



Overview of Monkeypox virus tests

The Partnership for Supply Chain Management

What is Monkeypox?

Monkeypox is a viral zoonotic disease caused by infection with the Monkeypox virus. It is transmitted to humans through close contact with an infected person or animal, or with material contaminated with the virus. It is also transmitted from one person to another by close contact with lesions, body fluids, respiratory droplets and contaminated materials such as bedding. The infection usually presents as rash and may often be associated with other symptoms such as fever, headache, muscle and backaches and swollen lymph nodes.

What tests are available for Monkeypox virus?

Available diagnostic tests for Monkeypox virus (MPXV) include polymerase chain reaction (PCR), rapid antigen and antibody, enzyme-linked immunosorbent assay (ELISA) and other serologic tests. PCR is the preferred laboratory diagnostic test for MPXV given its accuracy and sensitivity. Serology and antigen detection methods are of limited use because viruses to which Monkeypoxvirus is closely related to, Orthopoxvirus (OPXV), are serologically cross reactive, meaning they can give false positive reactions for Monkeypox virus, so do not provide Monkeypox virus specific confirmation.

What is a PCR test?

The term PCR stands for polymerase chain reaction. PCR or nucleic acid amplification tests (NAATs), as they are sometimes referred to, are used to detect genetic material from a specific organism, such as a virus. PCR tests are effective given their accuracy and sensitivity (i.e. the ability to detect very small concentrations of the genetic material that may be present in clinical specimens).

What is a Monkeypox PCR test?

The PCR test for Monkeypox virus analyses dry swab specimens collected from rashes or skin lesions (roof or fluid from vesicles and pustules, and dry crusts), looking for genetic material (Deoxyribonucleic acid or DNA) of the Monkeypox virus.

Scientists use the PCR technology to amplify (replicate) small amounts of viral DNA, if present in the sample, until it is detectable. In other words, PCR tests create copies of specific strands of DNA to enable the test to detect even the smallest possible amounts of virus present in a specimen.

WHAT ARE THE KEY STEPS IN MONKEYPOX VIRUS PCR TESTING?



Sample collection/ Transport

A healthcare provider uses a dry swab to collect material and fluid from rashes or skin lesions. After collection, the swab is placed in a tube containing viral transport media (VTM). Alternatively, the swab can be stored in a dry, sterile tube with no VTM.

Specimens need to be refrigerated or frozen within an hour of collection and submitted to the laboratory as soon as possible after collection



Extraction

In the laboratory, the scientist receives the specimen and uses nucleic acid extraction kits to isolate or extract the Monkeypox virus' genetic material from the specimen.

The extraction process can be done using either a manual or an automated process. The automated extraction process requires the use of an analyzer.



PCR

Special chemicals and enzymes and a PCR machine called a thermal cycler or thermocycler are used to make millions of copies of a small portion of the Monkeypox virus' genetic material (amplification) in the test tube. One of the chemicals in the tube produces a fluorescent light, if Monkeypox virus is present in the specimen. Once amplified enough, the PCR machine can detect this signal. Scientists use special software to interpret the signal as a positive test result.

What are the types of Monkeypox virus PCR test kits?

Broadly, there are two types of Monkeypox virus PCR test kits, namely automated and manual test kits.



AUTOMATED TEST KITS

These are kits packaged with reagents for both nucleic acid extraction and PCR (amplification). These reagents are placed in a PCR machine together with specimens to be tested. The testing process occurs on the machine and results are made available by the machine at the end of the process.



MANUAL TEST KITS

These are kits packaged with reagents for PCR amplification. Separate reagent kits for extraction are needed to be used together with the manual test kits to complete the process of PCR testing. Even in manual testing, the amplification step in PCR testing is still performed using a thermocycler.

Variations in testing protocols for Monkeypox virus PCR tests?

While tests are available for detection of Monkeypox virus, some testing protocols for involve two steps, in which the first PCR reaction detects Orthopox virus, but does not identify which species. This step is then followed by a second step, which can be PCR-based or utilize sequencing, to specifically detect Monkeypox virus. It is recommended that, before an assay is utilized to test clinical specimens, each laboratory should validate the test and/or verify the testing protocol.

What are the main considerations when requesting Monkeypox virus PCR test kits?

1

Select PCR test kits that are available in the Global Fund's Wambo ordering system.

2

Check the compatibility of the PCR test kit selected with the nucleic acid extraction kit/equipment and PCR analyzer.

3

When requesting for manual PCR test kits, ensure that you have enough extraction kits to match the number of PCR tests requested.

4

Check that you have all the other consumables that will be required for testing to be performed.



References

<https://www.cdc.gov/poxvirus/monkeypox/index.html>

<https://www.who.int/publications/i/item/WHO-MPX-laboratory-2022.1>

<https://www.finddx.org/mpx-test-directory/>