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Testing MicroLYSIS-RNA with Saliva

Introduction:
MicroLYSIS RNA is a lysis buffer that can work with the following three sample types and take samples through a rapid lysis method and then directly into RT-qPCR.

1. Dry Swabs
2. Viral Transport Medium
3. Sputum / saliva

Diagnosis of Sars-Cov-2 (COVID-19) antigen is hampered by numerous pinch points. These include availability of extraction reagents, plasticware, high-end liquid handling systems, and finances depending on where in the world testing is taking place. There is a need to overcome some if not all of these pinch points. This paper demonstrates the viability of going straight to RT-qPCR post lysis using MicroLYSIS-RNA from Microzone that retains a high level of sensitivity while being simple, and considerably cheaper than many extraction methods.

Using the MicroLYSIS RNA it is possible to go straight from a simple lysis procedure directly to RT-qPCR.

Method:
The protocol is short and simple. Take 100µl of saliva add 100µl of MicroLYSIS-RNA, vortex briefly. Incubate at room temperature for 5 minutes. Heat the sample to 95^{o}C for 10 minutes (this can be in a regular thermocycler if using PCR strips or a heating block). Cool. Add the resulting lysate to the RT-qPCR reaction. We used the Co-Diagnostics Logix Smart COVID-19 RT-qPCR kit. The kit uses a total reaction volume of 10µl. We added 5µl of the lysis supernatant to 5µl of the RT-qPCR reagent. We then followed the Co-Diagnostics' instructions for amplification: Reverse Transcriptase 15 minutes at 45^{o}C followed by one incubation at 95^{o}C for 2 minutes, followed by 50 cycles of 95^{o}C for 3 seconds and 55^{o}C for 32 seconds. We used the MIC thermocycler from BMS that was labelled as Co-Dx Box form Co-Diagnostics. The MIC is a small, rapid, 48 position qPCR thermocycler.

Samples: We used the Vircel Amplirun Total Sars-Cov-2 Control that was rehydrated with 50% saliva and 50% water. We diluted the saliva to be comparable with a commercial collection device. Data from the collection device not shown here.

Sensitivity Testing:

We rehydrated the control from Vircel to create a concentration of approximately 21.8 copies/µl. We then diluted this in molecular grade water to 1:2, 1:4 and 1:8 to test for inhibition. Figure 1 below, shows strong amplification of the neat and diluted samples. We can see the neat sample came up first followed by the 1:2. The 1:4 and 1:8 amplified at the same time and rate. The amplification of the dilutions demonstrated better efficiency. There is, however, no doubt about the amplification of any of the samples.

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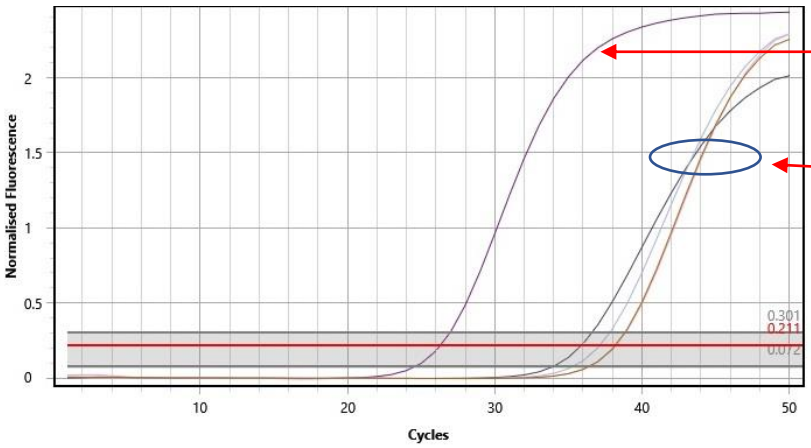

Positive control

Samples: 21.8 copies/µl then dilutions of 1:2, 1:4 and 1:8 in molecular biology grade water.

Figure 1

Validation Trial:

Very limited validation on COVID positive samples at this time. We have demonstrated the product works on a COVID postive sample using saliva collected in a commercial collection vessel. This required a 1:4 dilution to amplify effectively due to, what we believe to be, inhibitors in the collection fluid.

Discussion:

Our testing demonstrated the capacity for MicroLYSIS RNA to release viral antigen from saliva in a ready to use format. We were able to go straight from lysis to RT-qPCR. Diluting the lysate resulted in higher RT-qPCR efficiency. This suggests that something in the lyophilised sample inhibits RT-qPCR. We are confident in the product's ability to release SARS-CoV-2 antigen from saliva samples. Samples collected without the use of commercial devices amplify without further dilution. The fluids in collection devices appear to inhibit RT-qPCR when using MicroLYSIS RNA. We intend to invetigate ways to work with commercial collection devices.

All testing was carried out using the Co-Diagnostics Logix Smart COVID-19 RT-qPCR kit. This kit uses CoPrimer technology and virtually eliminates the production of primer dimers. The kit runs at 50 cycles without development of background in samples.

The Co-Diagnostics kit has been validated and granted FDA-EUA appoval in the USA for use with Saliva samples.

MciroLYSIS RNA combined with RT-qPCR using the Co-Diagnostic kits demonstrated a quick and sensitive method for detecting SARS-CoV-2 in saliva samples in this trial. We have not tried to replicate our findings with other kits at this tme.

Conclusion:

MicroLYSIS RNA potentially offers a rapid, cost effective alternative to RNA extraction.

Reagents Used:

- **Microzone, Stourbridge, UK** – MicroLYSIS RNA. A strong lysis buffer that leaves the sample ready for direct use in RT-qPCR. Supplied by Clent Life Science Ltd.
- **Co-Diagnsotics, Salt Lake City, USA:** Logix Smart COVID-19 RT-qPCR Detection Kit (CE-IVD and FDA-EUA approved).
- **Vircel, Spain:** Amplirun Total Sars-Cov-2 Control