Speedy Breedy - Lab Memo 22

Experiments to Investigate the Relationship between Time to Detection and Microbial Load in Contaminated Ice Cream Samples – *Listeria monocytogenes*

Author: Darren Hermes Date: October 2013

Principle & Background

As with all food manufacturing processes, Quality Control (QC) and Quality Assurance (QA) are essential both to protect the consumer and to ensure that any product is of the standard of quality that is expected. Significant investment is made, both financially and in time, to ensure high standards are met and any quality failures are rapidly addressed.

In ice cream production, microbial contamination, from raw ingredients such as milk, from unclean handling by staff, from contaminated equipment or from failures of pasteurisation can lead to equipment downtime, prolonged holding of inventory whilst awaiting QA results (and so reducing shelf life for the vendor), reduction in quality of the finished product (texture, colour, taste) or even product recall in the event of any human-health associated contamination.

Hypothesis

Our hypothesis is that Speedy Breedy, a portable, microbial respirometer designed for rapid detection of contamination in samples will be able to quantify contamination levels by exhibiting a clear relationship between contamination level and time to detection, whereby increased contamination leads to increasingly rapid detection times.

Aim of Study

The aim of this study is to correlate data for detection of contaminating organisms in artificially contaminated samples of ice cream with increasing levels of contamination using Speedy Breedy. In this series of experiments, pathogens, coliforms and other enterobacteriaceae (often used as indicators of contamination in many industries) will be used and in this particular experiment, Listeria monocytogenes.

Materials & Methods:

In order to measure Time to Detection (TTD) against varying bacterial load in sample, a stock culture of L. monocytogenes was first required and through serial dilution, a number of samples of L. monocytogenes with decreasing bacterial load created. An initial culture was cultivated using L. monocytogenes Vitroid discs (RQC01901, Sigma-Aldrich). 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.

1ml of a L. monocytogenes dilution was added to 49ml of a 1 in 10 dilution of store-purchased ice cream to give a total working volume of 50ml. This 50ml contaminated ice cream solution was then used to inoculate

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a Speedy Breedy dehydrated MacConkey culture medium vessel. This process was repeated for a total of five different L. monocytogenes dilutions.

Inoculated culture vessels were run through Speedy Breedy instruments using a 48 hour test protocol with a 36°C incubation temperature. TTDs were then recorded following the completion of the incubation period.

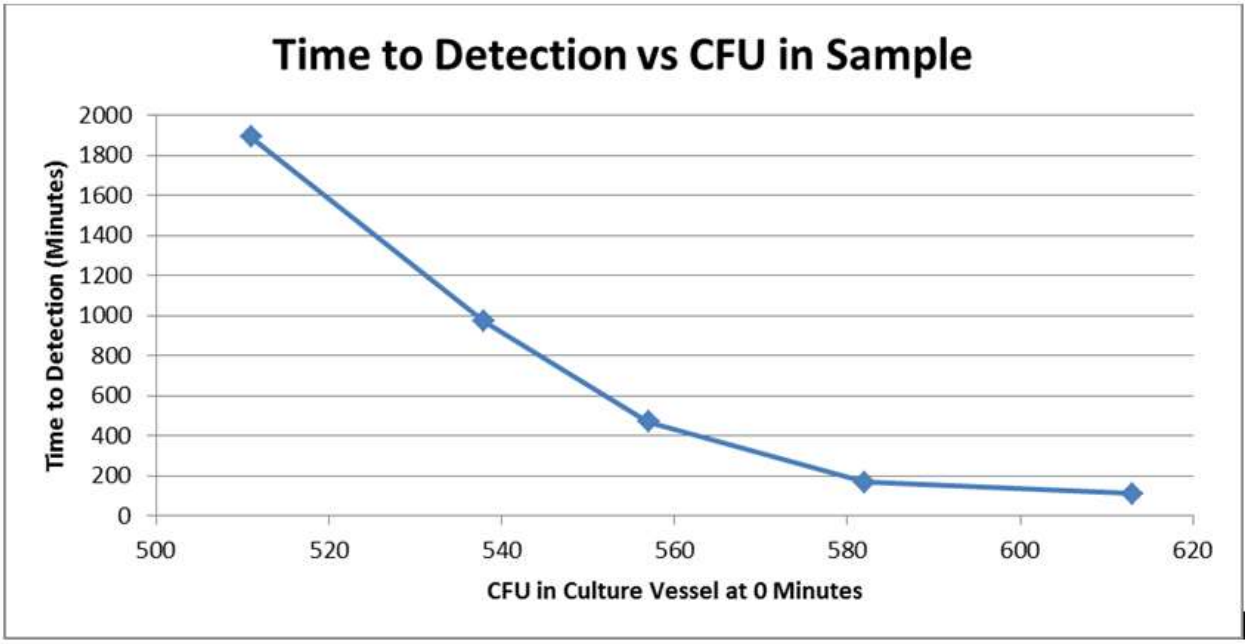
Results

Table 1 below shows data recorded for TTD with varying CFU loads 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 load in the culture vessel.

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

CFU in Vessel	1890	970	470	170	110
TTD (Minutes)	511	538	557	582	613

Figure 2: Initial sample bacterial load and corresponding Time to Detection (TTD).



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Interpretation

There is a marked reduction in Time to Detection with Speedy Breedy as contamination of the original sample is increased and there is a strong correlation between bacterial load and time to detection. Very low CFU levels (110) were detected within twelve hours of experimentation commencing.

Conclusions & Observations

As per our hypothesis, Speedy Breedy can be used to successfully detect L. monocytogenes contamination of ice cream samples. In addition, the strong correlation between Time to Detection and CFU levels in the inoculated sample suggest that Speedy Breedy can be used for quantitative analysis of samples based on Time to Detection recorded.

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