Experiments to Investigate a Selective Culture Medium for Rapid Detection of *Clostridium perfringens* in Speedy Breedy

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Background:

Clostridium perfringens is most commonly known as being a bacterium associated with food poisoning. Infection with *C. perfringens* is also associated with gas gangrene and other pathological symptoms.

As one of the most common bacterial causes of food poisoning, food producers are typically required to ensure that products are free of this organism. As a spore-forming species, *C. perfringens* is capable of surviving relatively high temperatures and so is of relevance both in the ready-to-eat food industry but also in production of foods that are intended for cooking.

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 *Clostridium perfringens* whilst selectively suppressing the growth of other bacterial species. We also hypothesised that Speedy Breedy would exhibit increasingly rapid detection times when challenged with increased *C. perfringens* contamination in samples.

Aim of Study:

The aim of this study was to correlate data for detection of *C. perfringens* 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 Shahidi-Ferguson Perfringens (SFP) medium modified to improve selectivity and to generate anaerobic conditions within the sealed culture vessel.

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) and Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis, Clostridium sporogenes*).

Materials & Methods:

In order to measure Time to Detection (TTD) against varying bacterial load in sample, stock cultures of *C. perfringens* as well as the organisms to be used for challenging the selectivity of the medium were first required. Through serial dilution, a number of samples of each organism with decreasing microbial load were created.

Initial cultures were cultivated using either Vitroid discs (ATCC 19404 *C. sporogenes*, ATCC 10240 *C. perfringens,* ATCC 9027 *P. aeruginosa*, ATCC 11175 *E. coli* Sigma-Aldrich) or Lenticule discs (NCTC 6571 *S. aureus*, NCTC 775 *E. faecalis,* 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 the cultures of *C. perfringens* and *C. sporogenes* this process was conducted under anaerobic conditions.

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

Control vessels containing 50ml sterile modified SFP 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 44°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 *C. perfringens* in culture vessels tested using Speedy Breedy as outlined above.

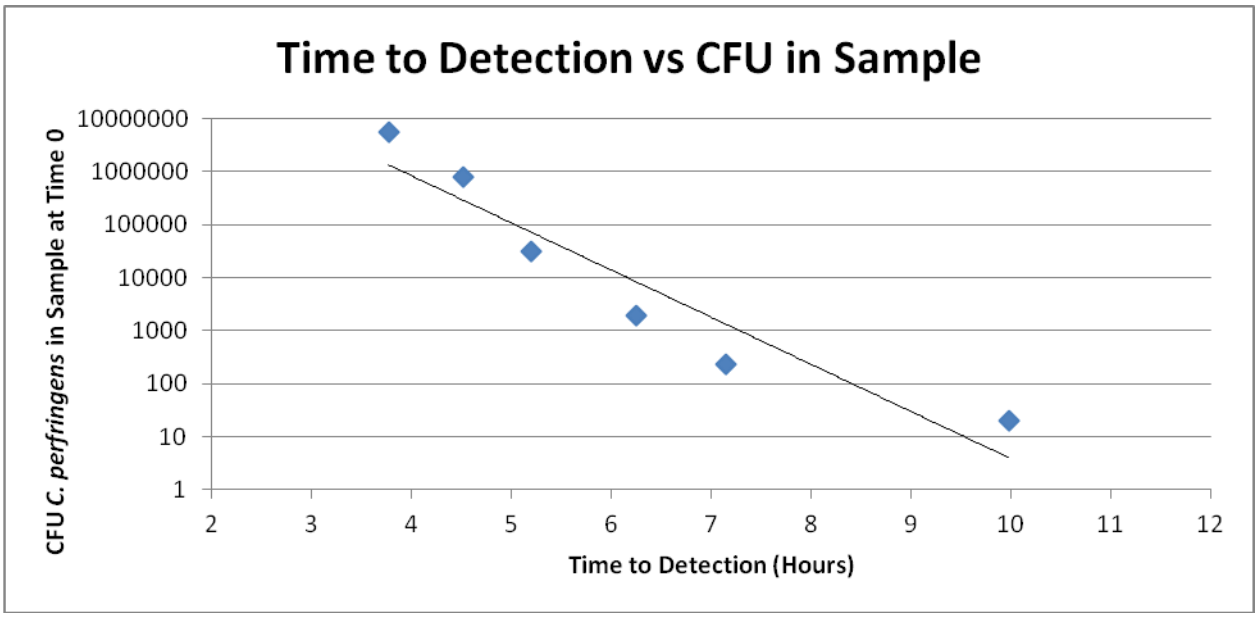
Figure 2 below shows the data from Table 1 plotted as a curve of TTD against CFU in the culture vessel.

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

CFU in Vessel	5.60×10^6	8.00×10^5	3.10×10^4	2.00×10^3	2.30×10^2	20
TTD (Minutes)	226	271	312	375	429	599
TTD (Hours)	3.77	4.52	5.20	6.25	7.15	9.98

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

Speedy Breedy -Lab Memo 32



Control vessels inoculated with non-*C. perfringens* 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-*C. perfringens* bacteria suggests that the modified SFP medium has selectively excluded the organisms. The viability of the inocula used was confirmed by successful agar plate culture.

Vessels inoculated with *C. perfringens* 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 *Clostridium perfringens*.

- The use of the modified SFP medium provides a good selective solution when wanting to screen samples for *C. perfringens*.
- 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 20 CFU in a 50 ml working volume (equating to less than 1 CFU per ml) in approximately 10 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 *Clostridium perfringens* detection.

Speedy Breedy
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