Speedy Breedy - Lab Memo 29

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

Author: Darren Hermes Date: March 2014

Principle & Background

Listeria monocytogenes is one the most significant food-borne pathogens, with listeriosis associated with a relatively high fatality rate. The clinical importance of this organism means that many food types, particularly the ready-to-eat foods, are screened for L. monocytogenes to ensure the absence of the bacterium as part of quality control procedures.

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 Listeria 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 L. monocytogenes in artificially contaminated samples of sterile water, with increasing levels of contamination. Detection of L. monocytogenes would be achieved using the portable microbial respirometer Speedy Breedy with culture vessels containing a modified Half Fraser Broth medium.

Half Fraser Broth is a routinely used (and ISO approved) enrichment medium for Listeria spp. identification. In these experiments, the addition of supplements, inhibitory to non-Listeria spp. would be used to improve the selectivity of the medium. This selectivity would then be challenged by artificially contaminating samples of sterile water with a selection of Gram positive and Gram negative organisms.

Materials & Methods

In order to measure Time to Detection (TTD) against varying bacterial load in sample, stock cultures of L. monocytogenes and 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) and Selectrol discs (NCTC 13376 P. mirabilis, TCS Biosciences).

Following serial dilution, 100µl of each dilution was used to create a spread plate culture (PB0122A Columbia Blood Agar, Oxoid / Thermo Scientific). After 24 hours 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 Half Fraser Broth. 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for six different dilutions of L. monocytogenes and for a single dilution of each of the non-Listeria spp. organisms.

Control vessels containing only 50ml sterile modified Half Fraser 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

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

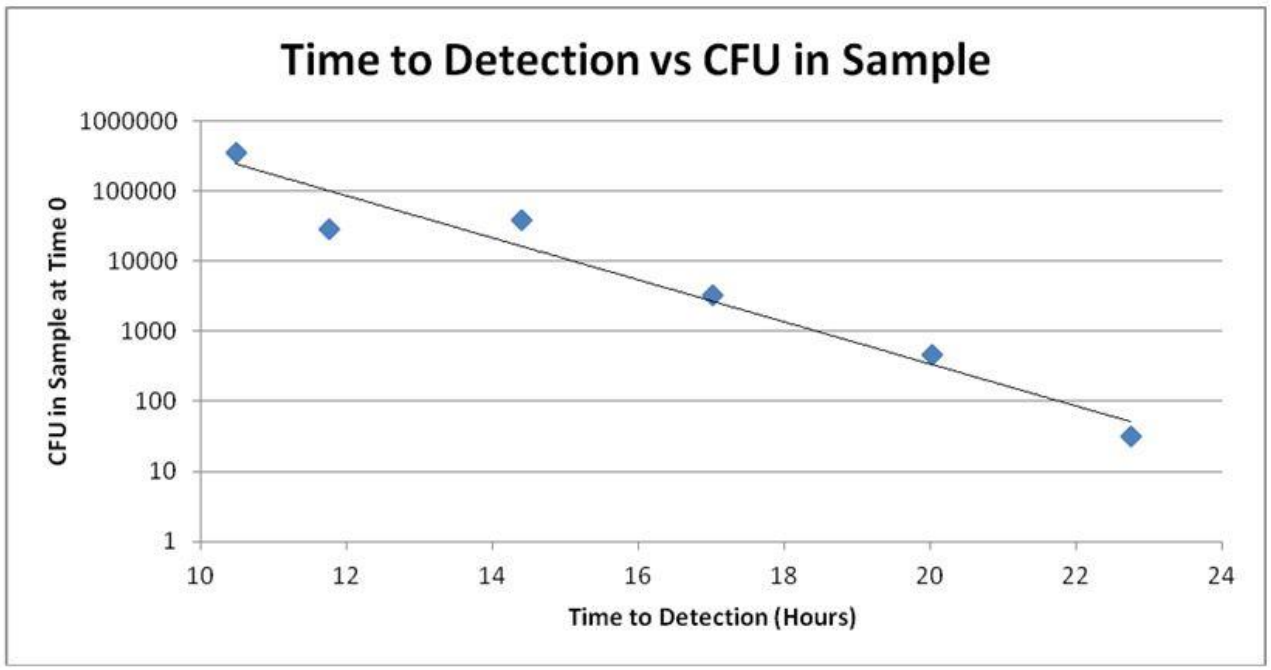
Figure 2 shows the data from Table 1 plotted as a curve of TTD against CFU in the culture vessel at the start of the experiment.

Table 1: Initial sample L. monocytogenes load and corresponding Time to Detection (TTD).

CFU in Vessel	3.60×10^6	2.90×10^5	3.80×10^4
TTD (Minutes)	628	705	864
TTD (Hours)	10.47	11.75	14.40

CFU in Vessel	3.30×10^3	4.60×10^2	30
TTD (Minutes)	1021	1201	1365
TTD (Hours)	10.47	20.02	22.75

Figure 2: Initial sample L. monocytogenes load (CFU) and corresponding Time to Detection (TTD).



All vessels inoculated with L. monocytogenes showed a distinct visual colour change of the culture medium from a dark brown-orange colour to turbid black colour.

Control vessels inoculated with non-Listeria 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 Half Fraser Broth medium has selectively excluded these organisms. The viability of the inocula used was confirmed by successful agar plate culture.

Vessels inoculated with L. monocytogenes show rapid detection and a strong correlation between microbial load and Time to Detection (R2 value for Figure 2 being 0.9506).

Conclusions & Observations

- As per our hypothesis, Speedy Breedy can be used to rapidly and selectively detect Listeria spp. whilst selectively controlling the activity of non-Listeria spp. organisms.
- The use of the modified Half Fraser Broth medium provides a good selective solution when wanting to screen samples for Listeria 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 30 CFU of L. monocytogenes in a 50 ml working volume (equating to less than 1.0 CFU / ml) in a little under 24 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.