Speedy Breedy - Lab Memo 31

Experiments to Investigate a Culture Medium for Selective, Presumptive Detection of *Vibrio spp.* Using Speedy Breedy

Author: Darren Hermes Date: March 2014

Background

The Vibrio spp. bacteria are a group of typically marine-inhabiting organisms. The group includes a number of known human pathogens but most notably the organism Vibrio cholerae – the bacterium associated with Cholera.

Speedy Breedy rapidly 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 an appropriate culture medium, Speedy Breedy would be able to selectively identify Vibrio spp. in samples. We also hypothesised that Speedy Breedy would exhibit increasingly rapid detection times when challenged with increased levels of contamination in samples.

Aim of Study

The aim of this study was to correlate data for detection of V. parahaemolyticus and V. vulnificus 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 modified Alkaline Peptone medium.

Materials & Methods

In order to measure Time to Detection (TTD) against varying bacterial load in sample, stock cultures of V. parahaemolyticus and V. vulnificus as well as the organisms to be used for challenging the selectivity of the medium (Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus faecalis and Staphylococcus aureus) were first required and through serial dilution, a number of samples of each organism with decreasing bacterial load created.

Initial cultures were cultivated using Vitroid discs (ATCC 9027 P. aeruginosa, ATCC 11175 E. coli, ATCC 19115 L. monocytogenes, Sigma-Aldrich), Lenticules (NCTC 6571 S. aureus, NCTC 775 E. faecalis, Public

Health England), Selectrol discs (NCTC 13376 P. mirabilis, TCS Biosciences) and freeze-dried cultures (ATCC 17802 V. parahaemolyticus, ATCC 27562 V. vulnificus, DSMZ).

Following serial dilution, enumeration using spread-plate technique was carried out over 48 hours with incubation at 37°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 50ml of modified Alkaline Peptone broth. 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for five different dilutions of V. parahaemolyticus and V. vulnificus and for a single dilution of each of the non-Vibrio spp. organisms.

Control vessels containing only 50ml sterile modified Alkaline Peptone broth were also tested.

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

Tables 1 and 2 below show data recorded for TTD with varying CFU loads of V. parahaemolyticus and V. vulnificus respectively, in culture vessels tested using Speedy Breedy as outlined above.

Figures 3 and 4 shows the data from Tables 1 and 2 respectively, plotted as a curve of TTD against CFU in the culture vessel at the start of the experiment.

Figure 5 shows the combined data from Tables 1 and 2, plotted as a single distribution.

Table 1: Initial sample V. parahaemolyticus load and corresponding Time to Detection (TTD).

CFU in Vessel	2.46×10^5	3.10×10^4	2.83×10^3	2.80×10^2	20
TTD (Minutes)	326	478	571	656	799
TTD (Hours)	5.43	7.97	9.52	10.93	13.32

Table 2: Initial sample V. vulnificus load and corresponding Time to Detection (TTD).

CFU in Vessel	1.84×10^5	1.03×10^4	9.80×10^3	1.10×10^3	84
TTD (Minutes)	416	498	517	615	778
TTD (Hours)	6.93	8.30	8.62	10.25	12.97

Figure 3: Initial sample V. parahaemolyticus load (CFU) and corresponding Time to Detection (TTD).

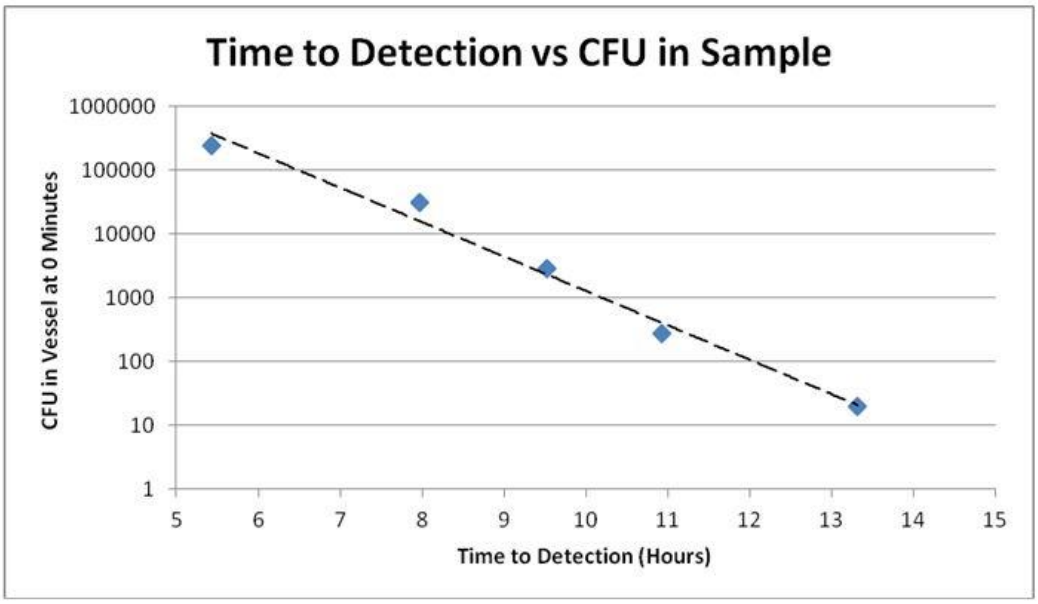


Figure 4: Initial sample V. vulnificus load (CFU) and corresponding Time to Detection (TTD).

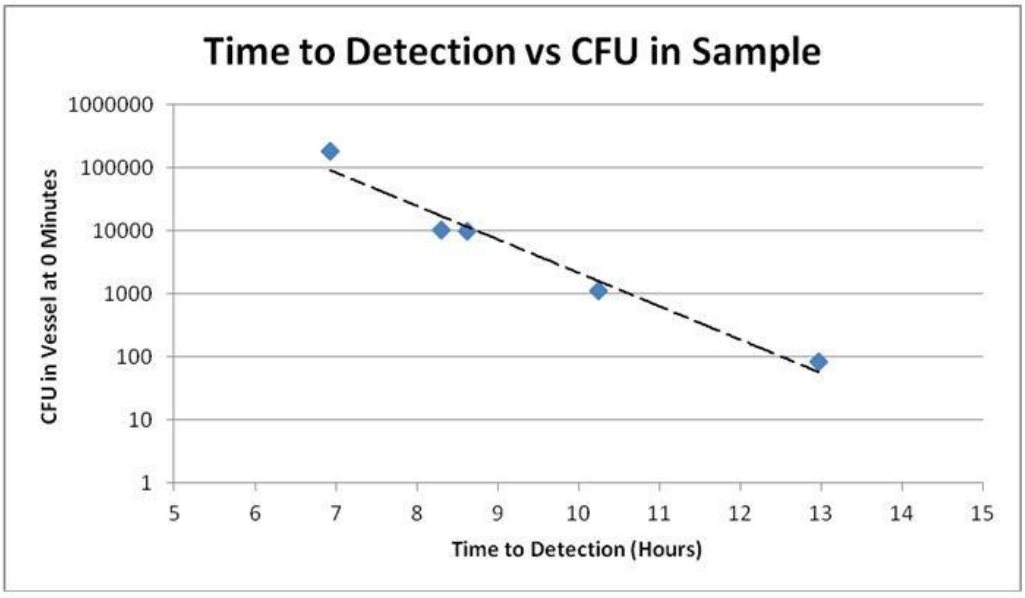
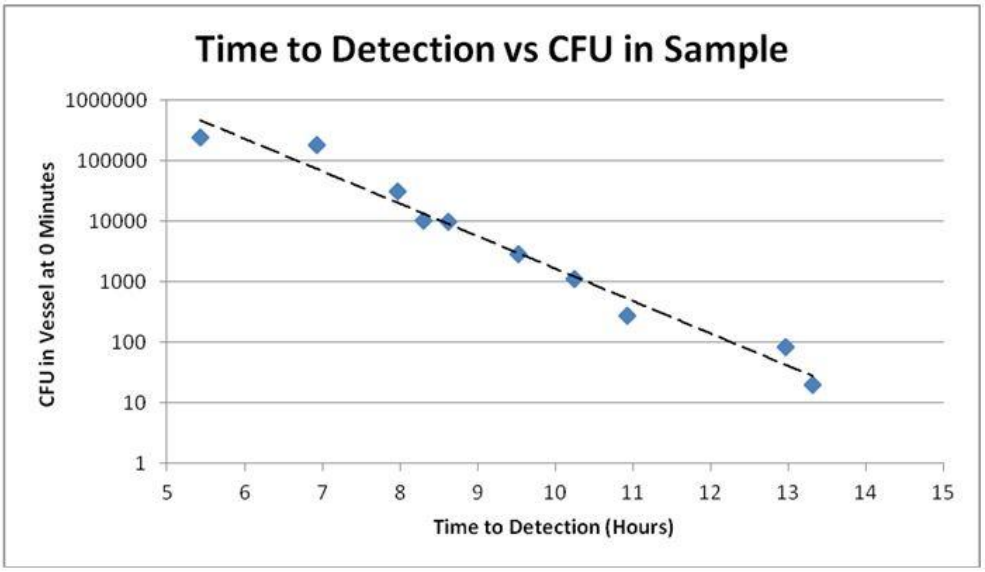


Figure 5: Initial sample Vibrio spp. load (CFU) and corresponding Time to Detection (TTD).



Control vessels inoculated with non-Vibrio spp. organisms as described above 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 suggests that the modified Alkaline Peptone medium has selectively excluded these organisms. The viability of the inocula used was confirmed by successful agar plate culture.

Vessels inoculated with Vibrio spp. show rapid detection and a strong correlation between microbial load and Time to Detection (R2 value for Figures 3, 4 and 5 being 0.9858, 0.9678 and 0.9720 respectively).

Conclusions & Observations

- As per our hypothesis, Speedy Breedy can be used to rapidly and selectively detect Vibrio spp. whilst selectively controlling the activity of non-Vibrio spp. organisms.
- The use of the modified Alkaline Peptone medium provides a good selective solution when wanting to screen samples for Vibrio spp.
- 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 < 100 CFU of Vibrio spp. in a 50 ml working volume (equating to less than 2.0 CFU / ml) in a little under 14 hours compared 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.

Speedy Breedy from BACTEST, St John's Innovation Centre, Cowley Road, Cambridge, CB4 0WS, UK
www.speedybreedy.com +44 01223 422312