Speedy Breedy – Lab Memo 23

Experiments to Investigate a Culture Medium for Selective Detection of *Pseudomonas aeruginosa* using Speedy Breedy for Rapid Detection

Background:

Capable of metabolising a wide range of organic compounds, *Pseudomonas aeruginosa* is a ubiquitous organism routinely found in soil and water, which whilst not typically pathogenic can act as an opportunistic pathogen in immunocompromised patients.

The high potential for the presence of *P. aeruginosa* in and on exposed surfaces that come into contact with water (such as taps) means that infection control monitoring in health care centres and maintenance work with water supplies in such environments should be mindful of the organisms importance. Effective monitoring for *P. aeruginosa* will be aided by easy access to a simple, reliable and rapid test for the bacterium.

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.

In this study the suitability of Speedy Breedy for rapid, selective detection of *P. aeruginosa* is assessed.

Hypothesis:

Our hypothesis is that using a selective medium, Speedy Breedy will be able to identify *P. aeruginosa* in samples whilst selectively excluding other bacterial species. We also hypothesise that Speedy Breedy will exhibit increasingly rapid detection times when challenged with increased *P. aeruginosa* contamination in samples.

Aim of Study:

The aim of this study is to correlate data for detection of *P. aeruginosa* in artificially contaminated samples of sterile water, with increasing levels of contamination. Detection of *P. aeruginosa* will be achieved using the portable microbial respirometer Speedy Breedy with culture vessels containing the medium Cetrimide broth. Cetrimide broth, as the name suggests, contains the antimicrobial compound cetrimide to which *P. aeruginosa* is resistant.

At the same time, selective detection will be challenged by artificially contaminating samples of sterile water with a selection of other organisms including coliforms and Gram positive organisms representative of commonly found water-borne organisms.

Materials & Methods:

In order to measure Time to Detection (TTD) against varying bacterial load in sample, stock cultures of *P. aeruginosa* and the organisms to be used for challenging the selectivity of the medium (*Escherichia coli*, *Citrobacter freundii, Enterobacter* aerogenes, *Enterococcus faecalis* and *Staphylococcus aureus*) were first required and through serial dilution, a number of samples of each organism with decreasing bacterial load created. These organisms were chosen as being representative of commonly found water-borne bacteria. Initial cultures were cultivated using Vitroid discs (RQC12002 *P. aeruginosa*, RQC01702 *E. coli*, RQC01652 *E. aerogenes*, Sigma-Aldrich) or Lenticules (NCTC 9750 *C. freundii*, 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 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 Cetrimide broth (M862, HiMedia). 1ml of prepared organism dilution was then used to inoculate the vessel. This process was repeated for five different dilutions for each organism.

Control vessels containing only 50ml sterile Cetrimide broth were also tested.

All vessels were incubated using Speedy Breedy instruments with a 24 hour test protocol at a 36°C incubation temperature. Pressure over time results from Speedy Breedy instruments were reviewed after the 24 hour test protocol completed to ascertain the TTD.

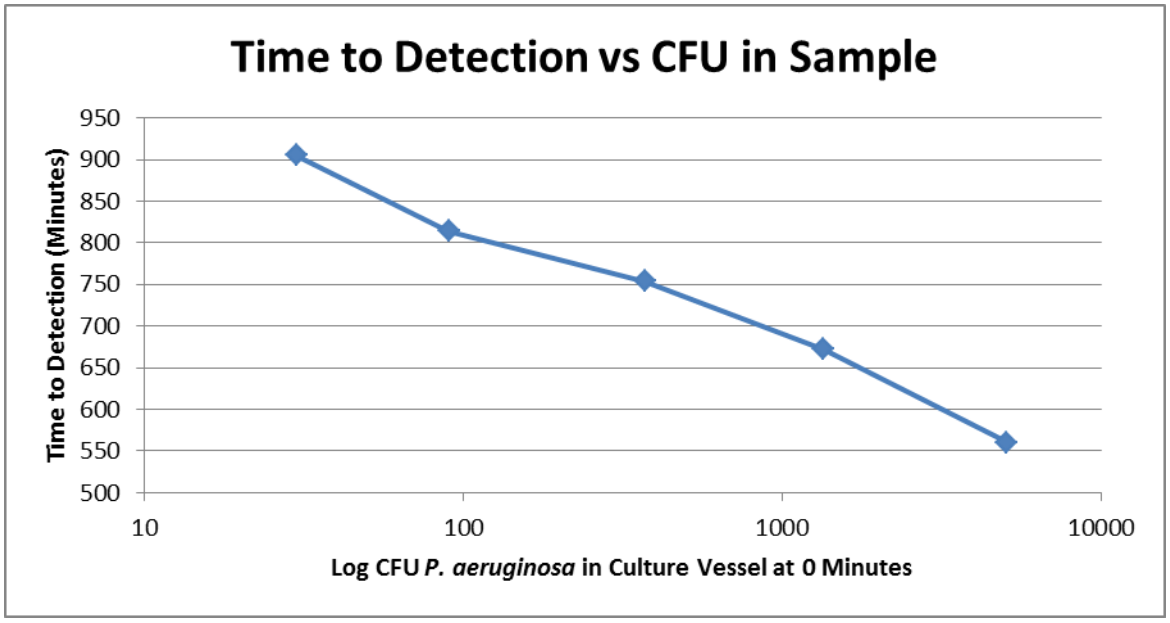
Results:

Table 1 below shows data recorded for TTD with varying CFU loads of *P. aeruginosa* 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 Log CFU load in the culture vessel.

Table 1: Initial sample P. aeruginosa load and corresponding Time to Detection (TTD).

CFU in Vessel	5050	1350	370	90	30
TTD (Minutes)	560	672	754	814	905
TTD (Hours)	9.33	11.20	12.57	13.57	15.08

Figure 2: Initial sample P. aeruginosa load (Log CFU) and corresponding Time to Detection (TTD).



Speedy Breedy – Lab Memo 23

Table 3 below shows a summary of results for the other organisms used to artificially contaminate sterile water samples.

Table 3: Initial sample contaminant and organism load and corresponding Time to Detection (TTD).

Organism	*E. coli*	*C. freundii*	*E. aerogenes*	*E. faecalis*	*S. aureus*
CFU in Vessel	1.12×10^6	5.10×10^5	4.20×10^5	9.80×10^5	1.28×10^6
TTD (Hours)	No Detection	No Detection	No Detection	No Detection	No Detection

Control vessels containing only sterile medium all showed no detection during the course of the experiment.

Interpretation:

There is a marked reduction in Time to Detection with Speedy Breedy as *P. aeruginosa* contamination of the original sample is increased and there is a strong correlation between bacterial load and time to detection. Very low CFU levels (30) were detected within sixteen hours of experimentation commencing.

The lack of microbial activity in vessels inoculated with non-*P. aeruginosa* species suggests that the Cetrimide medium has selectively excluded these organisms. In each case, a particularly high bacterial load was used to ensure effective challenge of the selective capability of the medium and in each case the non-*P. aeruginosa* species have failed to grow.

Conclusions & Observations:

As per our hypothesis, Speedy Breedy can be used to rapidly and selectively detect *P. aeruginosa* in water samples.

- The use of the Cetrimide medium provides a good selective solution when wanting to screen samples for *P. aeruginosa*. Despite challenge with particularly high concentrations of other common, water-borne organisms, only *P. aeruginosa* is successfully growing using this medium.
- 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 Time to Detection recorded.
- The successful detection of 30 CFU of *P. aeruginosa* in a 50 ml working volume (equating to less than 1.0 CFU / ml) in approximately 15 hours shows Speedy Breedy to be a rapid, sensitive and selective tool for *P. aeruginosa* detection when used in conjunction with Cetrimide medium.

Speedy Breedy
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