Speedy Breedy - Lab Memo 28

Experiments to Investigate a Selective Culture Medium for Selective Detection of *Staphylococcus aureus* in Speedy Breedy

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Background

The bacterium Staphylococcus aureus is typically isolated from the skin surface and respiratory tract in humans. Detection of S. aureus is important in food hygiene and healthcare environments where transmission of the organism can lead to skin infections, respiratory infections or food poisoning.

A rapid detection system that selectively identifies S. aureus may offer logistical and financial benefits in a variety of industries however typical laboratory detection techniques are labour intensive and require microbiology expertise.

Speedy Breedy confirms microbial contamination by the sensitive monitoring of pressure changes within a closed vessel. Vessels containing a culture medium facilitate microbial replication and subsequent microbial respiration leads to changes in pressure within the vessel which can be monitored. The length of time between inoculation and significant pressure activity, known as the Time to Detection (TTD) is indicative of the level of contamination present in the original sample.

Presented in an easy-to-use instrument, Speedy Breedy offers a simple, portable and rapid microbial detection system for multiple industries and without the need for formal scientific experience.

Hypothesis

Our hypothesis was that using a selective medium, Speedy Breedy would be able to identify Staphylococcus spp. in samples whilst selectively excluding other organisms. We also hypothesised that Speedy Breedy would exhibit increasingly rapid detection times when challenged with increased Staphylococcus spp. contamination in samples.

Finally, we hypothesised that by using an indicator system within the media, it would be possible to differentiate Staphylococcus aureus from other staphylococci.

Aim of Study

The aim of this study was to correlate data for detection of S. aureus in artificially contaminated samples of sterile water, with increasing levels of contamination. Detection would be achieved using the portable microbial respirometer Speedy Breedy with culture vessels containing a Mannitol Salt medium.

At the same time, selective detection would be challenged by artificially contaminating separate samples of sterile water with inocula of Escherichia coli, Enterococcus faecalis and Staphylococcus epidermidis.

Materials & Methods

In order to measure Time to Detection against varying bacterial load in the sample, stock cultures of S. aureus, S. epidermidis, E. coli and E. faecalis were first required and through serial dilution, a number of samples of each organism with decreasing bacterial load were created.

Initial cultures were cultivated using Lenticule discs (NCTC 6571 S. aureus, NCTC 775 E. faecalis, NCTC 9001 E.coli, Public Health England) or Selectrol discs (NCTC 13360 S. epidermidis, TCS Biosciences). Following serial dilution, 100µl of each dilution was used to create a spread plate culture (PB0122A Columbia Agar with Horse Blood, Oxoid / Thermo Scientific). After 24 hours incubation at 36°C, counts were taken of colony forming units (CFU) and from this, CFU / ml of serial dilution calculated.

Speedy Breedy culture vessels initially containing no culture medium were filled with 49ml of Mannitol Salt medium. 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for five different dilutions of S. aureus and separate, single, heavy inocula of known CFU concentration of S. epidermidis, E. coli and E. faecalis.

Control vessels containing 50ml sterile Mannitol Salt medium were incubated to demonstrate that no detection activity is derived from uninoculated vessels.

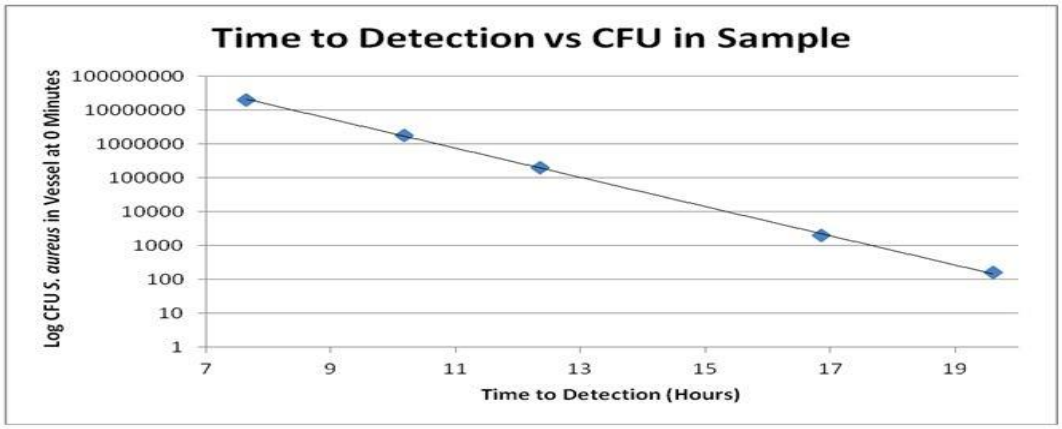
All vessels were incubated using Speedy Breedy instruments with a 48 hour test protocol at a 36°C incubation temperature. Pressure over time results from Speedy Breedy instruments were reviewed after the 48 hour test protocol completed to ascertain the TTD.

Results

Table 1 below shows data recorded for TTD with varying CFU loads of S. aureus in culture vessels tested using Speedy Breedy as outlined above.

Figure 2 shows the data from Table 1 plotted as a curve of TTD against CFU in the culture vessel.

Table 1: Initial sample S.aureus load and corresponding Time to Detection (TTD).



All vessels inoculated with S. aureus showed a distinct visual colour change of the culture medium from a red-orange colour to a bright yellow colour.

All vessels inoculated with S. epidermidis, despite generating a detection event, showed no visual colour change of the culture medium.

Control vessels inoculated with E. coli and E. faecalis and control vessels containing only sterile medium showed no detection event during the course of the experiment.

Interpretation

The lack of microbial activity in the control vessels inoculated with E. coli and E. faecalis suggests that the Mannitol Salt medium has selectively excluded these organisms. The viability of the inocula used was confirmed by successful agar plate culture.

Vessels inoculated with S. aureus show rapid detection and a strong correlation between microbial load and Time to Detection.

Vessels inoculated with S. epidermidis show a rapid detection however do not show the visual colour change of the culture medium that is observed with S. aureus.

Conclusions & Observations

- As per our hypothesis, Speedy Breedy can be used to rapidly and selectively detect S. aureus whilst selectively controlling the activity non-staphylococci organisms and differentiating from other staphylococci by means of a distinct colour change of the culture medium.

- The use of the Mannitol Salt medium provides a good selective, differential solution when wanting to screen samples for S. aureus.

- The strong correlation between Time to Detection and CFU levels in the inoculated samples suggests that Speedy Breedy can be used for quantitative analysis of samples based on the Time to Detection recorded.

- The successful detection of 160 CFU of S. aureus in a 50 ml working volume (equating to less than 4.0 CFU / ml) in a little under 20 hours in comparison to standard culture methods requiring up to 48 hours (this does not include time required for transportation of samples to a laboratory), shows Speedy Breedy to be a rapid, sensitive and selective tool.