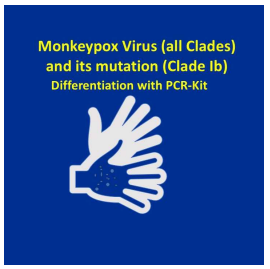
Oct 07, 2024

A protocol for the differentiation of the common monkeypox virus (all clades) and its mutation (1b clade) by real-time PCR



DOI
dx.doi.org/10.17504/protocols.io.5qpvokz4zl4o/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5qpvokz4zl4o/v1

Protocol Citation: Sudhir Bhatia, Gudrun Baersch 2024. A protocol for the differentiation of the common monkeypox virus (all clades) and its mutation (1b clade) by real-time PCR. **protocols.io https://dx.doi.org/10.17504/protocols.io.5qpvokz4zl4o/v1**

Protocol status: Working
We use this protocol and it's working

Created: October 01, 2024

Last Modified: October 07, 2024

Protocol Integer ID: 108740

Keywords: Monkeypox virus, Mpox, Realtime PCR, differentiation method, mutation clade Ib, smallpox virus, Mycobacterium leprae

Disclaimer

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Abstract

The monkeypox virus was first isolated in Copenhagen in 1958. It causes similar lesions to smallpox and should be distinguished from other pathogens such as *Mycobacterium leprae*, Varicella zoster virus and cowpox, as these viruses can also cause similar types of lesions. Smallpox was eradicated by vaccination in the early 1980s, so the new generation is not vaccinated; therefore, there is a risk that there is no protection against smallpox and related viruses. In Africa, this virus breaks out due to close contact between people. Such a viral infection can be contained by better hygienic conditions and the use of disinfectants, but in many African countries the hygienic conditions are so poor and poverty is probably another reason why the monkeypox virus cannot be controlled. The virus is spreading in other countries, such as the USA, where more than 30,000 positive cases have been reported with many deaths. This virus is also mutating and to control the spread of this virus, people need to be tested as the 1b clone is spreading in many countries. To control the spread of the virus, it is therefore necessary to differentiate the strains. Therefore, we present a protocol to distinguish between the monkeypox virus (all clades) and the 1b clade.

This test can be used on different isolated samples from different lesions like skin, plasma, serum, oral swabs, genital lesion samples.

Image Attribution

Genekam MPX PCR

Materials

FR550 - **Monkeypox Virus to differentiate between all clades and clade Ib -Double Check Realtime PCR-Manufacturer: Genekam Biotechnology AG, Germany**
optional:
FR118 - Human specific DNA - Internal Control (Realtime PCR-Kit) CE
-UDI-DI: 04262420430225

Composition:
• Tube A (1 Tube)
• Tube C (1 Tube)
• Tube B (1 Tube)
• Tube D1 (Positive Control) (1 Tube)
• Tube D2 (Negative Control) (1 Tube)

Please check them before you start. **Store them at -20°C in the dark.**

Equipment needed:
• Real time machine
• Laboratory centrifuge
• 96 well microplates for PCR or microtubes (0.2ml)
• Pipette-tips with and without filter (1-10µl, 10µl-100µl)
• Pipettes (quality pipettes)
• Vortexer
• DNA Isolation kit: **Genekam Biotechnology AG, Germany or equivalent**

Safety warnings

- The Kit is only for in vitro use.
- The Kit must be used through trained persons.
- The Kit should not be used after expiry date.
- During PCR, one should work very cleanly.
- All works must be done in different parts of laboratory.
- NEVER STORE THIS KIT WITH FOOD, VACCINE OR BACTERAL CULTURES.
- Decontaminate the instruments regularly (once a week).
- To dispose of the Kit and its contents, add 70% ethanol to the contents of the kit and then dispose of it.
- Thaw the tubes slowly. Never thaw in heating block or with hand heat.

Ethics statement

User may need ethics committee approval for conducting experiments with human samples, please check this.

Before start

-Consult the manual before start.
-Check the equipments needed before use.
-The use of internal control is highly recommended. This should be used as extra real time assay.
-First Probe: namely Carboxy-fluorescein (reporter, FAM) and 6-Carboxy tetramethyl rhodamine (quencher, TAM). The results will be shown as Ct -Values. Up to 40 Ct -Values should be taken positive. Ct -Values between 40-45 should be taken as marginal positive (doubtful). It indicates the presence of **all clades**
(Tube A).

2. Second probe: namely Carboxy-fluorescein (reporter; FAM) and 6-Carboxy tetramethyl rhodamine (quencher, TAM). The results will be shown as Ct -Values. Up to 40 Ct -Values should be taken positive. Ct -Values between 40-45 should be taken as marginal positive (doubtful). It indicates the **absence of clade Ib and the presence of all clades (Tube C).**

Tube A	Tube C	Sample results
+	**-**	**Positive for clade Ib**
+	**+**	**Positive for all clades**
-	**-**	**Negative**

1 Thaw one tube each: A, B, C, D1 and D2. If the kit is not in use, store them at -20°C. Keep tubes away from sunlight.

2 Mark your microtubes with a sample number, positive and negative Control.
All samples must be carried out for tube A and in parallel for tube C.

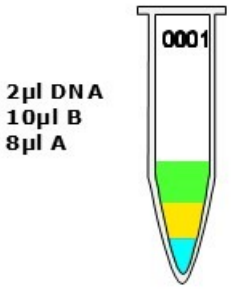
3 Thaw tube A. Add 8µl of Tube A to each tube. Otherwise use a 96 microwell plates.

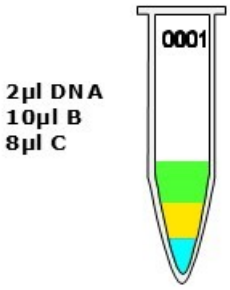
4 Thaw tube C. Add 8µl of Tube C to each tube. Otherwise use a 96 microwell plates.

5 Add 10µl of B to each microtube. Avoid touching the wall of the microtubes.

TIP: User can calculate the total requirement of chemicals needed. User can mix 8µl of A (C) + 10µl of B together in one tube for one reaction, but to have 10 reactions, there will be total volume of 180µl (80µl A (C) + 100µl B). From this, 18µl can be distributed into each tube. This step saves time and hardware.

6 Add 2µl of your DNA template (DNA isolated from samples) with a pipette tip with filter into each microtube according to your labelling, except positive- and negative Control (Do not touch the wall). Use a new pipette tip for each sample.





Important: All samples must be carried out for tube A and in parallel for tube C.

7 Use new pipette tip with filter. Add 2µl of Tube D1 to the positive Control. (Do not touch the wall).

8 Use a new pipette tip. Add 2µl of Tube D2 to the negative Control. (Do not touch the wall).

9 Check if everything is added correctly, as the volume of each microtube must be almost the same.

10 Now enter reporter (FAM) and quencher dye (TAM) to set up your software and run the following program:

15 seconds at 95°C
60 seconds at 60°C -- x 45 cycles

11 Before the start of the PCR program, check whether the plate or tubes are properly sealed. The wells of the plate or microtubes must be in contact with metal block (important!). There should be no air or lose contact with metal block of thermocycler. In case of 96-well plate, it should be sealed with adhesive cover. Now run your PCR.

12 After step 11 is finished take out the microtubes.

13 **STEP B**
Click the **Result**-Tab and the **Amplification**-Tab: Place the Threshold line above the background, then select: “Analyse”. Calculate the threshold cycle (Ct) for each well. There should be no signal in the negative control. Successful positive control and positive samples must give a curve in the software graphics.

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Tube A	Tube C	Sample results
+	-	**Positive for clade Ib**
+	+	**Positive for all clades**
-	-	**Negative**

Interpretation of results: A positive result in Tube A means that **all clades are present** in the sample. A positive result in Tube C indicates **the presence of all clades and the absence of**

clade Ib. This means that if a clade Ib mutation is present, the result in Tube A is positive and in Tube C negative. Negative samples must be negative in both tests.

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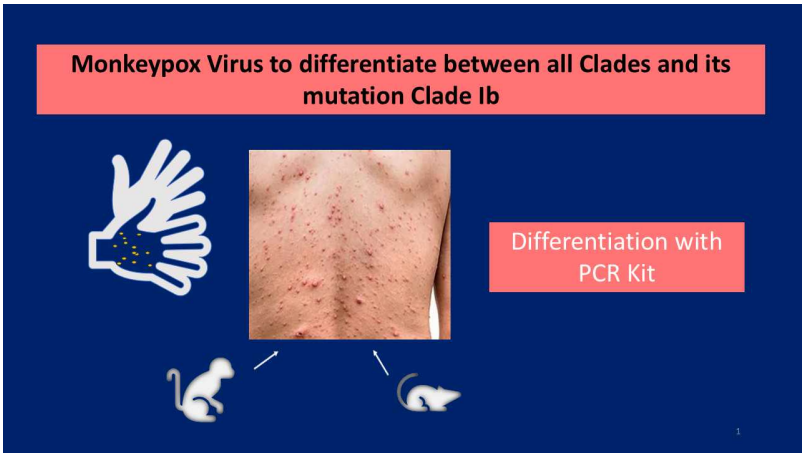

Protocol references

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