

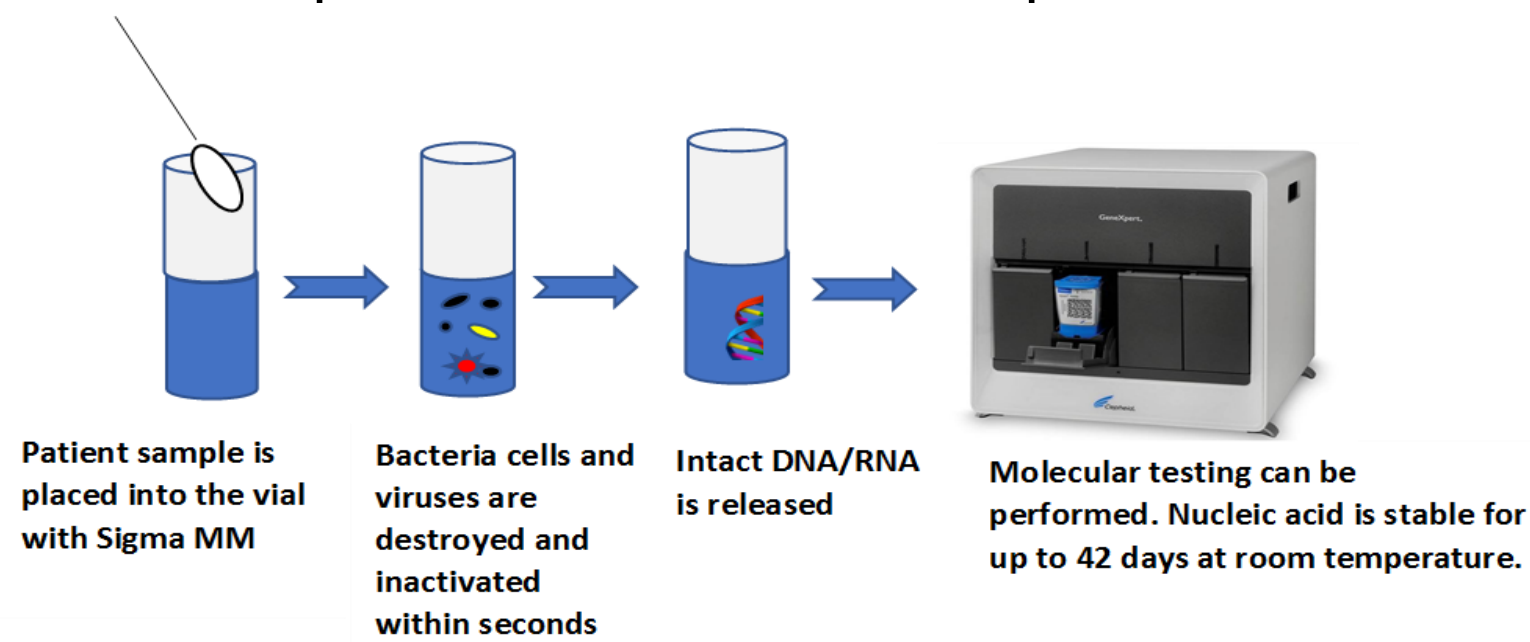
Evaluation of the new Molecular Transport Medium (Sigma MM) for the inactivation of bacterial pathogens and release of the intact DNA

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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.



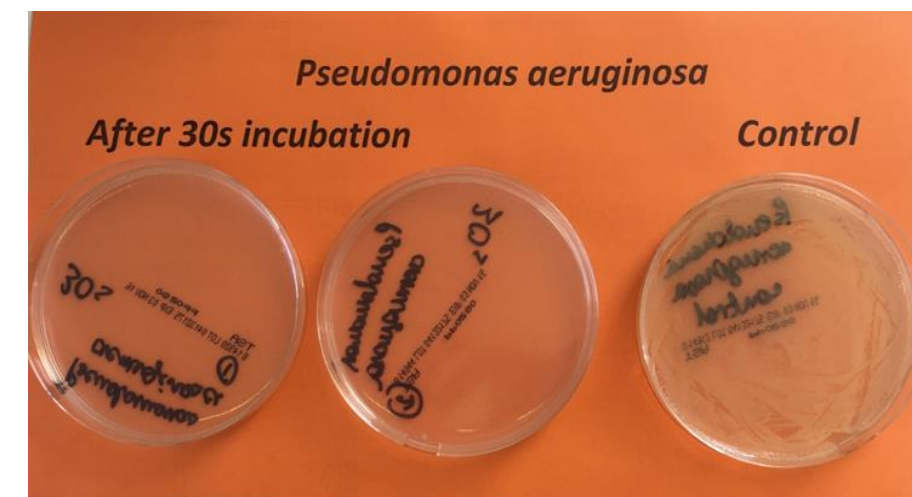
After 42 days, samples were tested for the presence of DNA using GeneXpert® MTB/RIF, Cepheid. For inactivation/killing properties of the Sigma MM 0.5McFarland bacterial concentrations of each strain (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Streptococcus 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.