Sales at (801) 438-1036 ext. 01 or at [www.co-dx.com/contact/](http://www.co-dx.com/contact/) to order.

## 5 COLLECTION, HANDLING, STORAGE, AND SHIPPING

See the following information for **Co-Dx Logix Smart™ Monkeypox RUO** collection, handling, storage, and shipping:

- The **Co-Dx Logix Smart™ Monkeypox RUO** is shipped on dry ice and should arrive frozen. Contact your distributor if one or more of the RUO components are not frozen upon arrival or are compromised during shipment.
- Upon receipt of RUO, follow internal laboratory procedures for quality control.
- Upon arrival, store all components at a temperature between -40°C and -16°C to prevent degradation of reagents.
- Avoid excessive thawing and freezing of components, specifically the master mix (MM), as this might affect the performance of the assay. Freeze reagents in multiple aliquots if they are to be used intermittently to ensure that less than 10 freeze-thaws are performed.
- Avoid storing components for more than 4 hours at between +2°C and +8°C.
- If you work in an area prone to power outages, keep a back-up generator for your freezer as well as a temperature data log system to ensure that the **Co-Dx Logix Smart™ Monkeypox RUO** remains frozen at a temperature between -40°C and -16°C.

- Protect the MM from light.
- Do not use expired products because the integrity of the components cannot be guaranteed.
- The product is not a biological waste. The Safety Data Sheet (SDS) can be retrieved from Co-Diagnostics website at the following link: [Safety Data Sheets | Co-Diagnostics, Inc. \(co-dx.com\)](#). Disposal should be in accordance with applicable regional, national, and local laws and regulations.

## 6 MATERIALS REQUIRED (NOT INCLUDED)

The following materials and devices are required but are not provided with this RUO:

- Appropriate 4-channel real-time PCR instrument, compatible with the fluorophores used in this test.
- Appropriate nucleic acid extraction system or kit
- Vortex mixer
- Centrifuge with a rotor for 2 mL reaction tubes
- Adjustable pipettes
- Disposable pipette tips with filters
- Disposable powder-free gloves
- Ice
- Biosafety cabinet, ideally BSL-2 facility



### WARNING!

Install, calibrate, and maintain all instruments properly according to the manufacturer's instructions and recommendations. Do **not** use instruments with outdated calibration.

## 7 BACKGROUND INFORMATION

Monkeypox is a viral zoonosis similar to smallpox, which is also part of the *Orthopoxvirus* genus. Monkeypox primarily occurs in central and west Africa, often in proximity to tropical rain forests, but also near urban areas. The monkeypox virus is an enveloped double-stranded DNA virus with a variety of mammalian hosts, including rope squirrels, Gambian pouched rats, dormice, non-human primates, among others. (Reference <https://www.who.int/news-room/fact-sheets/detail/monkeypox>)

Monkeypox virus is transmitted by close contact with lesions, body fluids, respiratory droplets, and contaminated materials such as bedding. The incubation period of monkeypox is usually from 6-13 days but can range from 5-21 days. The infection can be

divided into two periods: (Reference <https://www.who.int/news-room/fact-sheets/detail/monkeypox>).

The invasion period (lasts between 0-5 days) characterized by fever, intense headache, lymphadenopathy (swelling of the lymph nodes), back pain, myalgia (muscle aches) and intense asthenia (lack of energy). Lymphadenopathy is a distinctive feature of monkeypox compared to other diseases that may initially appear similar (chickenpox, measles, smallpox) (Reference <https://www.who.int/news-room/fact-sheets/detail/monkeypox>).

The skin eruption usually begins within 1-3 days of appearance of fever. The rash tends to be more concentrated on the face and extremities rather than on the trunk. It affects the face (in 95% of cases), and palms of the hands and soles of the feet (in 75% of cases). Also affected are oral mucous membranes (in 70% of cases), genitalia (30%), and conjunctivae (20%), as well as the cornea. The rash evolves sequentially from macules (lesions with a flat base) to papules (slightly raised firm lesions), vesicles (lesions filled with clear fluid), pustules (lesions filled with yellowish fluid), and crusts which dry up and fall off. The number of lesions varies from a few to several thousand. In severe cases, lesions can coalesce until large sections of skin slough off. (Reference <https://www.who.int/news-room/fact-sheets/detail/monkeypox>).

In May of 2022, cases of Monkeypox were reported to WHO from 12 member states across three WHO regions, where monkeypox virus is not endemic. These areas include the United States, Canada, Australia, United Kingdom, Spain, and Portugal. (Reference <https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385>) -This reference is different than the above links.

## 8 ACCESSORIES (NOT INCLUDED)

### 8.1 Thermocycler

Thermocyclers validated but not included with the test are displayed in Table 2.

**Table 2**

*Thermocyclers Validated but Not Included with the Test*

Thermocycler Machine	Catalog Number	Manufacturer
CoDx Box	MIC-4	Co-Diagnostics, Inc.

### 8.2 Extraction System

The extraction system required but not included with the test are displayed in Table 3. For serum samples, the Protocol: DNA Purification from Blood or Body Fluids (Spin Protocol) from the QIAamp DNA Mini Kit handbook should be used. For lesion exudate swab samples, use the Protocol: DNA Purification from Buccal Swabs (Spin Protocol) from the QIAamp DNA Mini Kit handbook.

**Table 3**
*Extraction and Automation Systems Validated with the Test*

Extraction Reagent		Manufacturer	Sample Input Volume	Sample Elution Volume
Name	Cat. Number			
QIAamp DNA Mini Kit (Qiagen)	51304 (50 extractions) 51306 (250 extractions)	Qiagen	Serum: 200 µL Lesion swab: 400 µL	Serum: 60 µL Lesion swab: 60 µL

## 9 WARNINGS AND PRECAUTIONS



**WARNING!**

Before performing any testing or running any sample, verify that all instruments have been properly installed, calibrated, and are well maintained. Do **not** use instruments with an outdated calibration.

Read this *Instructions for Use* document carefully before using the product. Before first use, check the components for the following:

- Integrity
- Frozenness upon arrival

Users should ensure the following:

- Limit use of this product to personnel instructed and trained in the techniques of real-time PCR.
- Always treat samples as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, lab coat, and eye protection when handling samples and always wear gloves when handling RUO components.
- Always use DNase/RNase-free disposable pipette tips with filters.
- Use segregated working areas for sample preparation, reaction setup, and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional workflow. To prevent contamination, change personal protective equipment (PPE) between areas.
- Store and extract positive materials (specimen, controls, and amplicons) separately from other reagents. Dedicate supplies and equipment to separate working areas and do not move them from one area to another.
- Consult the appropriate SDS for safety. The SDS for the master mix, positive control, and nuclease free water are provided with the shipment. If not provided

with the shipment, the SDS can be retrieved from Co-Diagnostics website at [Safety Data Sheets | Co-Diagnostics, Inc. \(co-dx.com\)](https://www.co-dx.com/SafetyDataSheets)

- Do not open the reaction tubes/plates post amplification.
- Do not autoclave reaction tubes/plates after the PCR, since this will not degrade the amplified nucleic acid and will pose a risk to the laboratory area to contamination.
- Do not use components that have passed expiration date.
- Discard sample and assay waste according to your local safety regulations.

## 10 SAMPLE INFORMATION

### 10.1 Sample Type

The **Co-Dx Logix Smart™ Monkeypox** RUO was validated using serum and swabs of simulated lesion exudate as the sample matrices. The sample requires processing with a nucleic acid extraction kit before being used with the test. See the Extraction System section of this document for information on the extraction kit validated with the test.

### 10.2 Sample Storage

Ensure the following when storing samples:

- Process all specimen types within 48 hours after collection, if storage is needed after 48 hours, store the samples frozen, preferably at -70°C (ECDC, 2020).
- Avoid repeated freezing and thawing of any specimen. If you need to keep a specimen for retesting, aliquot the specimen in different tubes to avoid freezing and thawing cycles.
- Monitor the temperature in the storage areas and recorded temperatures regularly to identify potential fluctuations.
- Do not use domestic refrigerators/freezers with wide temperature fluctuations. Domestic refrigerators/freezers are not suitable for the storage of frozen specimens (CDC, 2020).

### 10.3 Sample Handling

Laboratory workers should wear appropriate PPE, which includes disposable gloves, laboratory coat/gown, and eye protection when handling potentially infectious specimens.

Conduct samples suspected to be or confirmed to be infected with Monkeypox under a certified Class II biosafety cabinet in a BSL-2 containment facility. More details are provided in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) (CDC, 2009) or the WHO Laboratory Biosafety Manual (WHO, 2004).

For specific instructions on the handling of specimens for the Monkeypox virus, see also the CDC's webpage for the Preparation and Collection of Specimens – Monkeypox (CDC, 2022) and *Interim Guidance for Collection Diagnostic Specimens from Persons with Suspect Monkeypox* (CDC, 2003).

The quality of the extraction of the DNA from the samples is essential for the performance of **Co-Dx Logix Smart™ Monkeypox RUO**. Perform extraction by following manufacturer's instructions or an internally validated protocol. The suitability of the nucleic acid extraction procedure for use with **Co-Dx Logix Smart™ Monkeypox RUO** must be validated by the user.

**WARNING!**

If your sample preparation system uses washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

**11 PROCEDURE****11.1 High-Sensitivity Testing**

11.1.1 The **Co-Dx Logix Smart™ Monkeypox RUO** was tested in both 10  $\mu$ L and 20  $\mu$ L total reaction sizes. The 20  $\mu$ L reaction size works to increase the overall sensitivity of the test by increasing the volume of sample in the reaction. If increased sensitivity is desired, the user can increase the volume in the procedure in section 11.2 from 5  $\mu$ L to 10  $\mu$ L for the master mix (MM), PC, sample extract, and nuclease-free water. Additionally, ensure the appropriate reaction volume is selected for the thermocycler.

**11.2 Real Time PCR Setup**

11.2.1 Perform the nucleic acid extraction according to the manufacturer's instructions.

11.2.2 Set up the reagents.

11.2.3 Perform the steps below to set up the reagents.

11.2.3.1 Clean all working surfaces with a fresh 10% bleach solution followed by a molecular-grade alcohol or another equivalent method of cleaning that disinfects and degrades nucleic acids.

- 11.2.3.2 Thaw all reagents and samples on ice, or a cold block, before starting the setup.
- 11.2.3.3 Vortex all **Co-Dx Logix Smart™ Monkeypox** RUO MM, PC, nuclease-free water (used as a no-template control [NC]), and all sample tubes for 3 seconds before using.
- 11.2.3.4 Briefly spin the MM, PC, NC down before using to ensure reagents are properly mixed and to ensure removal of any condensation or residue from the lids.

#### 11.2.4 Set Up the Reaction

Perform the steps below to set up the reaction.

- 11.2.4.1 Collect enough reaction wells for each of the following:

- One for each NC,
- One for each sample you want to test, and
- One (or more) for each PC

**Note:** The example below displays the minimum number of wells needed for 5 samples.

PC	1
NC	1
Samples	5

<b>Total wells required</b>	<b>7</b>
-----------------------------	----------

- 11.2.4.2 Pipet 5 µL of MM into each well collected.
- 11.2.4.3 Pipet 5 µL of the NC into the appropriate wells (in addition to the 5 µL of MM already in the well).

**Note:** Ensure that at least one NC is included in each run and that enough space remains for at least one PC.

**Important:**

- Pipette on ice, if possible.
- Perform PC pipetting and sample setup in a separate area, or at a separate time from the MM and NC.

- Change pipette tips between samples and change pipette tips after pipetting each component.
- Pipet the PC last, if possible, to avoid contamination events.

- 11.2.4.4 Pipet 5 µL of sample or PC into the appropriate well.
- 11.2.4.5 Seal the reaction plate with an optical adhesive film or seal each reaction tube with its appropriate lid.
- 11.2.4.6 Place the plate or tubes into the RT-PCR instrument in the correct orientation and start the run.

### 11.3 PCR Instrument and Thermocycler Setup

- 11.3.1 For programming instructions questions regarding the use of other real-time PCR instruments, contact the Laboratory (801) 438-1036 ext. 03 or at [www.co-dx.com/contact/](http://www.co-dx.com/contact/).
- 11.3.2 If using Co-Diagnostics Inc. CoDx Box, contact the Laboratory (see contact information in Section 7.4.2) for the template file for download. The template file comes pre-programmed with the PCR instrument setup described in this section. When not using a template, or using another device, use the settings outlined below to program the PCR instrument.
- 11.3.2.1 To achieve optimal performance from the test, it is important to make sure that the instrument is compatible with the conditions outlined below.
- 11.3.3 Define the settings as displayed in Table 4.

**Table 4**

*Recommended Instrument Settings*

Item	Setting
Reaction Volume	10 µL (20 µL for high sensitivity protocol)
Ramp Rate	Default
Passive Reference	None

Program PCR instrument with the cycling conditions displayed in Table 5.

**Table 5**
*Recommended Cycling Condition Settings*

	Stage	Cycles	Temperature	Time
Initial Denaturation	Hold	1	95°C	5 minutes
Amplification	Cycling	50	95°C	3 seconds
			55°C	32 seconds

11.3.4 Ensure that the PCR instrument being used is compatible with the fluorophores below. Some devices may not have options for the quencher. If you need help or have questions, contact Co-Diagnostics Inc. Technical Support at (801) 438-1036 ext. 02 or at: [support@co-dx.com](mailto:support@co-dx.com).

11.3.5 Define the fluorescence detectors (dyes) as displayed in Table 6.

**Table 6**
*Fluorescence Detector Definitions*

Target	Detector Name	Reporter	Quencher
Monkeypox F8L	MPX	Quasar® 670	BHQ® - 2
Human RNaseP (IPC)	RNaseP (IPC)	CAL Fluor® Red 610	BHQ® - 2

11.3.6 When the run is finished, ensure that the run file is saved.

**12 DATA ANALYSIS**

For basic information regarding data analysis on specific qPCR instruments, refer to the user manual of the respective instrument.

**12.1 Positive Controls**

Validate the test run by checking to see that the PC has passed and that the control conditions displayed in Table 7 are met.

**12.2 No-Template Controls**

Validate the test run by checking to see that no-template control has passed and that the control conditions displayed in Table 7 are met.

**Table 7**
*Required Control Conditions*

Control Type	Control Name	Purpose of Control	MPX Result	RNaseP (IPC) Result
Monkeypox Positive Control	MPX (Quasar®670)	Verifies the performance of the master mix	+	+
	RNaseP (IPC) (CF®610)			
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-

12.2.1 If controls pass, interpret the sample results.

12.2.2 Invalid Test Run

12.2.2.1 If any of the controls fail, the run is invalid.

12.2.2.2 Document the run and initiate troubleshooting.

### 12.3 Interpretation of Results

Once the controls have passed, the unknown samples can be interpreted based on one of the following three possible outcomes:

- Positive
- Negative
- Invalid

A Positive result will show an amplification curve or cycle threshold value for Monkeypox. The cut-off value should be determined by in house validation testing. However, internal studies have shown rare primer-dimer formation or other non-specific amplification at 45 cycles. This fact can be attributed to the nature of the CoPrimers™ (Satterfield, 2014), (Poritz & Ririe, 2014). The amplification of the RNaseP (IPC) shows that the extraction was successful.

A Negative result shows no amplification for Monkeypox; The absence of a curve for monkeypox indicates a negative result ONLY when the RNaseP (IPC) marker is positive.

An Inconclusive result occurs if any of the controls fail. See the Troubleshooting section. See Table 8 for results translation.

**Table 8**
*Results Translation*

	Sample Result		Co-Dx Logix Smart™ Monkeypox RUO Positive Control	No Template Control (NC) (Master Mix + Water)	Interpretation of Results
	Monkeypox Virus Q670 Channel	Internal Positive Control (RNaseP) CF610 channel			
Instrument Reading	+	+	+	-	<b>*Monkeypox Virus DNA +</b>
	-	+	+	-	<b>Monkeypox Virus DNA -</b>
	<b>Any Result (+/-)</b>	-	+	-	<b>INVALID: See Troubleshooting</b>
		+	-	-	
		+	+	+	

Anything before 45 cycles is considered a positive analyte reading (+). Anything at or after 45 cycles is considered a negative analyte reading (-).

\*A positive result indicates a sample contains either monkeypox or other orthopoxvirus DNA.

### 13 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and we would like to be informed of any issues with the **Co-Dx Logix Smart™ Monkeypox** RUO even if the recommended steps for troubleshooting solve the issue. To give feedback complete the Customer Feedback form by visiting [www.co-dx.com/contact/feedback/](http://www.co-dx.com/contact/feedback/).

#### 13.1 Stability

Real-time and accelerated shelf-life and in-use stability studies are currently under testing. Currently, the expiration date of this product has been established as 12 months. Do not use expired reagents, because doing so may lead to inaccurate results.

Always use the most recent version of this document for updates as more stability information will be added when studies are completed.

#### 13.2 User Errors

Good Laboratory Practices for Molecular Biology Diagnostics (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel.

To help prevent errors such as splashes, crossover contamination, and volume selection, it is essential that users have some molecular biology experience and be familiar with proper pipetting technique. Pipette tips must be replaced after every pipetting. Gloves must be replaced often. Equipment, such as pipettes and real-time PCR instruments, should be calibrated when applicable.

A 90-minute online training for Good Laboratory Practices for Molecular Genetics Testing (Centers for Disease Control and Prevention, 2017) is available at the CDC website at the following link <https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html>.

### 13.3 Invalid Results

13.3.1 Co-Dx Logix Smart™ Monkeypox RUO PC not Amplifying

13.3.2 No amplification from the PC could be the result of one or multiple factors, such as one or more of the following:

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent)
- Incorrect placement of plates or tubes into the real-time PCR instrument
- **Co-Dx Logix Smart™ Monkeypox RUO MM or PC degradation** (result of reagents being stored at a temperature above -16°C for an extended period)
- Use of expired reagents
- Wrong reagents being used

When this occurs, it is best to disregard the results from the samples and re-test by re-amplification. If the PC fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction (or not, depending on the investigation results and risks identified in the process). If failure of the PC occurs a third time after re-extraction and re-amplification, open a new **Co-Dx Logix Smart™ Monkeypox RUO PC or MM**, and retest. If the test is still failing, call Technical Support at (801) 438-1036 ext. 02 or visit [support@co-dx.com](mailto:support@co-dx.com).

13.3.3 IPC RNaseP is not Amplifying in Samples

13.3.4 No amplification from the RNaseP channel could be the result of one or multiple factors, such as:

- Not enough nuclear material in the sample
- PCR inhibitors such as ethanol and heparin

- Incorrect extraction
- Extraction system used is not compatible or has a step that eliminates RNaseP DNA

**Note:** Positive amplification in the Monkeypox channel indicates a positive analyte result despite the lack of concurrent amplification in the IPC channel. The IPC amplification is dependent on the presence of human genomic DNA (gDNA) in the extraction sample, the amount of which is governed by the type of the sample and the extraction procedure used. Samples obtained from culture or sterile/pure sites (e.g., CSF, urine, or cell lysates) may not contain the human RNaseP gene.

When this occurs, the results should be interpreted as invalid and re-testing by re-amplification should be performed. If the IPC fails again, then samples should be re-extracted and re-amplified. If it fails a third time an investigation should be conducted to identify possible causes for the error. If the cause for the error is clear, the test can either be singled out as invalid due to either PCR inhibitors being present or not enough nuclear material being present. If the cause for an error is unclear, contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02 or contact us at [support@co-dx.com](mailto:support@co-dx.com).

### 13.3.5 No Template Control Showing Amplification

- 13.3.5.1 Amplification of monkeypox in a No Template Control indicates contamination in one or more of the reagents, incorrect placement of plate or tube into the real-time PCR instrument, or pipetting errors.

When this occurs, none of the results can be trusted and re-testing by re-amplification should be performed. If the NC fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in the process. If failure of the NC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02 or by visiting [support@co-dx.com](mailto:support@co-dx.com).

## 14 REFERENCES

- CDC. (2009). *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*. Retrieved from CDC Laboratories: <https://www.cdc.gov/labs/BMBL.html>
- CDC. (2017, Oct 27). *CDC Laboratory Training: Good Laboratory Practices for Molecular Genetics Testing*. Retrieved Mar 5, 2019, from Centers for Disease Control and Prevention: <https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html>
- Poritz, M., & Ririe, K. (2014, Mar). *Getting things backwards to prevent primer dimers*. *Journal of Molecular Diagnosis*, 159-62. doi:10.1016/j.jmoldx.2014.01.001
- Satterfield, B. (2014, Mar). *Cooperative primers: 2.5 million-fold improvement in the reduction of nonspecific amplification*. *Journal of Molecular Diagnosis*, 163-73. doi:10.1016/j.jmoldx.2013.10.004
- Viana, R. V., & Wallis, C. L. (2011). *Good Clinical Laboratory Practices (GLCP) for Molecular Based Tests Used in Diagnostic Laboratories*. In D. I. Akyar, *Wide Spectra of Quality Control* (pp. 29-52). InTech. Retrieved from <http://www.intechopen.com/books/wide-spectra-of-quality-control/goodclinical-laboratory-practice-gclp-for-molecular-based-tests-used-in-diagnostic-laboratories>
- CDC. (2003, Jun 23). *Interim Guidance for Collection of Diagnostic Specimens from Persons with Suspect Monkeypox*. Retrieved June 10, 2022, from Centers for Disease Control and Prevention: <https://www.aphl.org/programs/preparedness/Smallpox/pdf/diagspecimens.pdf#:~:text=The%20Centers%20for%20Disease%20Control%20and%20Prevention%20%28CDC%29,collection%20of%20human%20clinical%20specimens%20for%20monkeypox%20testing.>
- WHO. (2004). *Laboratory Biosafety Manual*. Retrieved from Emergencies preparedness, response: [https://www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_11/en/](https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/)
- WHO. (2022, May 21). *Multi-country monkeypox outbreak in non-endemic countries*. Retrieved from WHO website: <https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385>
- WHO. (2022, May 19) *Monkeypox*. Retrieved from WHO website: <https://www.who.int/news-room/fact-sheets/detail/monkeypox>
- CDC. (2022, Jun 9). *Preparation and Collection of Specimens-Monkeypox*: <https://www.cdc.gov/proxvirus/monkeypox/clinicians/prep-collection-specimens.html>

## 15 LEGEND OF PACKAGE SYMBOLS

See Table 9 for the legend of package symbols.

**Table 9**

*Legend of Package Symbols*

Icon	Definition
	Catalog number
	Batch Code
	Cap color
	Component
	Content/Volume
	Number
	Use-by-date
	Contains sufficient for x tests/reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
	Non-Sterile product – Do not sterilize
	Manufacturer
	Research Use Only