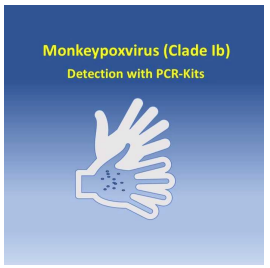
Aug 19, 2024

A simple and rapid protocol for real-time PCR detection of monkeypox virus (all clades including Ib)



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We use this protocol and it's working

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Disclaimer

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Abstract

Monkeypox virus is re-emerging. It is causing outbreaks again in many countries, especially in African countries such as Congo, and poses a threat to other countries as immunity from the smallpox vaccine is not present in the entire world population. This is why the World Health Organization has declared a state of emergency because its clade Ib is spreading worldwide. It is essential to test various samples, especially skin lesions, for this virus and perform differential diagnosis, e.g. cowpox virus, varicella zoster virus and *Mycobacterium leprae*, which cause similar symptoms. We show the protocol for monitoring this virus for research studies with our real-time PCR that detects all clades including recent clade Ib. The user can analyze its old pathological samples too.

Developing a highly specific test for monkey pox virus is a challenge as most of tests are likely to cross react with very closely related pathogens like cow pox virus, varicella zoster virus, variola etc, so they can deliver false positive results. Even many tests available in the literature cannot properly detect this virus, as they target the region affected by mutations. Such tests give false negative results. The user must take care of this point.

Image Attribution

Genekam Mpox PCR

Materials

FR547 - Monkeypox Virus (Realtime PCR-Kit)
-UDI-DI: 04262420430348
optional:
FR118 - Human specific DNA - Internal Control (Realtime PCR-Kit) CE
-UDI-DI: 04262420430225

Composition:
• Tube A (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.
- Real time PCR is based on fluorogenic dyes. There are 2 dyes, namely Carboxy-fluorescein (reporter, FAM) and 6-Carboxy tetramethyl rhodamine (quencher, TAMRA). The results will be shown as Ct -Values. Up to 40 Ct should be taken positive. Value between 41-45 Ct should be taken as marginal positive (doubtful).

1 Thaw **one tube** each: A, B, D1 and D2. If the kit is not in use, store them at -20°C. Keep tubes away from sunlight. 5m

2 Mark your microtubes with a sample number, positive and negative Control. 2m

3 Thaw tube A. Add 8µl of Tube A to each tube. Otherwise use a 96 microwell plates. 2m

4 Add 10µl of B to each microtube. Avoid touching the wall of the microtubes. 2m

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

5 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.** 3m

6 Use new pipette tip with filter. Add 2µl of Tube D1 to the positive Control. (Do not touch the wall). 1m

7 Use a new pipette tip. Add 2µl of Tube D2 to the negative Control. (Do not touch the wall). 1m

8 Check if everything is added correctly, as the volume of each microtube must be almost the same. 1m

9 **Now enter reporter (FAM) and quencher dye (TAM) to set up your software and run the following program:** 1h 30m

15 seconds at 95°C
60 seconds at 58°C -- x 45 cycles

10 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. 1m

11 After step 10 is finished take out the microtubes. 1m

12 **STEP B** 4m

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.

13 It is recommended to perform the presence of the monkey virus itself as well as a differential diagnosis as shown in the figure below.

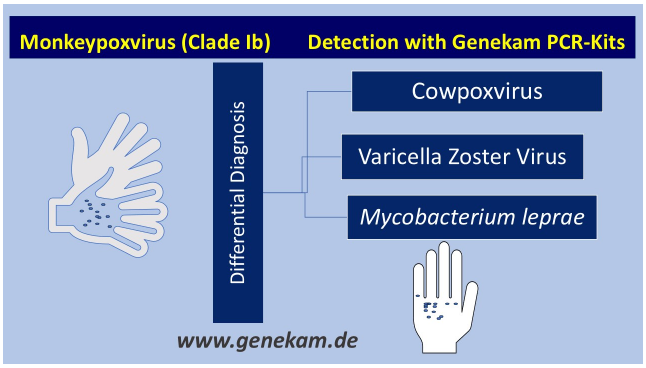

Figure 1: Detection of monkeypox virus and other viruses, e.g. cowpox virus, varicella zoster virus and *Mycobacterium lepre*, that cause similar symptoms, for differential diagnosis purposes.

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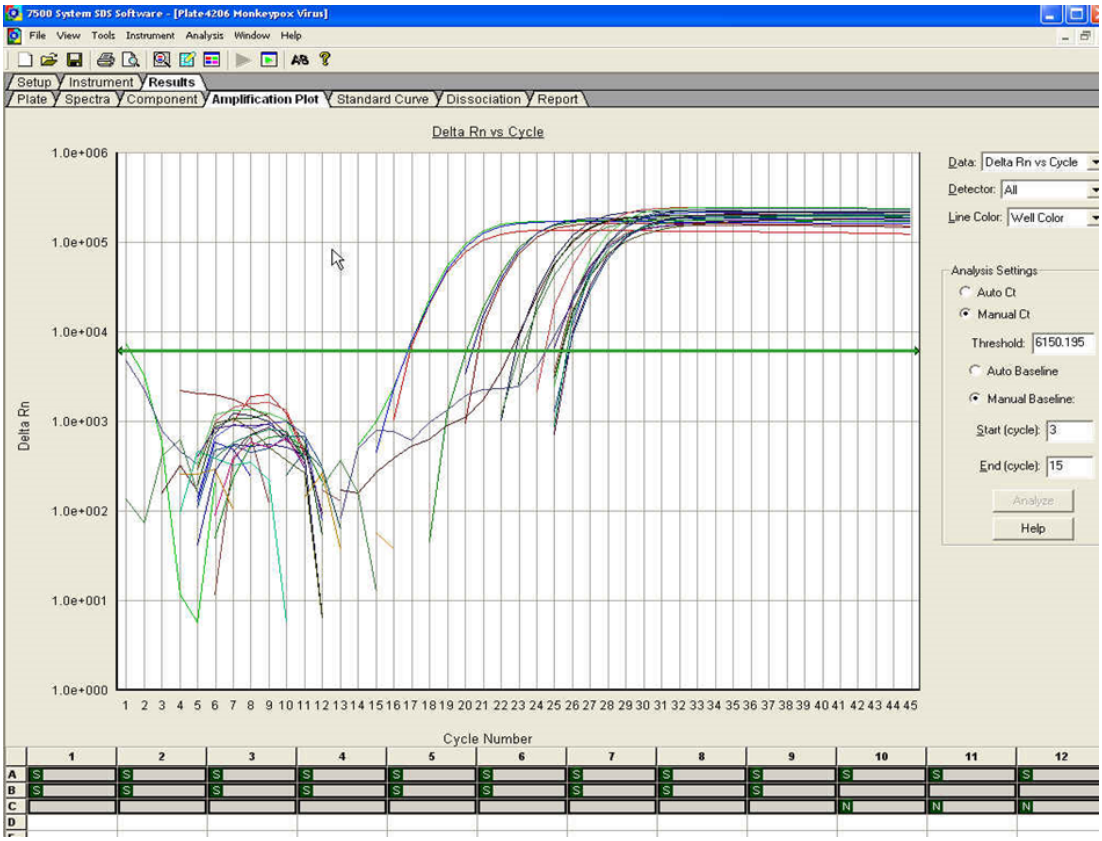

Figure 2: Results of monkey pox virus on ABI7500 real time machine.

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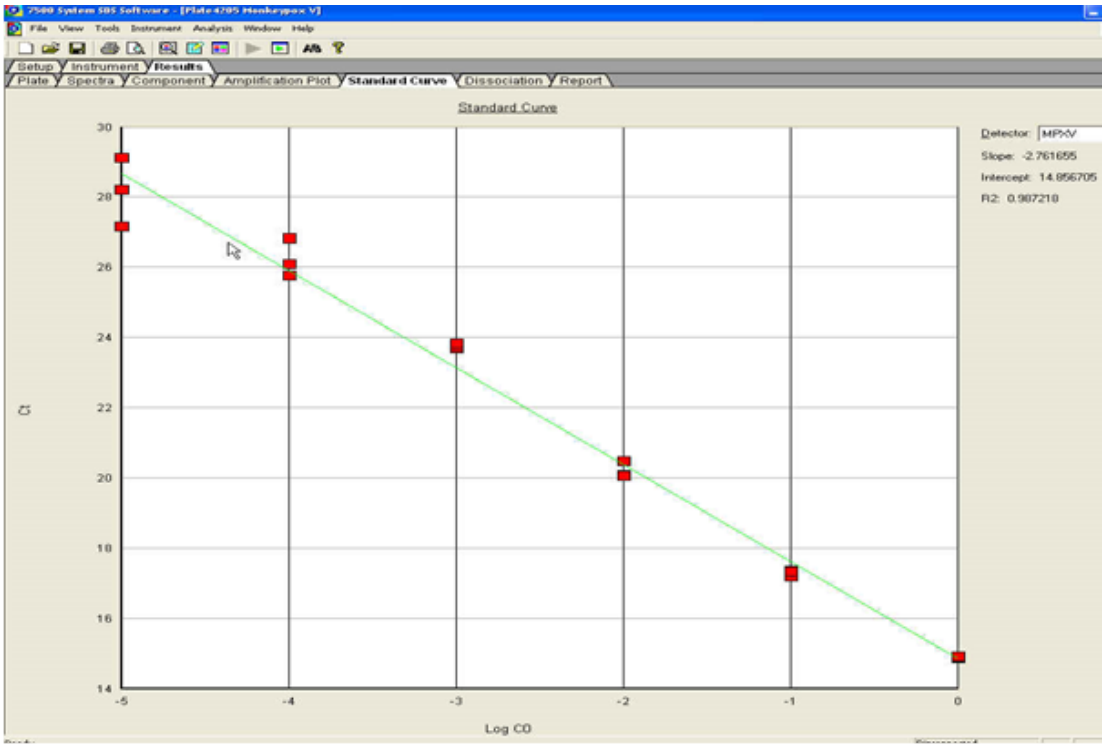

Figure 3: Standard curve of monkeypox virus on ABI7500 real time machine.

16 Sensitivity: 0,0004pg/µl

Protocol references

-**Bhatia**, S, Baersch, G. Detection of SARS CoV-2 in different samples with a singleplex and mutation resistant real time PCR assay. May 31, 2024, DOI **dx.doi.org/10.17504/protocols.io.5qpvokkkzl4o/v1**

-**Bhatia**, S, Baersch, G. Detection of avian Influenza Virus H5N1 with a Real time PCR kit. May 28, 2024, DOI **dx.doi.org/10.17504/protocols.io.5qpvok38bl4o/v1**

-Bhatia S, Baersch G. Isolation of DNA from spots of old microscopic glass slides with mini column isolation kit for molecular analysis. Microbes Infect Dis 2024; 5(1): 4-10. DOI: **10.21608/mid.2023.239256.1625**