Poster 7

SARS-CoV-2 stability testing using the Sigma MM™ deactivating media

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Introduction

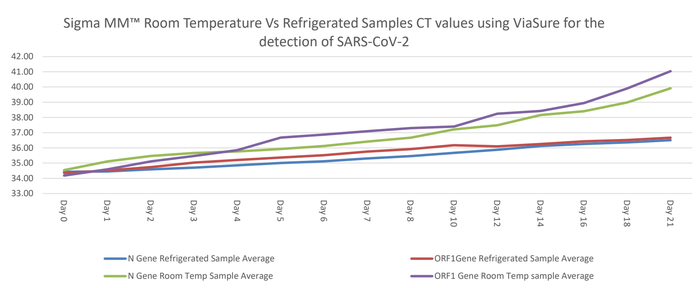
With the emergence of SARS-CoV-2 Sigma MM™ deactivating and stabilising media became a key part in laboratory testing. To ensure safe and viable testing on multiple platforms such as Roche® Liat®, Cepheid® GeneXpert® and the Mic-4® using Viasure® testing kits. Sigma MM™ tubes were introduced when point of care Covid testing was implemented, a requirement for Covid-19 deactivated samples became essential to protect staff and when further testing was required, such as; sequencing or typing. These specialist tests were essential for variant of concern studies. These tests were required to be sent away which often caused a delay in processing and therefore a deactivating and stable media such as Sigma MM™ played a key role in this process.

Method

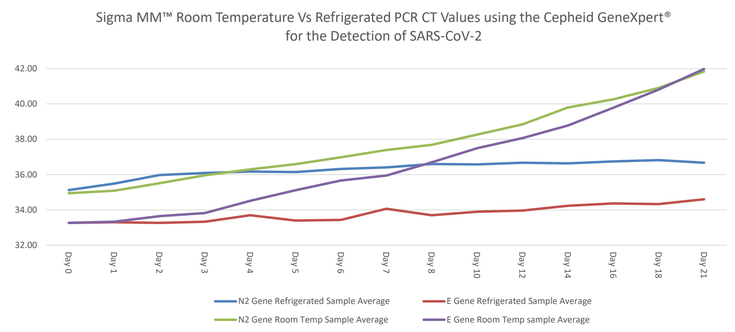
1ml of Virocult® and Sigma MM™ medium were spiked with 100µl AccuPlex™ SARS-CoV-2 reference material kit whole genome control (Ref: 0505-0126), into stock solutions. The control contained 5000 copies/ml when this control is diluted down, a CT value is determined near the threshold. If the genetic material (RNA) degraded over time the result would become negative. Controls are always run at the threshold. These were produced in triplicate and tested by three different methods to detect qualitative and quantitative SARS-CoV-2 RNA by PCR analysis.

Results

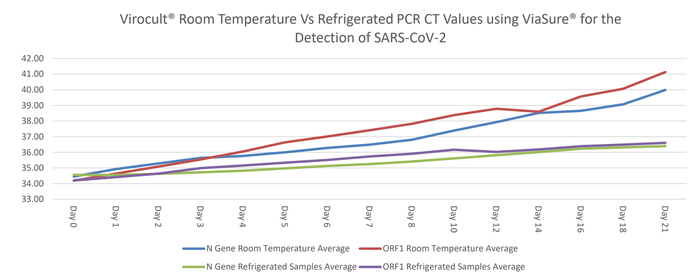
Graph 1 Showing the results of PCR testing from Sigma MM™ samples at both room temperature and refrigerated samples using the ViaSure SARS-CoV-2 assay identifying both the N and ORF1 gene regions.



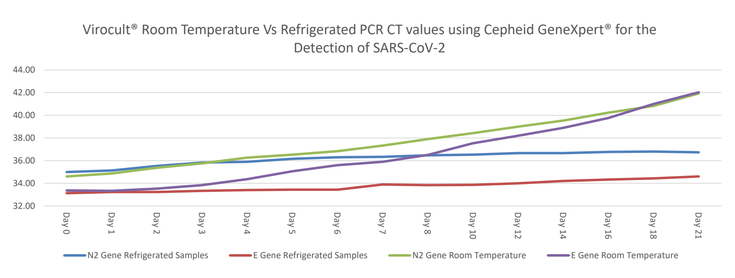
Graph 2 Showing the results of PCR testing from Sigma MM™ samples at both room temperature and refrigerated samples using the Cepheid GeneXpert® for the detection of SARS-CoV-2 assay identifying both the N2 and E gene regions.



Graph 3 Showing the results of PCR testing from Virocult® samples at both room temperature and refrigerated samples using the ViaSure® SARS-CoV-2 assay identifying both the N and ORF1 gene regions.



Graph 4 Showing the results of PCR testing from Virocult® samples at both room temperature and refrigerated samples using the Cepheid GeneXpert® for the detection of SARS-CoV-2 assay identifying both the N2 and E gene regions.



Results

Sigma MM™ was compared directly at room temperature using both the ViaSure® PCR assay and the Cepheid GeneXpert® assay and the graphs of the average CT values show a clear difference in the start and end point of the PCR results for the two variables of room temperature and refrigerated sample protocols. Graph 1 shows the start point of the N Gene and ORF gene to be 34.59 and 35.11 at room temperature and 34.52 and 34.50 at the refrigerated temperatures. After 21 days the CT value for the room temperature N Gene and ORF were 41.04 and 39.91 in comparison the refrigerated end point remained lower at 36.67 and 36.40. This indicates a notable difference in the two temperature holding conditions and suggests that with Sigma MM™ media, SARS-CoV-2 RNA maintains stability better at refrigerated temperatures when compared to a room temperature holding condition. This was also supported by Graph 2 which shows PCR results from the Cepheid GeneXpert®, the CT values remain stable at refrigerated temperatures. The end points for the two temperatures showed a drift with the N2 and E genes producing CT values after 21 days of 41.84 and 41.98 respectively. When the CT values were plotted, the 21-day values remained lower in comparison than the room temperature samples at 36.67 and 34.60.

Virocult® media performed similarly to Sigma MM™ with some additional drift in CT values. PCR CT values observed using the ViaSure® assay signalled at 34.45 and 34.19 for the N Gene and ORF gene and ended at 36.40 and 36.61 at refrigerated temperature. The room temperature signalled at 34.55 and 34.20 and the end point was 39.99 and 41.13, this drift was still notable however some drift was also observed at refrigerated temperatures. The Cepheid results showed a similar drift pattern which suggests the RNA is still viable with the Virocult® medium which is therefore a suitable preservative media.

Conclusion

In conclusion; the results show that the ability of both Virocult® and Sigma MM™ transport medium to preserve the viral RNA from SARS-CoV-2 enables it to remain detectable by PCR for at least 21 days. In fact the specimens refrigerated at 4^{O}C demonstrated very stable CT values, and SARS-CoV-2 is likely to have remained detectable for even longer.

Summary

Both Sigma MM™ and Virocult ® medium are able to maintain SARS-CoV-2 RNA integrity and stability to a provide positive PCR result for at least 21 days at both room temperature and 4^{O}C . While Sigma MM™ offers the additional safety of virus inactivation, both products are suitable for the transport and storage of specimens for SARS-CoV-2 PCR analysis.