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Confidential report for:**Bactest**

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Report on:**Application of Speedy Breedy to determine the microbiological quality of ice-cream with respect to coliforms**

Work performed by Campden BRI (Chipping Campden) Limited
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1. INTRODUCTION

Bactest has developed an instrumental method for detection of microorganisms. The Speedy Breedy system offers a rapid test for the detection of microbiological contaminants based on changes in pressure caused by microbial respiration. The system can detect minor changes in negative or positive pressure and so has application to detection of many different bacterial species with different respiration patterns.

Previous tests done on behalf of the Client has shown that the system was able to detect a range of clinical microorganisms and microbial populations in water samples. Studies have shown equivalent or faster detection times than other rapid growth detection systems and thus the Speedy Breedy shows promise for the detection of microbial populations in foods and drinks.

The aim of the studies reported here was to investigate the potential application of Speedy Breedy to determine the levels of coliforms present in ice-cream.

Detection times in the Speedy Breedy were compared to plate count results obtained using conventional ISO standard methods in order to determine the correlation between the two approaches. The Speedy Breedy performed well and was able to provide detection times of around 7 to 11 hrs for coliform counts in ice-cream ranging from <10 cells per ml of ice-cream to 10,000 cells per ml of ice-cream.

The data provided in this report is intended to provide demonstration data that the Speedy Breedy can be used to determine the microbiological quality of ice-cream. Users of the system would need to demonstrate it was fit for purpose for their own products as they would have to do for any analytical method.

2. METHODS

2.1 Products

The products used in the trial are shown below

Table 1: Products

Product		Sample code
Vanilla Ice-cream	Fat g/100ml: 2.6 Protein g/100ml: 1.3 Carbs g/100g: 9.6	MB/131801/1
Chocolate Ice-cream	Fat g /100ml: 2.7 Protein g/100ml: 1.5 Carbs g/100g: 9.4	MB/1318012

The samples were purchased on 08/12/2013 and were in satisfactory condition.

All samples were labelled with appropriate sample code. Samples were stored in a domestic freezer prior to use.

Testing was carried out between 10/12/2013 and 16/01/2014.

2.2 Organisms

The following microbial strains were used in this trial

Organism	CRA number
<i>Hafnia alvei</i>	509
<i>Enterobacter cloacae</i>	16677
<i>Citrobacter freundii</i>	6759
<i>Escherichia coli</i>	16041

Prior to each experiment, the culture was grown in Nutrient Broth at 37°C for 18-24hours. The numbers of cells present were estimated microscopically using a haemocytometer and the culture was diluted to achieve the correct level for direct inoculation into the ice-cream samples.

2.3 Experimental matrix

Contamination levels:

It was intended to cover the approximate range of <10 cfu/g, 10¹ cfu/g, 10² cfu/g, 10³ cfu/g.

Each of the 4 contamination levels were analysed in duplicate for the two ice-cream types making a total of 16 analyses of Speedy Breedy.

20g samples of ice-cream were taken and inoculated with 0.1ml of inoculum and mixed well. A sample (10g) was taken and added to 90ml Sterile distilled water. 50ml of this was used to fill a MaConkey vessel and the remainder was used for conventional testing using ISO 4832:2006. Serial dilutions were made in MRD and 1ml samples of each dilution were transferred to 90ml Petri dishes and levels of coliforms enumerated.

Organism	Test method	Method Summary*
Coliform enumeration	TES-MB-005 (ISO 4832:2006)	Pour plate plus over layer with VRBA. Incubation at 37±1°C for 24±2h

For the Speedy Breedy, the chambers were set to run at 36°C for 48hours but were stopped once a significant event was recorded.

2.4 Analysis of results

For the conventional test, the numbers of cfu per ml of product were calculated.

For the Speedy Breedy, the time at which a significant event was registered was recorded as the detection time (DT) in minutes. This was converted to DT in hours.

The log₁₀ number of cfu/ml were plotted against the log₁₀ DT in hours and the correlation was calculated. For counts of <10, the limit of detection/square root of 2 was used as the most likely count.

3. RESULTS

Table 1 contains the data for the ice-cream samples as cfu/ml, detection time in minutes and detection time in hours. This is also shown in Figure 1 as \log_{10} cfu/ml versus \log_{10} detection time in hours.

The data in Table 1 show that the Speedy Breedy is capable of detecting low levels of coliforms in ice-cream. Sample of ice-cream containing coliforms at a level of <10 cfu/ml to 1.2×10^4 cfu/ml were detected within 7.67 to 11.27 hours.

Table 1: Data for coliforms in ice-cream

Set up date	Product	cfu/ml coliforms	DT min	DT hour
17-Dec 2013	Vanilla	40	513	8.55
17-Dec 2013	Vanilla	340	490	8.17
17-Dec 2013	Vanilla	2400	488	8.13
17-Dec 2013	Vanilla	12000	460	7.67
17-Dec 2013	Chocolate	20	623	10.38
17-Dec 2013	Chocolate	120	615	10.25
17-Dec 2013	Chocolate	2000	531	8.85
17-Dec 2013	Chocolate	13000	465	7.75
07-Jan 2014	Vanilla	<10	481	8.02
07-Jan 2014	Vanilla	50	529	8.82
07-Jan 2014	Vanilla	600	471	7.85
07-Jan 2014	Vanilla	4090	497	8.28
07-Jan 2014	Chocolate	<10	676	11.27
07-Jan 2014	Chocolate	30	668	11.13
07-Jan 2014	Chocolate	490	607	10.12
07-Jan 2014	Chocolate	5450	530	8.83

It can be seen from Figure 1 that there is some variation in the detection times for the different inoculum levels. Also there was a noticeable difference in the detection times for vanilla and chocolate ice-cream. The detection times in chocolate are generally longer than in vanilla. There are no obvious reasons for this based on fat or sugar content of the two varieties but it would appear that chocolate ice-cream is more inhibitory than vanilla.

Figure 2a shows the regression line for vanilla ice-cream only. There is some scatter in the points and there is an obvious outlier which influences the correlation. If the outlier is removed the correlation is improved (Figure 2b)

Figure 3 shows the regression line for chocolate ice-cream only. There is a very good correlation between detection time and bacterial count for chocolate ice-cream

Figure 1: Scatter plot of log₁₀ cfu/ml coliforms versus log₁₀ detection time in hours

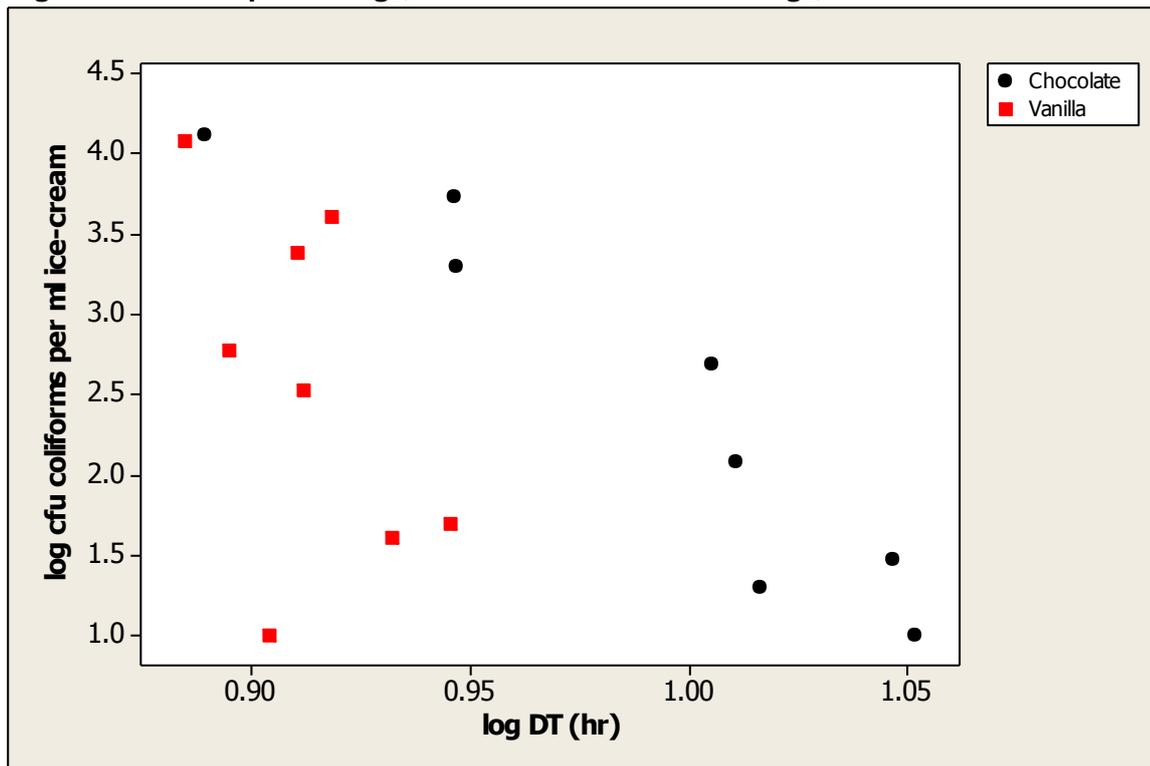


Figure 2a: Fitted line plot of log₁₀ cfu/ml coliforms in Vanilla ice-cream versus log₁₀ detection time in hours

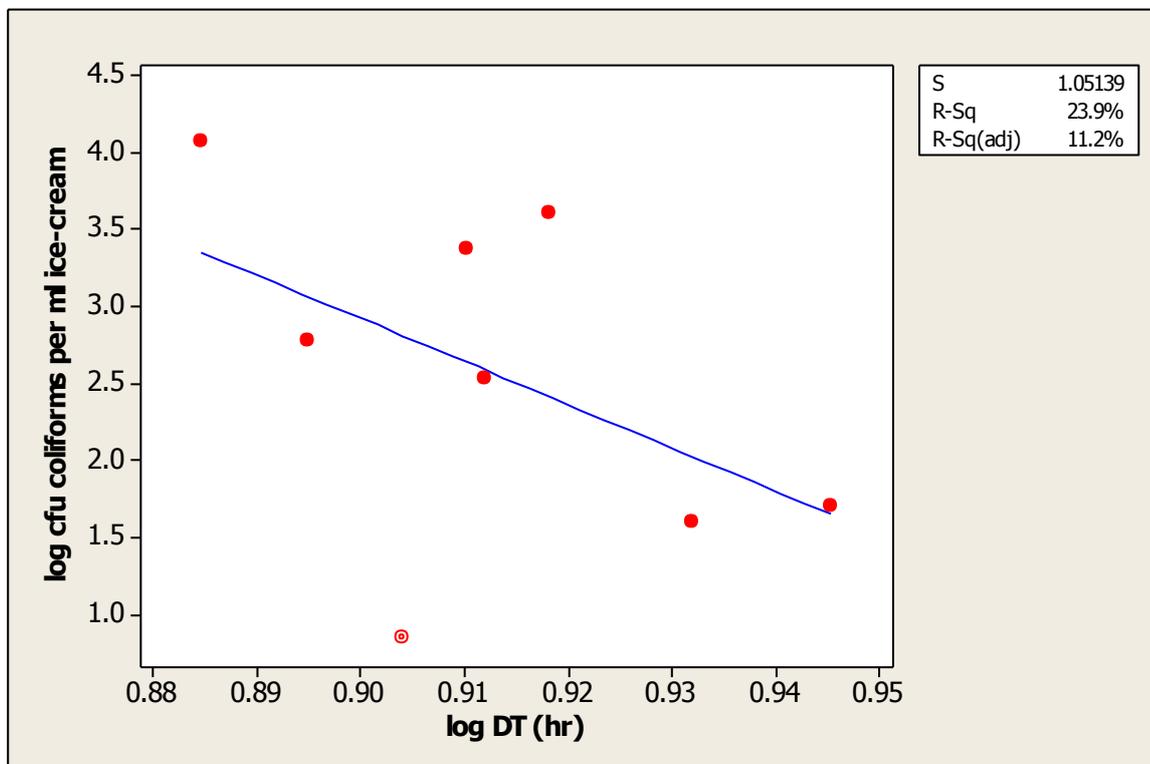


Figure 2b: Fitted line plot of \log_{10} cfu/ml coliforms in Vanilla ice-cream versus \log_{10} detection time in hours with outlier removed.

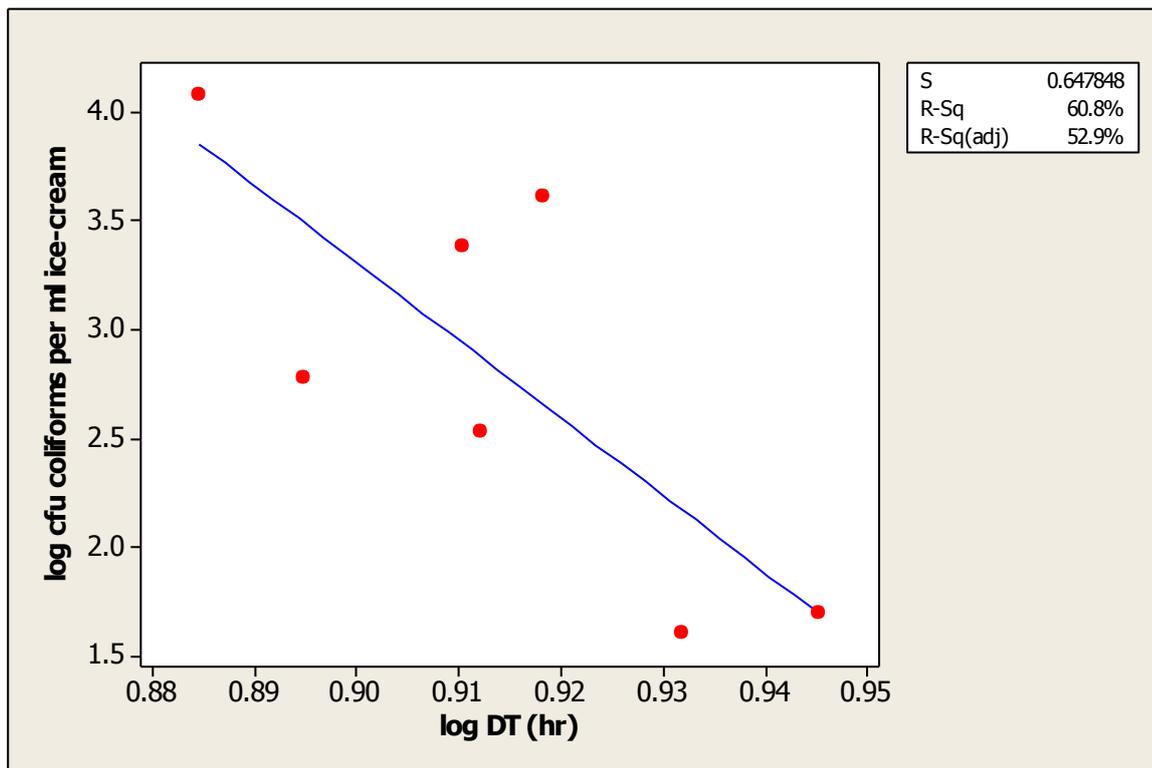
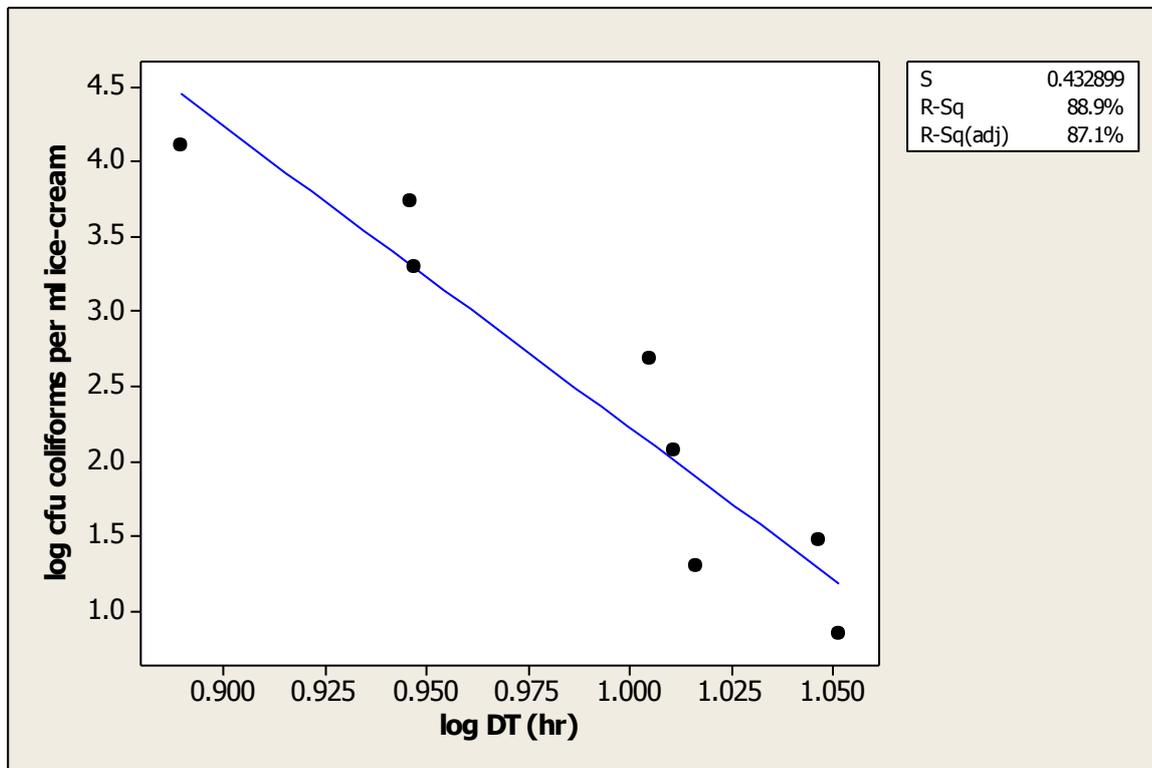


Figure 3: Fitted line plot of \log_{10} cfu/ml coliforms in Chocolate ice-cream versus \log_{10} detection time in hours



4. CONCLUSION

The data from this study has shown

- The Speedy Breedy shows promise for use as a screening tool where samples of ice-cream containing coliform bacteria can be tested to see if they detect within a threshold time. The times to detection were affected by the variety of ice-cream tested with chocolate ice-cream showing good correlation between detection time and bacterial numbers. Users of the system would need to demonstrate it was fit for purpose for their own products as they would have to do for any analytical method.
- Based on the data presented here a detection time of 7.5 hr or greater would equate to a level of between 10 and 10⁴ cfu/g ice-cream. Faster detection times would indicate a higher level of contamination than 10⁴ cfu/g
- Speedy Breedy was fast compared with current techniques, taking less than a day to determine the level of contamination compared with two days or more for plate counts.
- Speedy Breedy can be used at the site of ice-cream manufacture, removing the need for samples to be shipped to a laboratory, further reducing the time to achieve a result.

In summary, all samples tested in this project were found to be positive by the Speedy Breedy respirometer technology and detection was more rapid than by traditional microbiology in all cases.

This new methodology was also found to be very sensitive and able to detect very low cell concentrations.

The Speedy Breedy staff provided excellent training and technical support. The device was easy to use.