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PRESERVATION MEDIUM FOR CULTURE OR MOLECULAR TESTING

INDEX

SARS-COV-2 Stability Testing using the ∑-MM™ Deactivating Media

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SYNOPSIS

Sigma Virocult® and Sigma MM™ were assessed for stability of RNA across several platforms (Roche Liat®, GeneXpert® and the Mic-4® using the ViaSure® kits) and at both refrigerated and ambient temperatures. Both Sigma MM™ and Sigma Virocult® medium 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°C .

INTRODUCTION

With the emergence of SARS-CoV-2 Σ-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. Σ-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 Σ-MM™ played a key role in this process.

METHOD

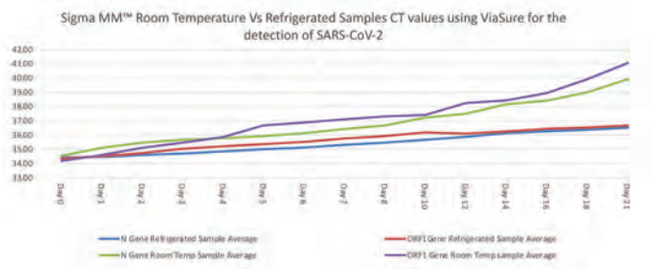
1ml of Virocult® and Σ-MM™ medium were spiked with 100µl AccuPlex™ SARS-CoV-2 reference material kit whole genome control (Ref: 0505-0126), into stock solutions. The control contained 5000 copies/ml when this control is diluted down, a CT value is determined near the threshold. If the genetic material (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

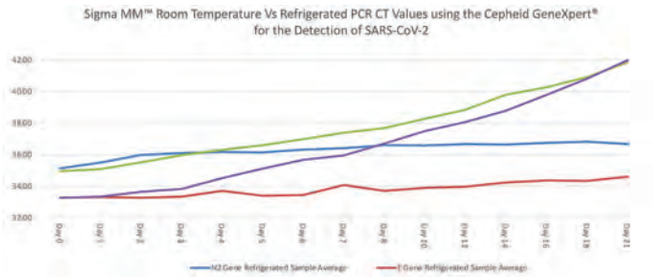
Σ-MM™ was compared directly at room temperature using both the ViaSure® PCR assay and the Cepheid GeneXpert® assay and the graphs of the average CT values show a clear difference in the start and end point of the PCR results for the two variables of room temperature and refrigerated sample protocols. 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 Σ-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 Σ-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.

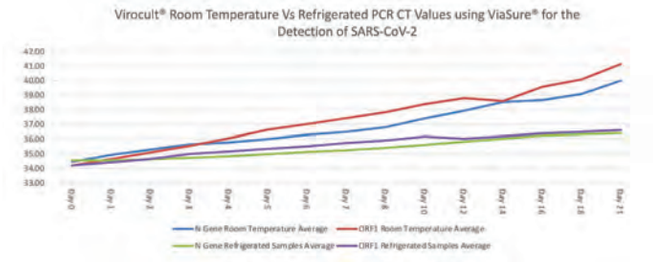
Graph 1 Showing the results of PCR testing from Σ-MM™ samples at both room temperature and refrigerated samples using the ViaSure SARS-CoV-2 assay identifying both the N and ORF1 gene regions.



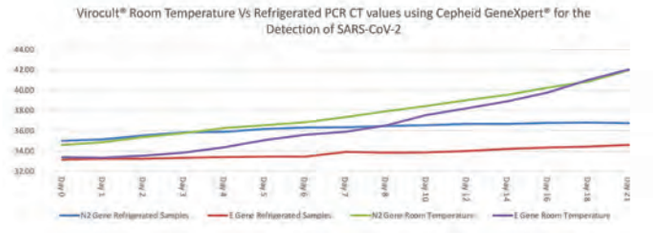
Graph 2 Showing the results of PCR testing from Σ-MM™ samples at both room temperature and refrigerated samples using the Cepheid GeneXpert® for the detection of SARS-CoV-2 assay identifying both the N2 and E gene regions



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.



CONCLUSION

In conclusion; the results show that the ability of both Virocult® and Σ-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°C demonstrated very stable CT values, and SARS-CoV-2 is likely to have remained detectable for even longer.

SUMMARY

Both Σ-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°C. While Σ-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.

APPENDIX 1

Σ-MM						
Analyser: Mic-4 from ViaSure Temperature: Refrigerated						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N Gene	Orf Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	34.58	34.21	34.49	34.22	34.21	34.62
Day 1	34.48	34.32	34.51	34.41	34.38	34.82
Day 2	34.69	34.61	34.58	34.61	34.49	34.97
Day 3	34.71	34.92	34.69	34.96	34.69	35.21
Day 4	34.79	34.99	34.79	35.20	34.99	35.41
Day 5	34.86	35.13	35.02	35.32	35.13	35.64
Day 6	34.94	35.25	35.14	35.41	35.27	35.89
Day 7	35.18	35.32	35.37	35.89	35.37	36.05
Day 8	35.34	35.47	35.42	36.06	35.61	36.23
Day 10	35.52	35.63	35.67	36.32	35.83	36.57
Day 12	35.76	35.79	35.89	35.79	35.98	36.72
Day 14	35.92	35.87	36.22	36.15	36.22	36.72
Day 16	36.03	36.21	36.34	36.31	36.41	36.76
Day 18	36.13	36.31	36.48	36.39	36.48	36.84
Day 21	36.19	36.42	36.60	36.59	36.74	36.99

Σ-MM						
Analyser: Mic-4 from ViaSure Temperature: Room Temperature						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N Gene	Orf Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	34.54	34.18	34.53	34.17	34.54	34.19
Day 1	34.79	34.54	35.55	34.48	34.99	34.75
Day 2	35.31	34.99	35.64	34.99	35.45	35.35
Day 3	35.66	35.39	35.67	35.24	35.65	35.78
Day 4	35.78	35.63	35.78	35.72	35.73	36.21
Day 5	35.98	36.89	35.90	36.55	35.89	36.59
Day 6	36.12	37.21	36.14	36.71	36.11	36.70
Day 7	36.41	37.56	36.39	36.86	36.43	36.85
Day 8	36.71	37.89	36.56	36.99	36.74	37.02
Day 10	36.92	37.97	37.78	37.11	36.93	37.12
Day 12	37.39	39.21	37.96	38.24	37.12	37.28
Day 14	38.18	38.35	38.12	38.48	38.19	38.43
Day 16	38.41	38.43	38.38	39.83	38.43	38.58
Day 18	39.67	39.65	38.60	40.41	38.69	39.65
Day 21	39.94	40.69	39.87	41.64	39.93	40.80

Σ-MM						
Analyser: GeneXpert from Cepheid Temperature: Room Temperature						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N2 Gene	E Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	35.4	33.2	34.3	33.4	35.1	33.2
Day 1	34.4	33.2	35.2	33.5	35.5	33.2
Day 2	34.9	33.6	35.6	33.6	35.8	33.6
Day 3	35.5	33.8	35.9	33.6	36.3	33.9
Day 4	35.8	34.3	36.5	34.6	36.5	34.5
Day 5	36.2	34.6	36.8	34.9	36.6	35.7
Day 6	36.4	34.9	37.4	35.3	36.9	36.7
Day 7	36.8	35.3	37.8	35.6	37.4	36.8
Day 8	36.9	35.9	38.2	36.2	37.8	37.8
Day 10	37.7	36.4	38.5	37.2	38.4	38.8
Day 12	38.3	36.8	39.3	37.9	38.8	39.3
Day 14	39.6	37.2	40.3	38.8	39.3	40.2
Day 16	39.9	38.5	40.8	39.3	39.9	41.4
Day 18	40.6	39.9	41.3	40.1	40.7	42.3
Day 21	41.4	40.8	42.2	42.0	41.8	42.9

Σ-MM						
Analyser: GeneXpert from Cepheid Temperature: Refrigerated						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N2 Gene	E Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	35.2	33.3	35.4	33.2	34.6	33.3
Day 1	35.7	33.3	35.8	33.3	34.8	33.3
Day 2	36.3	33.3	36.5	33.2	35.1	33.3
Day 3	36.5	33.3	36.6	33.4	35.1	33.3
Day 4	36.6	33.4	36.7	33.5	35.2	34.2
Day 5	36.6	33.4	36.6	33.4	35.2	33.4
Day 6	36.7	33.4	36.8	33.5	35.4	33.4
Day 7	36.7	33.7	36.7	33.8	35.8	34.7
Day 8	36.7	33.7	37.1	33.7	35.9	33.7
Day 10	36.8	33.9	36.9	33.9	36.0	33.9
Day 12	36.8	33.9	36.9	34.1	36.3	33.9
Day 14	36.8	34.2	36.8	34.2	36.3	34.3
Day 16	36.9	34.4	36.9	34.3	36.4	34.4
Day 18	36.9	34.2	36.9	34.4	36.6	34.4
Day 21	36.9	34.9	36.4	34.8	36.7	34.1

Σ-MM			
Analyser: Liat from Roche Temperature: Refrigerated			
Time	Replicate 1	Replicate 2	Replicate 3
0	Detected	Detected	Detected
Day 2	Detected	Detected	Detected
Day 6	Detected	Detected	Detected
Day 10	Detected	Detected	Detected
Day 14	Detected	Detected	Detected
Day 21	Detected	Detected	Detected

Σ-MM			
Analyser: Liat from Roche Temperature: Room Temperature			
Time	Replicate 1	Replicate 2	Replicate 3
0	Detected	Detected	Detected
Day 2	Detected	Detected	Detected
Day 6	Detected	Detected	Detected
Day 10	Detected	Detected	Detected
Day 14	Detected	Detected	Detected
Day 21	Detected	Detected	Detected

APPENDIX 1 CONTINUED

VIROCULT			
Analyser: Liat from Roche Temperature: Refrigerated			
Time	Replicate 1	Replicate 2	Replicate 3
0	Detected	Detected	Detected
Day 2	Detected	Detected	Detected
Day 6	Detected	Detected	Detected
Day 10	Detected	Detected	Detected
Day 14	Detected	Detected	Detected
Day 21	Detected	Detected	Detected

VIROCULT			
Analyser: Liat from Roche Temperature: Refrigerated			
Time	Replicate 1	Replicate 2	Replicate 3
0	Detected	Detected	Detected
Day 2	Detected	Detected	Detected
Day 6	Detected	Detected	Detected
Day 10	Detected	Detected	Detected
Day 14	Detected	Detected	Detected
Day 21	Detected	Detected	Detected
Day 10	Detected	Detected	Detected
Day 14	Detected	Detected	Detected
Day 21	Detected	Detected	Detected

VIROCULT						
Analyser: MIC-4 ViaSure Temperature: Refrigerated						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N Gene	Orf Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	34.60	34.19	34.48	34.19	34.58	34.22
Day 1	34.61	34.28	34.51	34.29	34.52	34.68
Day 2	34.69	34.60	34.59	34.41	34.58	34.90
Day 3	34.73	34.91	34.67	34.90	34.78	35.16
Day 4	34.80	34.99	34.75	35.18	34.91	35.30
Day 5	34.80	35.05	35.01	35.29	35.11	35.68
Day 6	34.97	35.25	35.14	35.40	35.27	35.89
Day 7	35.10	35.35	35.29	35.84	35.34	36.01
Day 8	35.32	35.47	35.32	36.02	35.59	36.23
Day 10	35.52	35.62	35.60	36.29	35.71	36.59
Day 12	35.69	35.80	35.85	35.59	35.90	36.69
Day 14	35.90	35.85	36.03	35.99	36.12	36.71
Day 16	36.03	36.22	36.29	36.18	36.38	36.76
Day 18	36.13	36.30	36.39	36.29	36.42	36.88
Day 21	36.19	36.40	36.51	36.41	36.50	37.01

VIROCULT						
Analyser: MIC-4 ViaSure Temperature: Room temperature						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N Gene	Orf Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	34.41	34.18	34.50	34.21	34.43	34.19
Day 1	34.81	34.51	35.01	34.64	34.98	34.78
Day 2	35.29	34.97	35.38	35.01	35.19	35.29
Day 3	35.66	35.48	35.67	35.38	35.59	35.78
Day 4	35.76	35.71	35.79	35.78	35.76	36.65
Day 5	35.99	36.68	35.99	36.49	36.01	36.75
Day 6	36.34	37.32	36.28	36.71	36.23	37.01
Day 7	36.45	37.89	36.58	37.01	36.43	37.32
Day 8	36.71	37.99	36.71	37.28	36.99	38.19
Day 10	37.01	38.81	37.91	37.99	37.25	38.34
Day 12	37.42	39.31	38.48	38.37	37.89	38.68
Day 14	38.38	38.48	38.98	38.52	38.21	38.79
Day 16	39.01	39.39	38.47	39.83	38.48	39.50
Day 18	39.49	39.76	38.99	40.43	38.71	39.99
Day 21	39.98	40.69	39.99	41.89	39.99	40.80

VIROCULT						
Analyser: Cepheid GeneXpert Temperature: Refrigerated						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N2 Gene	E Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	35.1	33.1	35.4	33.1	34.5	33.2
Day 1	35.2	33.2	35.6	33.2	34.6	33.3
Day 2	35.7	33.3	35.8	33.2	35.1	33.2
Day 3	36.2	33.3	36.2	33.4	35.1	33.3
Day 4	36.3	33.3	36.3	33.4	35.1	33.5
Day 5	36.6	33.4	36.6	33.5	35.3	33.4
Day 6	36.7	33.4	36.8	33.5	35.4	33.4
Day 7	36.7	33.6	36.7	33.7	35.6	34.4
Day 8	36.8	33.7	36.8	33.9	35.8	33.9
Day 10	36.8	33.8	36.9	33.9	35.9	33.9
Day 12	36.8	33.9	36.9	34.2	36.3	33.9
Day 14	36.9	34.2	36.8	34.2	36.3	34.2
Day 16	36.9	34.4	36.9	34.3	36.5	34.3
Day 18	36.9	34.7	36.9	34.4	36.6	34.2
Day 21	37.1	34.9	36.4	34.8	36.7	34.1

VIROCULT						
Analyser: Cepheid GeneXpert Temperature: Room temperature						
	Replicate 1 CT Value		Replicate 2 CT Value		Replicate 3CT Value	
Time	N2 Gene	E Gene	N2 Gene	E Gene	N2 Gene	E Gene
0	34.6	33.5	34.2	33.4	35.0	33.2
Day 1	34.4	33.2	34.9	33.5	35.3	33.3
Day 2	34.8	33.5	35.5	33.5	35.8	33.6
Day 3	35.3	33.8	35.8	33.8	36.2	33.9
Day 4	35.8	34.5	36.3	34.3	36.7	34.3
Day 5	36.2	34.7	36.8	34.9	36.6	35.6
Day 6	36.4	34.9	37.2	35.2	36.9	36.7
Day 7	36.8	35.2	37.9	35.8	37.3	36.7
Day 8	37.3	35.9	38.5	36.2	37.9	37.4
Day 10	37.7	36.6	39.1	37.1	38.5	38.9
Day 12	38.4	37.1	39.8	37.9	38.8	39.6
Day 14	39.3	37.9	40.1	38.6	39.2	40.2
Day 16	40.1	38.7	40.7	39.2	39.9	41.4
Day 18	40.8	39.9	41.1	40.8	40.6	42.3
Day 21	41.8	41.1	42.2	42.0	41.8	43.0

Inactivation of SARS-COV-2 in MWE's ∑-MM™ Inactivation Medium

Medical Research Council & University of Glasgow's Centre for Virus Research

SYNOPSIS

The inactivation of SARS-CoV-2 achieved in specimens collected using Sigma MM™ was assessed using cell culture to look for viable virus. The virus was inactivated in 1 minute across all 6 of 6 samples tested, showing reductions in titres of greater than 5log10.

INTRODUCTION

The extensive testing and surveillance response to the SARS-CoV-2 pandemic has highlighted the need to render specimens safe for transportation, and for testing in circumstances with limited biological containment facilities. A plethora of unregulated collection and transportation devices has come to market, many claiming to inactivate coronavirus, but with little supporting evidence. It seems to be assumed that all that is required is a claim that the transport medium is "guanidine based" is sufficient, but various studies have demonstrated that claims to inactivation properties are often exaggerated, and that many samples are likely to retain infective virus.

Σ-MM™ has been on the market for a number of years and has consistently been shown to effectively eliminate infectious microorganisms from specimens, including mycobacteria, bacteria and viruses. During the pandemic it was demonstrated to be compatible with most PCR platforms, so was capable of rendering specimens safe, but preserving the viral RNA for accurate diagnostic reporting.

A study was designed in cooperation with the Medical Research Council & University of Glasgow's Centre for Virus Research to measure the inactivation of SARS-CoV-2 achieved in specimens collected using Σ-MM™. According to BS EN 14476 1, there should be a titre reduction of more than 4 log10 for effective virucidal activity, so this has been used as a benchmark in this study, as in a similar study by Public Health England 2.

MATERIALS

Assays were performed in Vero E6 MESO cell line, which is a subclone of Vero E6 cell line based on susceptibility to SARS-CoV-2.

SARS-CoV-2-CVR-Gla-1 strain used in this study was originally isolated from a patient's sputum sample, and it contains D614G mutation in Spike gene (GISAID accession: EPI_ISL_461705).

Inactivation buffer: Σ-MM™, Ref MWMM, Lot.20M16, Exp 2021/12

METHODS

SARS-CoV-2-Gla-1 virus isolate was mixed with the Σ-MM™ Medium at predetermined ratios and times (Table 1). Each condition has been performed in a technical triplicate. 100 µl or 500 µl of SARS-CoV-2 was added to 1.5 ml of Σ-MM™ medium and incubated for 1 min & 5min. Untreated virus sample was used as the control, where the Sigma buffer was replaced with PBS. After inactivation the cytotoxic component of the medium was removed using the PEG precipitation method.

Briefly, PEG 8000 was added to the final concentration of 30% to the inactivated virus solution. After overnight incubation at 4°C the virus was pelleted by centrifugation for 1h at 1500 rpm. Then the pellets were washed twice by addition of PBS and centrifugation for 10 min at 1500 rpm each time. Samples were resuspended in 500 µl DMEM supplemented with 2% FBS. Survival of the virus in the samples was assessed by plaque assay on Vero E6 MESO cell line. Titre reduction was calculated by subtracting the mean logarithmic virus titre for Σ-MM™ buffer-treated and PEG-pelleted sample from the logarithmic virus titre for the PBS-treated input virus, with standard errors of the mean calculated.

	Buffer		Virus Amount	inactivation time
	Buffer	Buffer Volume		
1	Σ-MM™	1.5ml	100 µl	1 min
2	Σ-MM™	1.5ml	100 µl	5 min
3	Σ-MM™	1.5ml	500 µl	1 min
4	Σ-MM™	1.5ml	500 µl	5 min
5	PBS	1.5ml	100 µl	30 min
6	PBS	1.5ml	500 µl	30 min

Table 1. Inactivation conditions used in this study.

RESULTS

Σ-MM™ Molecular Medium was tested for the ability to inactivate SARS- CoV-2-Gla-1 isolate. Two virus-to-buffer ratios (1.5 to 0.5 and 1.5 to 0.1), and two inactivation times (1 min and 5 min) were used. As a control PBS was used to replace the buffer. After treatment, the cytotoxic component was removed using PEG precipitation. Following washing and resuspension of the pellet, all the samples in their entirety were titred by plaque assay on Vero E6 MESO cells starting with a neat dilution. Input virus stock was also titred to assess recovery of virus following PEG precipitation. Titre reduction was calculated by subtracting the mean logarithmic virus titre for Σ-MM™ buffer-treated and purified sample from the logarithmic virus titre for the PBS-treated input virus, with standard errors of the mean calculated.

We observed with the PBS-treated sample that there was a loss of approximately 1 Log10 pfu/ml of virus during the PEG precipitation. Following treatment with Σ-MM™ for all the samples, reduction in titre was over 6 log10 when compared to input virus, or over 5 log10 when compared to recovered virus. (Table 2)

	Buffer		Virus Amount	inactivation time	Virus detectable in titration [A,B]	Titre Reduction [C,D] (log10 [±SE])
	Buffer	Buffer Volume				
1	Σ-MM™	1.5ml	100 µl	1 min	0/3	5.89 [± 0.0]
2	Σ-MM™	1.5ml	100 µl	5 min	0/3	5.89 [± 0.0]
3	Σ-MM™	1.5ml	500 µl	1 min	0/3	5.78 [± 0.0]
4	Σ-MM™	1.5ml	500 µl	5 min	0/3	5.78 [± 0.0]
5	PBS	1.5ml	100 µl	30 min	2/2	1.01 [±0.89][E]
6	PBS	1.5ml	500 µl	30 min	2/2	1.11 [±0.15][E]

Table 2. Virus inactivation results.

A Number of positive samples per number of replicates

B The limit of detection in plaque assay was 1 pfu/ml

C Titre reduction calculated in comparison to PBS-treated input virus (5.89 log10 for 100ul virus samples, and 5.78 log10 for 500ul virus samples)

D Standard Error (SE) = Standard deviation / square root of total number of samples

E Titre reduction calculated in comparison to input virus

CONCLUSION

BS EN 144761,3, requires that there should be a titre reduction of more than 4 log10 for virucidal suspension tests. Σ-MM™ consistently exceeded this requirement for both the time points and concentrations used in the study. In fact, within 1 minute of inoculation there was no detectable virus at all in 6 out of 6 samples tested. Given that the test concentrations were higher than would be the case for clinical specimens4, the study demonstrates that Σ-MM™ can be used as a safe transport system for SARS-CoV-2 specimens, offering rapid inactivation. The results are consistent with other studies using different inactivation reagents and methods2,5. Another standard, ASTM E1052-206 requires that one part of virus suspension is added to nine parts of the test substance before holding at the desired temperature for the required contact time, and then assayed for viable virus in an appropriate host system. In this study the 100ul and 500ul inocula represent dilutions below and above the ASTM requirement, so the results can also be interpreted as meeting this standard, although further specific dilutions should be assayed.

Regardless of these results, it is essential that safe handling procedures continue to be observed for this emerging pathogen until the epidemiology is more fully understood.

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Validation of the ∑-MM™ Medium for the Detection of SARS-COV-2

Helen Jones, Kamran Khan, Paula Rutland
& Mohamed Abushugra

SYNOPSIS

A cohort of 20 patients who had tested positive for SARS-CoV-2 were swabbed using Sigma MM™ and Sigma Virocult® Transport media. These duplicate swabs were then tested on the Roche Liat®, Biofire® film array, GeneXpert® and the Mic- 4® using the ViaSure® kits. The results demonstrated that both Sigma Virocult® and Sigma MM™ swabs were capable of recovering SARS CoV-2 via PCR on multiple platforms without any cross reactivity or invalid results.

Poster 6

NHS Bedfordshire Hospitals NHS Foundation Trust

Validation of the Sigma MM™ Medium for the detection of SARS-CoV-2.

Helen Jones, Kamran Khan, Paula Rutland & Mohamed Abushugra

Introduction

With the emergence of SARS-CoV-2 in 2019, Sigma MM™ came to the forefront of Covid-19 testing because of its key performance characteristics. This media deactivates SARS-CoV-2 in 1 minute from samples taken direct from the patient. This media does this while retaining the ability to do PCR testing from the sample on multiple platforms and the ability to sequence and type using this deactivated primary sample. Sigma MM™ helped facilitate the key implementation of SARS-CoV-2 testing within the A&E department which allowed the Covid-19 PCR results to be available within 25 minutes using the Roche® Liat®. This allowed for safe testing of Covid-19 samples in a point of care setting without the use of a safety cabinet or a category 3 facility. In addition to this, confirmatory testing could be performed within the laboratory on platforms such as the GeneXpert®, the Mic-4® using the ViaSure® kit and the Biofire® film array.

Multi-platform Approach The use of multiple analysers with Sigma MM™ demonstrated its versatile use and provided the laboratory with resilience and a flexible approach to patient management.

Method

A cohort of 20 patients who had tested positive for SARS-CoV-2 were swabbed in duplicate using Sigma MM™ and Virocult® Transport media . These duplicate swabs were then tested on the Roche Liat®, Biofire® film array, GeneXpert® and the Mic-4® using the ViaSure® kits. 10 negative patients were also tested using duplicate swabs and 10 blank duplicate swabs were also tested. Patients who had a primary positive result were re-swabbed on the same day using the Sigma MM™ de-activating medium.

Results

From the 20 patients swabbed, both the Sigma MM™ and the Virocult® swabs tested positive on all the platforms. The 20 negative swabs all produced negative results indicating no cross-reactivity with the media. The results, demonstrated that both Virocult® and Sigma MM™ swabs were capable of recovering SARS-CoV-2 via PCR on multiple platforms without any cross reactivity or invalid results. The results for the SARS-CoV-2 positive cohort patients with Sigma MM™ are shown in Table 1.

Table 1 shows the results from multiple platforms for the detection of SAR-CoV-2 from Sigma MM™.

Patient	Primary Patient result Using Virocult and ViaSure SAR-CoV-2	Cepheid GeneXpert SAR-CoV-2 Sigma MM Result	Viasure SAR-CoV-2 Sigma MM Result	Roche Liat SAR-CoV-2 Sigma MM Result	BioFire SAR-CoV-2 Sigma MM Result
1	Detected	Detected	Detected	Detected	Detected
2	Detected	Detected	Detected	Detected	Detected
3	Detected	Detected	Detected	Detected	Detected
4	Detected	Detected	Detected	Detected	Detected
5	Detected	Detected	Detected	Detected	Detected
6	Detected	Detected	Detected	Detected	Detected
7	Detected	Detected	Detected	Detected	Detected
8	Detected	Detected	Detected	Detected	Detected
9	Detected	Detected	Detected	Detected	Detected
10	Detected	Detected	Detected	Detected	Detected
11	Detected	Detected	Detected	Detected	Detected
12	Detected	Detected	Detected	Detected	Detected
13	Detected	Detected	Detected	Detected	Detected
14	Detected	Detected	Detected	Detected	Detected
15	Detected	Detected	Detected	Detected	Detected
16	Detected	Detected	Detected	Detected	Detected
17	Detected	Detected	Detected	Detected	Detected
18	Detected	Detected	Detected	Detected	Detected
19	Detected	Detected	Detected	Detected	Detected
20	Detected	Detected	Detected	Detected	Detected

POCT Application

The impact on patient pathways as a result of the Implementation of Sigma MM™ was significant allowing point of care testing to help triage patients based on the results of rapid near patient PCR tests within 25 minutes of sampling. This was key when patients admitted from A&E were segregated in to Green, Amber or Red pathways. This infection control process reduced the risk for patients and allowed the efficient and safe flow of patients through A&E. The application of Sigma MM™ also contributed to effective patient management where patient segregation was not possible such as pediatric's, Rapid POCT testing allowed early detection of SAR-CoV-2 and effective management of patients.

Conclusion

The results show both the Sigma MM™ and Virocult® were able to detect SARS-CoV-2 in patient samples. The negative results demonstrated no cross-reactivity with the media and PCR chemistry across all platforms. The ability to use Sigma MM™ in A&E and paediatrics has been a significant aspect of providing safe and effective patient management due to the effective use of the deactivating medium.

Special Thanks to the Microbiology team at Luton & Dunstable Hospital the support and dedication during the pandemic to patients and staff has been exemplary and the hard work is a shining example.

Evaluation of Microbial Viability and Nucleic Acid Integrity after Exposure to New Molecular Transport Medium (∑-MM™)

Ellena Elcocks, Sheikh Qadir & Emmanuel Adukwu

SYNOPSIS

A range of organisms (bacteria and yeasts) were inoculated into Sigma MM™. The inoculum was then aliquoted into TSB broth at 1 minute and 2 minutes to assess for turbidity indicating growth over a 10 day period. A separate aliquot was also removed for molecular assessment. No turbidity was noted at 10 days for any of the organisms tested. This study demonstrated that the Sigma MM™ molecular medium is able to rapidly inactivate bacteria and yeast whilst preserving bacterial DNA.

P1590
ECCMID 2019

Evaluation of Microbial Viability and Nucleic Acid Integrity After Exposure to New Molecular Transport Medium (Sigma MM™)

Ellena Elcocks, Sheikh Qadir & Emmanuel Adukwu
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INTRODUCTION

Sigma MM™ is a new molecular medium described as revolutionary due to its ability to kill bacteria and viruses whilst releasing intact nucleic acids for molecular diagnostics. As sample collection often occurs outside the laboratory and transport of the specimen requires safe and robust handling, ensuring the integrity of the sample is preserved and maintained is of utmost importance.

The aim of this study was to evaluate the new molecular transport medium (Sigma MM TM) for its ability to kill microorganisms whilst preserving and maintaining DNA integrity.

METHODS

RAPID INACTIVATION ASSAY

Organisms used - *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Methicillin resistant Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus pyogenes,Klebsiella pneumoniae, Candida auris, Candida albicans, Haemophilus influenzae.*

↓

Cultures grown on appropriate agar and under appropriate conditions then used to inoculate phosphate buffered saline (PBS) using the 0.5 McFarland standard as reference.

↓

100 µL of culture used to inoculate Sigma MM™. Sample was incubated at room temp for 1 min and 2 min after which a 100 µL aliquot was removed and plated. Aliquot was also removed at 1 minute

and used to inoculate 10 mL of TSB which was incubated at room temperature for 10 days and observed for turbidity indicating growth.

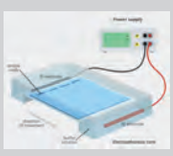
NUCLEIC ACID INTEGRITY ASSAY

Overnight bacterial culture centrifuged at 5000 x *g* for 10 min

DNA analysis using Nandrop One and agarose gel electrophoresis

DNA extraction using QIAGEN DNEasy Blood and Tissue Kit

Pellet resuspended in Sigma MMTM and incubated at room temp for 10 min



This study was supported by MWE https ://www.mwe.co.uk

RESULTS AND DISCUSSION

RAPID INACTIVATION ASSAY

The Sigma MM™ molecular medium was effective at killing all the organisms (Gram positive, Gram negative and yeast) within 2 minutes of exposure. Also no turbidity was observed in the microtitre wells after a 10 day holding period (Table 1).

Table 1. Rapid inactivation of bacteria and fungi (within 1 minute) following incubation with Sigma MM TM molecular medium. Data obtained from 3 independent experiments.

Microorganisms	Untreated control CFU/ml	Treated (1 min) CFU/ml	Treated (2 min) CFU/ml	10 day Holding period
Escherichia coli	1.31E+08	0	0	No turbidity
Enterococcus faecalis	9.71E+07	0	0	No turbidity
Staphylococcus aureus	8.51E+07	0	0	No turbidity
MRSA	1.40E+08	0	0	No turbidity
Pseudomonas aeruginosa	7.98E+07	0	0	No turbidity
Salmonella typhimurium	1.56E+08	0	0	No turbidity
Streptococcus pyogenes	1.69E+08	0	0	No turbidity
Klebsiella pneumoniae	6.54E+07	0	0	No turbidity
Candida auris	2.00E+07	0	0	No turbidity
Candida albicans	1.57E+07	0	0	No turbidity
Haemophilus influenzae	6.94E+07	0	0	No turbidity

DNA extraction after incubation of the test organisms (*E. coli* and *S. aureus*) in SigmaMM™ was successful. The DNA extract was of good quality and high purity demonstrated by A_{260}/A_{280} ratios between 1.8 2.0. Using gel electrophoresis, we were successful at visualising DNA extracted from *E. coli*, though poor yield of MRSA resulted in faint DNA bands (Figure 1).

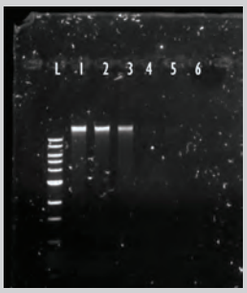


FIGURE 1 - Agarose gel electrophoresis of DNA extracted from bacteria using the QIAGEN DNeasy Blood and Tissue Kit. Lane L, 1 kbp DNA Ladder; lane 1 3, E. coli replicates; lane 4 6, MRSA replicates.

CONCLUSION

This study demonstrated that the Sigma MM™ molecular medium is able to rapidly inactivate bacteria and yeast whilst preserving bacterial DNA. Following an extended exposure time. Gel electrophoresis was suitable in visualising intact DNA of E. coli however low yields observed indicate the importance of PCR to amplify DNA. Further studies are ongoing to assess DNA integrity of other organisms over an extended holding period using PCR based methods.

An Investigation of the Compatibility of a New Molecular Transport Medium (∑-MM™) with a PCR Analytical Platform

Kamran Khan & Jehad Al-Nashash

SYNOPSIS

Sigma Transwabs® and Sigma MM™ were inoculated with control strains of MRSA and a range of organisms containing genes for Carbapenem resistance. This study has shown that both the Sigma MM™ devices and the culture based Sigma Transwab® devices are compatible with the GeneXpert PCR analyser for both MRSA and CRE. There was no interference with the chemistry. The Sigma MM™ devices were also completely effective at killing the MRSA and CRE at all concentrations, rendering the specimens non-infective.

European Meeting on Molecular Diagnostics
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October 09 - 11, 2019
Noordwijk, Netherlands

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NHS Foundation Trust

An investigation of the compatibility of a new molecular transport medium (Sigma MM™) with a PCR analytical platform

Kamran Khan
Luton and Dunstable University Hospital, Luton, United Kingdom

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that causes severe morbidity and mortality worldwide (Cookson *et al.* 2011). In 2017/2018, a mandatory population-based bacteraemia surveillance scheme reported a total of 12,784 MRSA bacteraemia and methicillin-susceptible *S. aureus* (MSSA) cases in England alone (PHE 2018). Alongside this, CRE (Carbapenem-resistant *Enterobacteriaceae*) are an emerging threat to public health producing high levels of resistance to antibiotics. These CRE bacteria contain resistant genes, known as; KPC, OXA-48, NDM, VIM AND IMP-1 which can be detected via PCR. MRSA and CRE specimens represent a significant proportion of routine microbiological specimens and are very important for the management of critically ill patients. In recent years the introduction of self-contained cartridge systems for the rapid identification of target pathogens using nucleic acid amplification has transformed the ability of clinical microbiology laboratories to provide accurate and timely diagnostic data, allowing correct treatment to be commenced immediately, and also preventing unnecessary treatment. When culture is not required, it can be convenient and safer to transport the specimen in a medium which effectively inactivates the pathogen without disrupting the DNA or RNA so that analysis is still possible. If such a system is used, however, it is essential to know that the medium will be compatible with such an analysis.

For this study we investigated the ability of Sigma MM™, a liquid transport medium developed by Medical Wire and Equipment (MWE), to recover analytical quality DNA from specimens spiked with MRSA and CRE specimens, while demonstrating that the biological pathogen had been inactivated and lysed. The PCR analysis was performed using Cepheid GeneXpert ® PCR analyser and Cepheid Xpert MRSA and CARBA-R kits. The results for both PCR and culture were compared with those for identical specimens inoculated into Sigma-Transwab ® (liquid Amies medium), or Sigma MM™ as appropriate.

Methods

MRSA

Samples were spiked with known positive MRSA strain NCTC 12493. A concentration of 0.5 McFarland bacterial was prepared in sterile saline and diluted 10^{-1},10^{-2}, and 10^{-3}. Aliquots of 100µl for each dilution were inoculated directly into the tubes of 6 Sigma Transwabs® and 6 Sigma MM™, giving a total of 18 inoculated devices. A further 2 devices of each type were inoculated with 100µl of sterile saline as negative controls.

CRE

The same method was used for the CRE testing with strains *Klebsiella pneumoniae* NCTC13438 (KPC), *Pseudomonas aeruginosa* NCTC13437 (VIM), *Klebsiella pneumoniae* NCTC13443 (NDM), *E.coli* NCTC13476 (IMP-1) and *Klebsiella pneumoniae* NCTC13442 (OXA-48) 100µl of each bacterial suspension being inoculated into 2 Sigma Transwabs® and 2 Sigma MM™, giving a total of 60 inoculated devices. After a holding time of 24 hours all devices were tested by inoculating into the test kits for GeneXpert, and by plating onto MRSA Chromogenic agar plates, and CRE chromogenic agar as appropriate. Specimens for GeneXpert were processed according to the manufacturer's instructions. All agar plates were incubated at 37^{O}C for 18 hours.

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This study was supported by MWE

mwe medical wire
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Results

Table 1. CRE findings on Cepheid GeneXpert ® PCR using Sigma Transwab ®

Dilution	Samples tested for each organism	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours
		IMP-1		VIM		OXA-48		KPC		NDM	
10-1	2	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth
10-2	2	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth	Detected	Moderate Growth
10-3	2	Detected	Scanty Growth	Detected	Scanty Growth	Detected	Scanty Growth	Detected	Scanty Growth	Detected	Scanty Growth

Table 2. CRE findings on Cepheid GeneXpert ® PCR using Sigma MM™

Dilution	Samples tested for each organism	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours	Target	Incubation culture growth results after 18 hours
		IMP-1		VIM		OXA-48		KPC		NDM	
10-1	2	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth
10-2	2	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth
10-3	2	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth	Detected	No Growth

Table 3. MRSA findings on Cepheid GeneXpert ® PCR using Sigma Transwab®

Dilution	Samples Tested	Target	Incubation culture growth results after 18 hours
		MRSA	
10-1	6	Detected	Moderate Growth
10-2	6	Detected	Moderate Growth
10-3	6	Detected	Scanty Growth

Table 4. MRSA findings on Cepheid GeneXpert ® PCR using Sigma MM™

Dilution	Samples Tested	Target	Incubation culture growth results after 18 hours
		MRSA	
10-1	6	Detected	No growth
10-2	6	Detected	No Growth
10-3	6	Detected	No growth

All negative controls gave negative PCR results and no growth on culture

Discussion and Conclusion

With reports of multi-drug resistant organisms continuing to increase and therapeutic options decreasing, infection control methods are of increasing importance. The launch of Sigma MM™, a commercial specimen inactivation/transport medium enables downstream molecular processing of MRSA sample. This screening strategy uses a testing modality with a rapid turnaround time (less than one hour) whilst inactivating the biological pathogen but preserving its DNA and RNA, therefore increases the capacity of laboratory technicians to execute more demanding tasks.

This study has shown that both the Sigma MM™ devices and the culture based Sigma Transwab® devices are compatible with the GeneXpert PCR analyser for both MRSA and CRE. There was no interference with the chemistry. The Sigma MM™ devices were also completely effective at killing the MRSA and CRE at all concentrations, rendering the specimens non-infective.

Evaluation of Sigma Molecular Medium for use in a Routine Diagnostic Virology Laboratory

Rachel Turnbull

SYNOPSIS

Anonymised clinical samples which had tested positive for viruses were retained for this study. Samples included Nose & Throat (NT) swabs, Nasopharyngeal Aspirate (NPA), Rectal Swab, Mouth swab, Lip swab. The samples were spiked into Sigma MM™ and tested for a range of targets. In all sample types, all types of virus NA was detectable over extended timeframes without any signs of degradation over time.

European Meeting on Molecular Diagnostics
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Evaluation of Sigma Molecular Medium for use in a routine diagnostic Virology Laboratory

Rachel Turnbull, Senior lecturer Healthcare Science, University of Sunderland
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Background:

Accurate detection of pathogenic nucleic acid in clinical samples is crucial for diagnosis of many infectious diseases in patients. A timely and reliable diagnosis is important to enable initiation of appropriate treatment and high quality patient care. (1) This relies on quality clinical samples being collected and transported to the laboratory promptly without any degradation of sample.

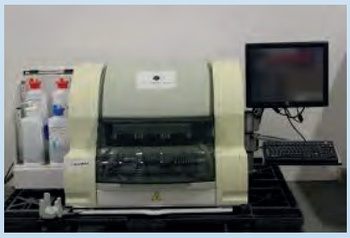
PCR is now the gold standard for testing for many infections and often the first line assay in many routine laboratories, not just Specialist Centres. The repertoire and accessibility of commercially available diagnostic PCR tests has dramatically increased over recent years and with the development of Point of Care Testing, the clinical setting where samples may be handled and processed has evolved. (2)

Sigma MM is a sample collection and transport medium, designed to safely inactivate infectious virus in clinical samples whilst maintaining viability of nucleic acid (NA) to enable detection for diagnostic purposes. This project investigated its suitability for routine clinical use, with a range of sample types harboring different, common viral pathogens. If successful Sigma MM would be a suitable medium for safe collection and transport of clinical samples. This would be particularly beneficial when collecting and testing respiratory samples where there is an increased risk of exposure to the healthcare worker in settings outside of the laboratory, during POCT or an outbreak situation.

Materials & Methods:

Anonymised clinical samples identified as harboring virus were collected and stored at 4°C. Sample types included Nose & Throat (NT) swabs, Nasopharyngeal Aspirate (NPA), Rectal Swab, Mouth swab, Lip swab.

250µl of positive viral material from each sample was spiked to Sigma MM, vortexed and stored at room temperature. Aliquots of medium were extracted using the Biomerieux easyMag® after varying time periods and extracts stored at -20°C prior to testing.



Extracts were tested for a range of targets using the ABI 7500 standard and the Cepheid Smartcycler®.

Samples in Sigma MM were tested directly, without extraction, using the Cepheid GeneXpert® FluRSV assay.

Additionally, HSV spiked Sigma MM was tested without extraction using the Altona RealStar® HSV assay.

Sigma MM was also spiked with multiple viruses (RSV, Enterovirus and Adenovirus), extracted and tested to determine performance in cases of multiple infections.

Aim:

- **To determine suitability of Sigma MM media for routine use for detection of nucleic acid from clinical samples**

Objectives:

- Test multiple sample types, collected in Sigma MM for extracted NA by RT-PCR
- Investigate stability of NA in samples stored over extended timeframes
- To determine stability and detectability of NA in Sigma MM from patients with multiple infections
- To determine if extraction of NA is necessary prior to testing

Results:

- In all sample types, all types of virus NA was detectable over extended timeframes without any signs of degradation over time (Table 1).

Sample	Target	Day1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 10
NT swab	RSV	20.25	19.83	/	20.65	/	20.72	/	20.97
NPA	RSV	24.82	24.94	/	25.02	/	24.49	/	24.69
NT swab	Influenza Virus	28.49	28.13	/	28.50	/	29.78	/	/
Rectal swab	Entero virus	29.3	/	28.0	/	28.3	/	30.3	/
Mouth swab	Entero virus	31.7	/	31.6	/	32.1	/	32.3	/
Lip	HSV	19.93	/	20.06	/	20.11	/	19.83	/

Table 1 CT values of samples tested over extended time frames, up to 10 days stored at room temperature

- Sigma MM was capable of preserving viral NA in samples with multiple virus present, which was detectable after extraction.

- All samples which were unextracted prior to being tested gave unresolved results by RT-PCR.

Conclusions:

In this study nucleic acid from all virus types was detectable by RT-PCR following collection and long term storage at room temperature in Sigma MM. No signs of depletion of NA were evident as CT values remained fairly constant over time. Sigma MM was a suitable collection medium for all sample types tested, with no signs of inhibition evident in extracted samples. The medium performed well using different commercial assays on multiple platforms, however samples required extraction prior to testing. It may be that with unextracted samples, with no washing step polymerase is degraded by residual Sigma MM. In which case the results could be improved by a washing step.

Sigma MM is a suitable transport medium for safe inactivation and preservation of viral NA to enable reliable detection of multiple virus types from clinical samples. Accurate detection of pathogens remains a key step in diagnosis of infection in patients, enabling prompt initiation of appropriate treatment and relevant patient management.

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Acknowledgements:

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- Karl Hubbert, Pathology, South Tees Hospitals NHS Foundation Trust

Evaluation of the New Molecular Transport Medium (∑-MM™) for the Inactivation of Bacterial Pathogens and Release of the Intact DNA

Monika Stuczen & Jamie Laughlin

SYNOPSIS

Study revealed that Sigma MM™ rapidly kills bacteria, including mycobacteria, while releasing, stabilising and preserving nucleic acids. Tested bacterial strains were inactivated within 30 seconds of inoculation. TB was killed after 1 minute while the intact DNA was detected and identified after 42 days of incubation.

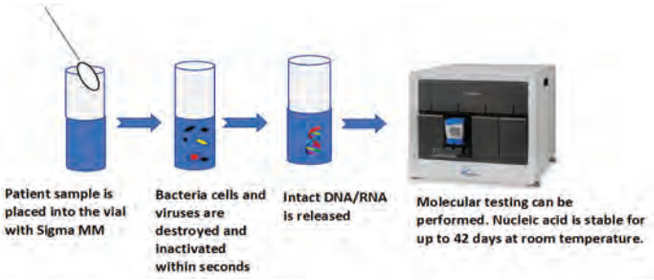
Evaluation of the new Molecular Transport Medium (Sigma MM) for the inactivation of bacterial pathogens and release of the intact DNA

Monika Stuczen [1] & Jamie Laughlin [2]

1. Medical Wire, Corsham, England 2. South West London Pathology, Microbiology, St George's University Hospitals NHS Foundation Trust, London, England

Introduction

Sigma MM is a novel molecular transport medium designed to inactivate/ kill bacteria and viruses, and release intact DNA/RNA for molecular diagnostics. Inactivated samples are safe for transportation, holding and processing. The medium disturbs/lyses lipid membranes, destroys proteins including enzymes and nucleases. Naked DNA/RNA is stable and well preserved within the sample.



Aims

The aim of this study was to evaluate the ability of the medium to inactivate bacteria including mycobacteria within 30 sec, 1 2 and 5 minutes, and release intact DNA. The ability of the medium to preserve intact DNA over 42 days was also assessed and tested using GeneXpert.

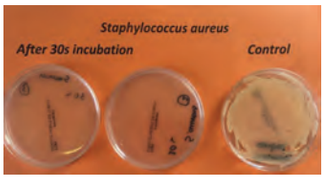
Methods

For *Mycobacterium tuberculosis* (TB) 15 known positive samples were transferred to culture bottles 5 after 1 min, 5 after 2 minutes and 5 after 5 minutes) They were put up on the Bactec MGIT 960 Mycobacterial Detection System and the presence of bacterial growth was observed over 42 days of incubation.

After 42 days, samples were tested for the presence of DNA using GeneXpert® MTB/RIF, Cepheid. For inactivation/killing properties of the Sigma MM 0.5McFarland bacterial concentrations of each strain *(Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Haemophilus influenzae, Streptococcus pneumoniae, Clostridium sporogenes, Enterococcus faecalis, Bacteroides fragilis, Streptococcus pyogenes, Listeria monocytogenes, Salmonella enteritidis, Proteus mirabilis and Bacillus subtilis)* were prepared in sterile saline and inoculated onto the culture media as a positive control.

Molecular media in triplicate were inoculated with 100µl of 0 5 McFarland concentration and vortexed for 5 seconds. After 30 seconds of incubation 100µl of the media was inoculated onto the appropriate culture media. Plates were incubated at 37°C for the time required for each particular strain. After incubation plates were assessed for any growth of bacteria in the culture media.

Molecular transport medium was effective in killing/inactivating all microorganisms tested after 30 seconds of incubation. There was no growth observed All positive control plates had too numerous to count bacterial growth.



Picture 1. Inactivation/killing of *S. aureus* in Sigma MM after 30 sec of incubation

Picture 2. Inactivation/killing of *P. aeruginosa* in Sigma MM after 30 sec of incubation

Results

For TB a single sample of a known TB positive sample after de-activation in Sigma MM suspension came up positive after 15 days on the MGIT liquid culture system. Other than this one sample all other known TB samples were de activated by the new medium. DNA was successfully detected using GeneXpert in all samples after 42 days of incubation and the smallest Ct difference between any pair of probes was less than 2.0.

Sample number	Incubation culture results after 42 days	GeneXpert results after 42 days incubation	Ct Values				
			Probe A	Probe B	Probe C	Probe D	Probe E
1	negative	positive	16.8	18.3	17.2	18.4	18.6
2	negative	negative	0	0	0	0	0
3	negative	positive	15.7	17.4	16.2	17.3	16.9
4	negative	positive	15.5	16.2	16.9	17.2	17.1
5	negative	positive	18.4	19.6	18.9	19.9	19.7
6	negative	positive	15.2	16.1	16.5	17.1	17.0
7	negative	positive	15.4	17.4	16.2	17.3	16.9
8	negative	positive	18.4	19.3	18.7	19.9	19.7
9	negative	positive	15.5	16.2	16.9	17.2	17.1
10	negative	positive	18.6	19.8	18.5	19.9	19.1
11	negative	positive	15.9	17.1	16.1	17.8	16.5
12	negative	positive	14.8	16.9	15.8	17.2	16.3
13	negative	positive	15.1	16.4	16.5	17.0	17.1
14	positive	positive	16.5	16.2	16.3	16.9	16.9
15	negative	positive	18.6	19.9	18.7	19.9	19.5

Conclusions

New molecular transport medium rapidly kills bacteria and stabilizes and preserves released nucleic acids. Tested bacterial strains were inactivated within 30 seconds of inoculation. TB was killed after 1 minute and intact DNA well preserved after 42 days of incubation. Sigma MM makes the sample safe for transportation, shipment and processing at ambient temperature and microbial DNA/RNA detectable using molecular methods. During pandemics and epidemics specific and effective molecular detection is a crucial part of microbial identification and epidemiological surveillance. Transport systems that maintain viability of pathogens may increase infectious disease risk and RNA/DNA degradation. Sigma MM is a new molecular transport medium which is specifically designed to preserve nucleic acids in order to process sample using molecular identification methods.

Evaluation of a New Medium for Molecular Detection of Influenza A & B Viruses in Clinical Specimens

Martine Valette, Gwendolyne Burfin,
Clio Socratous & Bruno Lina

SYNOPSIS

Sigma MM™ renders specimens containing influenza noninfectious and safe for handling and transport, while also allowing high quality extraction of nucleic acid for use in RT-PCR. Sigma MM™ could be used for the molecular diagnostic testing of clinical specimens for Influenza viruses.

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Evaluation of a new Medium for Molecular Detection of Influenza A & B Viruses in Clinical Specimens

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INTRODUCTION and PURPOSE

Influenza epidemics occur every winter in temperate countries but the epidemiology and the severity of the outbreak varies widely according to the circulating viruses. Laboratory diagnosis is an important tool for the control of the outbreak and the adaptation of health care services in hospitals. In France, the influenza surveillance in the general population relies on the “R seau Unique” network. The general practitioners or paediatricians collected clinical specimens from patients presenting with influenza like infection. The sampling consists of nasal-nasopharyngeal swab taken within 36 hours after the onset of the disease. The clinical samples collected during outpatient consultation are sent by post. Upon reception at The National Influenza Centre each clinical sample is tested for the detection of influenza viruses by real time RT-PCR and inoculated on MDCK cells for virus isolation and characterization. PCR is now the first choice laboratory test for influenza infection either for diagnostic or the virological surveillance. The molecular technique is rapid, specific and sensitive while cell culture requires the appropriate cell line and an average of several days for virus isolation, making them of limited use for the clinician. The purpose of this study was planned to assess the performance of a new collection medium for molecular analysis.

MATERIAL and METHOD

Clinical samples from surveillance in the general population were selected for this evaluation. The choice was made on positive specimens for A(H1N1) pdm09 and B-Victoria lineage both detected by RT-PCR on the original material and for which the virus was isolated on cell culture. The protocol also included the testing of MWE’s Sigma Molecular Medium (Sigma MM) for 2 contact times: 10 and 30 minutes. All the tests of this device will be carried out in parallel with the classical medium used in the laboratory (Eagle’s MEM).

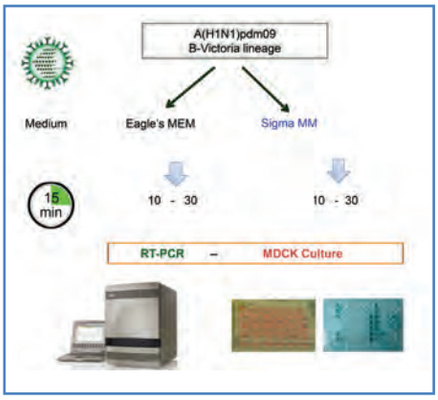
For an aliquot of 200µl of clinical sample the same volume of Sigma MM or Eagle MEM was added and stored at ambient temperature for 10 and 30 min before testing. Each experimental sample was tested according to the protocols for influenza diagnostic and virus isolation used for the clinical sample from the general population surveillance.

The RT-PCR diagnostic was an in-house one step real-time RT-PCR targeting the M gene for Influenza A and NS gene for Influenza B. Identical experimental samples were inoculated on MDCK for the control of infectivity at the various conditions. The viral growth was tested for cytopathic effect and confirmed by ELISA for influenza A & B virus on the supernatant after 2 days incubation at 33°C. All negative samples were seeded on fresh MDCK cells. After the second passage the presence of the virus was monitored by ELISA after 6 days of cell culture. The absence of detection from the second passage is considered as negative cell grown.

RESULTS - INFECTIVITY MDCK cell CULTURE

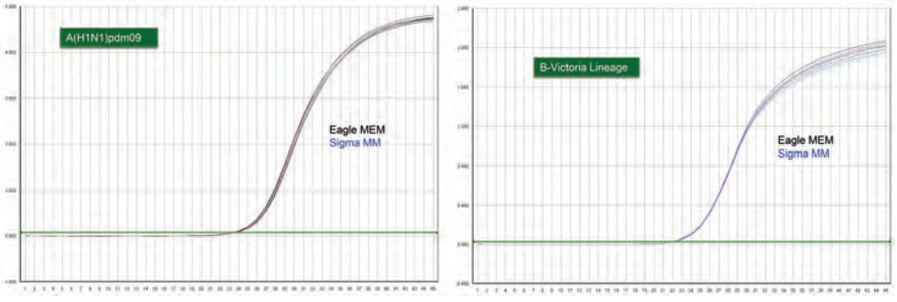
The influenza A(H1N1)pdm09 or B-Victoria lineage were inoculated on 24 well plates in the presence of trypsin. The viruses could be isolated on the first MDCK cells passage with Eagle MEM either by cytopathic effect and ELISA antigen detection in the supernatant. There was no viral growth with Sigma MM even for the short time contact of 10 min.
A second passage on fresh cells did not yield any viral growth for either virus, indicating that all infectivity had been eliminated.

PROTOCOL



RESULTS – RT-PCR

The graph represents the RT-PCR results for A(H1N1)pdm09 and B-Victoria lineage viruses tested in Eagle’s MEM or in Sigma MM under the different experimental conditions. The RT-PCR results for the tested Sigma MM were exactly identical to the currently used medium Eagle’s MEM



DISCUSSION

In France, the influenza surveillance in the general population is established on RT-PCR detection and MDCK cell inoculation on samples collected by the doctor and sent to the laboratory by post. In this study a new medium (Sigma MM) for safe transportation and molecular detection of microorganisms was evaluated and the performance compared with that of clinical specimens tested by current techniques.

The Sigma MM device showed the same performance for influenza A&B molecular diagnostic as eagle MEM. The RT-PCR results were similar in terms of sensitivity (detection level, Ct values) and efficacy (Fluorescence levels). The data were comparable for Influenza A(H1N1)pdm09 and B-Victoria lineage viruses.

The loss of infectivity was observed for both influenza viruses A&B on cell culture. The device is designed to kill microorganisms but preserve release of the nucleic acids. These results provide evidence that the new swab collection device Sigma MM is reliable for molecular analysis when compared to current Eagle’s medium.

The RT-PCR laboratory detection technique is essential for influenza diagnostic because of the continuous evolution and the emergence of new viruses. So the Sigma Molecular Medium should be suitable for influenza A&B molecular diagnostic. This safe device could be an advantage for the shipment of non-infectious viruses/ samples, as highly pathogenic species for example, to be used as positives for molecular diagnostic.

Conclusion : From this study, it has been shown that Sigma MM renders the clinical samples noninfectious and safe for handling and transport, while also allowing high quality extraction of nucleic acid for use in RT-PCR. Sigma MM could be used for the molecular diagnostic of clinical specimens for Influenza viruses.

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