Speedy Breedy - Lab Memo 26

Microbial Analysis of Water-Based Paint Samples, One believed to be 'Clean' and the second 'Contaminated', using Speedy Breedy

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Principle & Background

In this study we investigated potential contamination in two samples of water based paint provided by a well-known multi- national manufacturer. One sample was believed to be 'clean' and the second was suspected of having a bacterial contaminant.

The objective was to investigate whether Speedy Breedy can differentiate between the two samples and further whether it can determine the level of contamination (if present) in the sample.
The investigation aimed to show that the Time to Detection (TTD) is closely linked to the bacterial numbers in the sample since each generation in exponential growth (doubling time) will take a measurable duration (doubling time).
Further work was undertaken to assess the effect of the biocide within the sample in relation to Time to Detection of the contamination.

Speedy Breedy provides a rapid, portable, on-site solution to semi-quantitation (equivalent to agar plates) of contamination, with a very low limit of detection.
Speedy Breedy determines contamination by measuring sensitive pressure changes within a closed vessel due to microbial respiration and assigns a Time to Detection using an internal algorithm that defines a significant pressure event.

Experiment 1

Determining Best Sample Preparation

Samples of both 'clean' and 'contaminated' paint were cultured using standard agar plate techniques in the laboratory to identify any contaminating organisms present.

No contamination was seen from samples of the 'clean' paint.

In the 'contaminated' paint a single organism, a coliform, was found in heavy growth and following isolation, speciation using microbial ID kit was performed, revealing the organism to be Citrobacter freundii.

With this in mind, subsequent tests with Speedy Breedy used MacConkey Broth Culture Vessels as a selective medium for coliform growth with a 360 C incubation protocol.

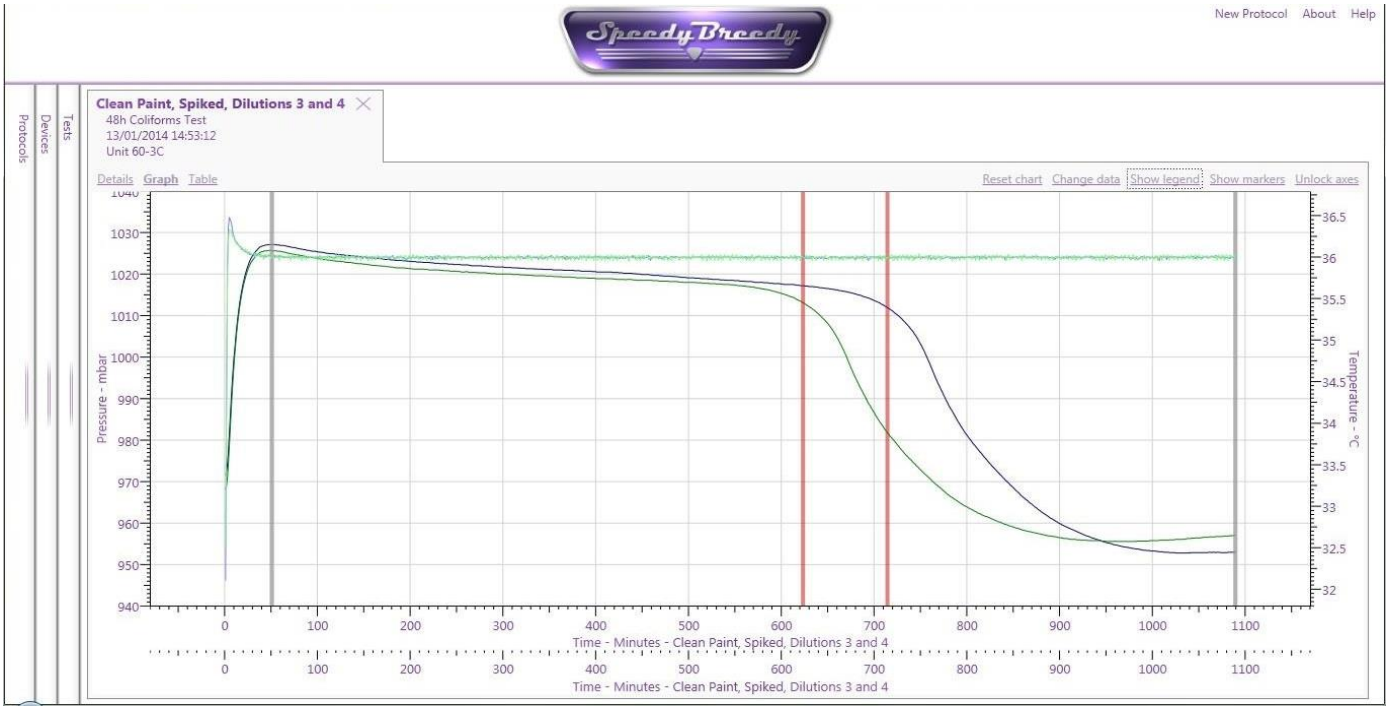
Experiment 2

Determining Best Sample Preparation

The presence of biocidal compounds in the sample material was expected to have an effect on the Time to Detection values that might be obtained from Speedy Breedy due to their antimicrobial properties.

It was hypothesised that diluting the sample material would allow for the effect of the biocidal compounds to be reduced to a point where dilution has been sufficient to remove any biocidal activity.

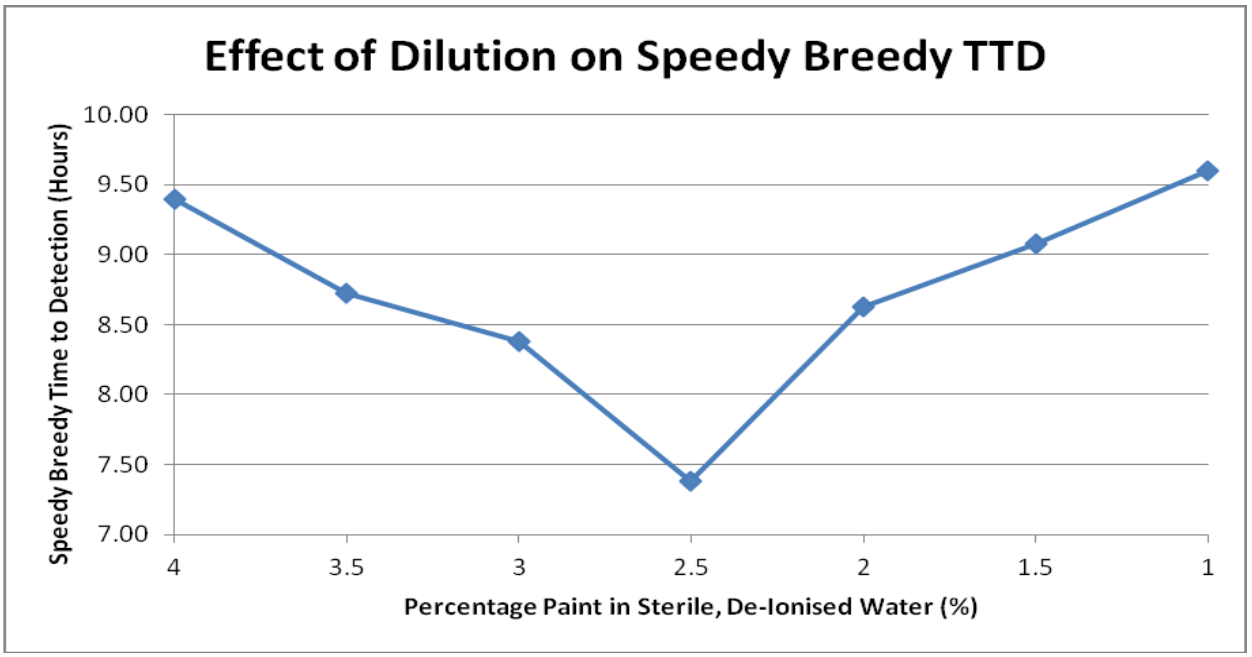
To determine the most appropriate dilution of sample to make for subsequent testing, where dilution of biocide is
Effective but dilution of sample does not excessively dilute the contaminating organisms present, samples of contaminated paint were diluted at varying percentages in sterile, de-ionised water and the Time to Detection determined in Speedy Breedy.



Results

Percentage paint	4.0	3.5	3.0	2.5	2.0	1.5	1.0
TTD (Minutes)	564	523	503	443	518	545	576
TTD (Hours)	9.40	8.72	8.38	7.38	8.63	9.08	9.60

The results obtained showed that increasing dilution of contaminated paint up to 2.5% paint in sterile, de-ionised water improved Time to Detection. Further dilution leads to increasing Time to Detection.



The 2.5% paint concentration is a level of dilution whereby the inhibitory effect of biocidal compounds has been removed. Where paint is more concentrated, biocide inhibits microbial growth and increases Time to Detection even though numbers of organisms are, in theory, greater.

As the 2.5% dilution represents an effective neutralisation of the biocidal Compounds, continued dilution merely acts to reduce the numbers of organisms present and so produce increased Time to detection.

Experiment 3

Speedy Breedy Time to Detection ('Clean' Paint)

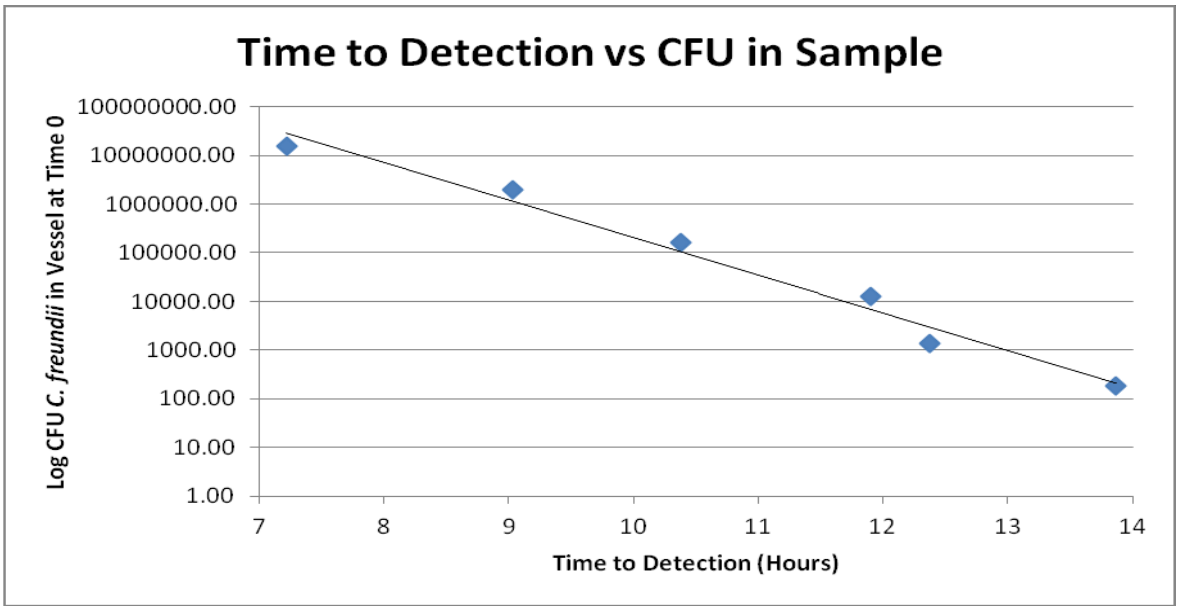
A 2.5% concentration of 'clean' paint was prepared and tested in Speedy Breedy.
No contamination was noted within a 48 hour period.

Experiment 4

Speedy Breedy Time to Detection ('Contaminated' Paint)

Having established a 2.5% paint dilution as that which gives optimum Time to Detection, samples of clean paint made to the same dilution were artificially contaminated with known numbers of Citrobacter freundii to establish if the level of microbial activity and Time to Detection correlate.

The numbers of organisms in this experiment were ascertained using standard spread plate and counting methods for bacterial enumeration.



The results curve, plotted above, shows a very strong correlation between Log CFU and Time to Detection (R^2 = 0.9796). The data above enables us to determine two key factors:
Referencing the Time to Detection result for 2.5% paint in Experiment 2 (7.38 hours), the calibration curve above would suggest that the level of bacterial contamination in the contaminated (2.5%) paint was approximately 1.0 x 10^7 CFU.

The calibration curve above can be extrapolated to estimate the time required to detect a single CFU. This would provide a cut-off time where, if in routine Speedy Breedy testing under the same conditions (2.5% paint, MacConkey broth) there is no positive result by the cut-off time, sample material can be considered clean.

It should be noted that the CFU values described above relate to CFU within a Culture Vessel of 50ml volume. Therefore
1.00 x10^7 CFU in 50ml of 2.5% paint solution equates to 2.00 x 10^5 CFU / ml of 2.5% paint solution.

Speedy Breedy has clearly demonstrated its ability to aid in the quality control of water based paint products.