microzone

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MicroLYSIS-RNA Direct to RT-qPCR

For samples suspected of COVID-19.

Samples are potentially infectious! All steps must be carried out using appropriate safety measures

This document outlines our current protocols. These are potentially subject to slight changes as further testing is carried out. We do not expect any radical change at this point.

Kit Content

1 x Bottle of MicroLYSIS-RNA

Protocols:

Sputum:

1. In an appropriate tube with cap, add 200µl of sputum and add 200µl MicroLYSIS-RNA. Close the tube.
2. Vortex the mixture
3. Allow to incubate at Room Temperature for 10 minutes.
4. Spin at 10,000 rpm for 5 minutes.
5. The supernatant is now ready for testing.

In a final reaction volume of 20µl, add 2µl of lysis supernatant to RT-qPCR reaction / test and make up to 20µl with RT-qPCR mastermix and water as per kit instructions.

Swabs in Media:

1. In an appropriate tube with cap, add 200µl of swab media and add 200µl MicroLYSIS-RNA. Close the tube.
2. Vortex the mixture.
3. Allow to incubate at Room Temperature for 10 minutes.
4. Spin at 10,000 rpm for 5 minutes.
5. The supernatant is now ready for testing.

In a final reaction volume of 20µl, add 2µl of lysis supernatant to RT-qPCR reaction / test and make up to 20µl with RT-qPCR mastermix and water as per kit instructions.

Dry Swabs:

1. In an appropriate tube with cap, add swab tip and 400µl of PBS to tube (less volume of PBS can be added depending on the swab construction).
2. Close tube and vortex briefly. Incubate for five minutes
3. Take a volume of PBS in to a new tube and add equal volume of MicroLYSIS-RNA. Close the tube.

4. Vortex the mixture
5. Incubate at Room Temperature for 10 minutes.
6. Spin at 10,000 rpm for 5 minutes.
7. The supernatant is now ready for testing.

In a final reaction volume of 20µl, add 2µl of lysis supernatant to RT-qPCR reaction / test and make up to 20µl with RT-qPCR mastermix and water as per kit instructions.

Potential Inhibition:

Due to make up of MicroLYSIS-RNA, we have seen limited inhibition of amplification with dry swabs and sputum collected in simple collection tubes. Where inhibition was expected or seen (such as sputum collection vessels) then we have two protocols that worked:

1. Using 1µl in the reaction instead of 2µl overcame inhibition.
2. A simple dilution of the lysis supernatant and addition of 2µl restored amplification.

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