Speedy Breedy -Lab Memo 27

Experiments to Investigate a Selective Culture Medium for Rapid Detection of *Salmonella enterica ssp. enterica* in Speedy Breedy

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Background:

Salmonella is a well-known cause of food-poisoning and production of many food and drink products require quality testing to ensure a complete absence of the bacterium. Such pathogen testing is particularly important for ready-to-eat foods that do not undergo any cooking process after distribution.

In an industry where laboratory work is often out-sourced, the time between taking samples for quality testing and receiving laboratory results can be upwards of 4-5 days. Food manufacturers face the dilemma of either shipping product before receiving their results (with the risk of a product recall should results return positive) or withholding stock until results are received (with the logistical implication of storage and financial implication on cash flow).

The availability of a reliable, accurate and sensitive screening tool that can be used alongside traditional testing techniques might offer a quality and financial benefit to a food producer.

Speedy Breedy confirms microbial contamination by the sensitive monitoring of pressure changes within a closed vessel. Containing a culture medium, vessels promote microbial replication. As part of a closed system, microbial respiration leading to changes in gas presence in the vessel can be monitored. An internal algorithm defines a significant pressure event associated with detection of contamination and the length of time from inoculation of sample to pressure event is the Time to Detection (TTD).

Hypothesis:

Our hypothesis was that using an appropriate medium, Speedy Breedy would be able to identify *Salmonella enteric subsp. enterica* (hereafter referred to as *S. enterica*) whilst selectively inhibiting the growth of other bacterial species. We also hypothesised that Speedy Breedy would exhibit increasingly rapid detection times when challenged with increased *S. enterica* contamination in samples.

Aim of Study:

The aim of this study was to correlate data for detection of *S. enterica* 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 Brilliant Green (BG) medium modified to improve selectivity.

At the same time, selective detection would be challenged by artificially contaminating samples of sterile water with heavy inocula of both Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Citrobacter freundii, Proteus mirabilis*) and Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*).

Materials & Methods:

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

Initial cultures were cultivated using either Selectrol discs (NCTC 13376 *P. mirabilis*, TCS Biosciences Ltd), Vitroid discs (NCTC 6017 *S. enterica,* ATCC 9027 *P. aeruginosa*, ATCC 11175 *E. coli* Sigma-Aldrich) or Lenticule discs (NCTC 6571 *S. aureus*, NCTC 775 *E. faecalis,* NCTC 9750 *C. freundii*, Public Health England).

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 48 hours incubation at 36°C, counts were taken of colony forming units (CFU) and from this, CFU / ml of serial dilution calculated. For confirming CFU values for dilutions of *P. mirabilis*, spread plate culture was performed using a different medium to prevent swarming and facilitate colony counts (PO0120 CLED Agar, Oxoid / Thermo Scientific).

Speedy Breedy culture vessels initially containing no culture medium were filled with 49ml of modified BG medium. 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for five different dilutions of *S. enterica* and for a single dilution of each of the bacteria used for selectivity testing.

Control vessels containing 50ml sterile modified BG 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:

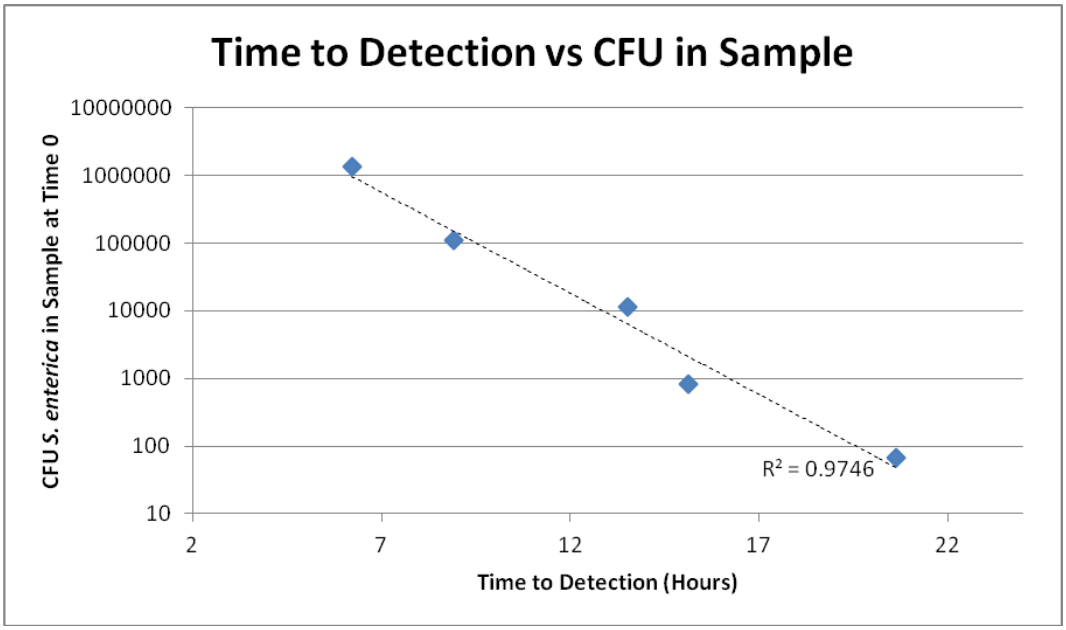
Table 1 below shows data recorded for TTD with varying CFU loads of *S. enterica* in culture vessels tested using Speedy Breedy as outlined above.

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

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

CFU in Vessel	1.36×10^6	1.08×10^5	1.12×10^4	8.30×10^2	68
TTD (Minutes)	373	534	811	908	1238
TTD (Hours)	6.22	8.90	13.52	15.13	20.63

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



Control vessels inoculated with non-*Salmonella spp.* bacteria at concentrations of greater than 1000 CFU all showed no detection event during the course of the experiment. Control vessels containing only sterile medium also showed no detection during the course of the experiment.

Interpretation:

The lack of microbial activity in vessels inoculated with non-*Salmonella spp.* bacteria suggests that the modified BG medium has selectively excluded the organisms. The viability of the inocula used was confirmed by successful agar plate culture.

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

Conclusions & Observations:

As per our hypothesis, Speedy Breedy can be used to rapidly and selectively detect Salmonella.

- The use of the modified BG medium provides a good selective solution when wanting to screen samples for Salmonella.
- 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 68 CFU in a 50 ml working volume (equating to less than 1.4 CFU per ml) in approximately 20 hours in comparison to standard culture methods requiring up to 2 days, shows Speedy Breedy to be a rapid, sensitive and selective screening tool for Salmonella detection.

Speedy Breedy
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