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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 Virology Department, James Cook University Hospital, South Tees Hospitals NHS Foundation trust
Rachel.Turnbull@sunderland.ac.uk

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.

MATERIAL AND 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.

Cepheid Smartcycler

ABI prism 7500 SDS

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	Day 1	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.

REFERENCES

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