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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 TVC**

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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 application of the Speedy Breedy to determine the levels of total mesophilic bacteria 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 very well and was able to provide detection times of around 2 to 7 hrs for total bacterial counts in ice-cream ranging from <10 cells per ml of ice-cream to over a million 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	MB/131801/1
	Protein g/100ml: 1.3	
	Carbs g/100g: 9.6	
Chocolate Ice-cream	Fat g /100ml: 2.7	MB/1318012
	Protein g/100ml: 1.5	
	Carbs g/100g: 9.4	

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

A range of both Gram positive and Gram negative organisms was used to inoculate the ice-cream as shown below:

Organism	CRA
<i>Hafnia alvei</i>	509
<i>Enterobacter cloacae</i>	16677
<i>Staphylococcus aureus</i>	1244
<i>Bacillus cereus</i>	16440
<i>Pseudomonas fluorescens</i>	15937
<i>Lactobacillus plantarum</i>	5903
<i>Escherichia coli</i>	16041

Prior to each experiment, the microbial strains were grown in the relevant broth and mixed into a cocktail for use in inoculation.

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 10^1 cfu/g, 10^2 cfu/g, 10^3 cfu/g, 10^4 cfu/g, 10^5 cfu/g and 10^6 cfu/g.

Each of the 6 contamination levels were analysed in duplicate for the two ice-cream types making a total of 24 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 TSB vessel and the remainder was used for conventional testing using ISO 4833:2003. 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*
TVC enumeration	TES-MB-005 (ISO 4833:2003)	Pour plate with PCA Incubation at $30\pm 1^\circ\text{C}$ for $48\pm 4\text{h}$

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.

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 log₁₀ cfu/ml versus log₁₀ detection time in hours.

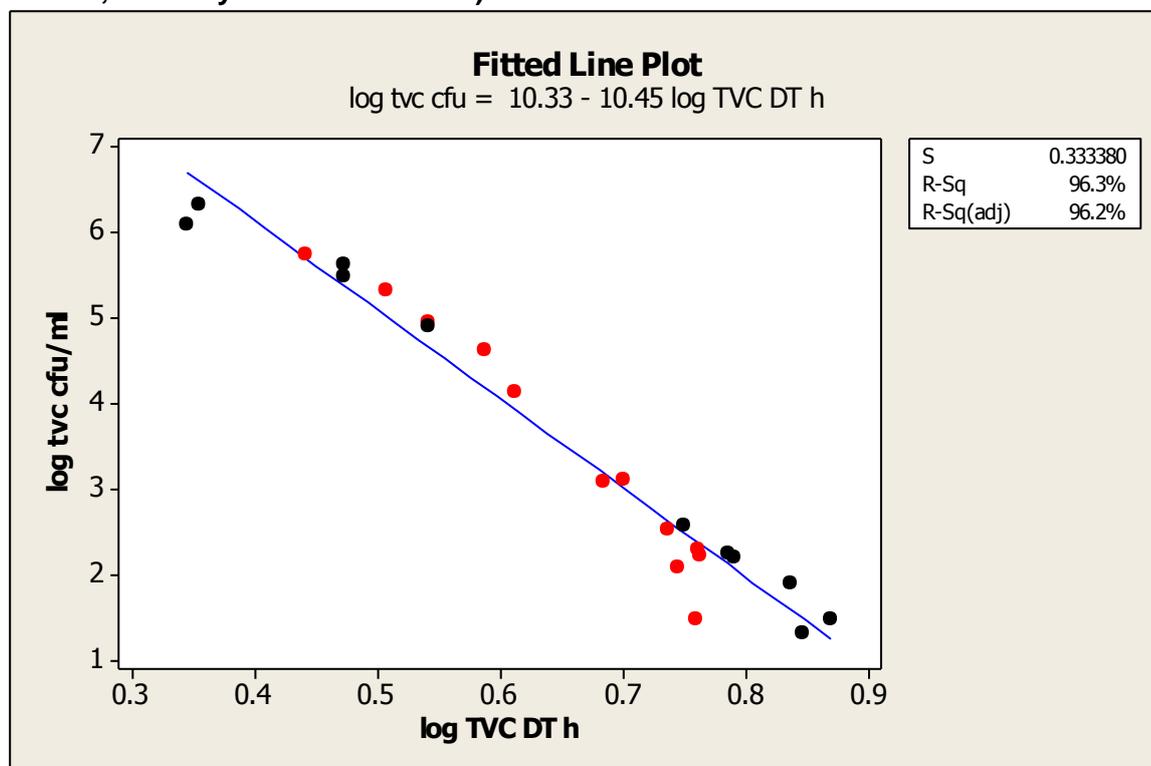
The data in Table 1 show that the Speedy Breedy is capable of detecting low levels of TVC in ice-cream. Sample of ice-cream containing TVC at a level of <10 cfu/ml to 2.2x10⁶ cfu/ml were detected within 2.2 to 7.4 hours.

Table 1: Data for TVC in ice-cream

Set up date	Product	cfu/ml TVC	DT min	DT hr
14th Jan 2014	Vanilla	<10	352	5.87
14th Jan 2014	Vanilla	30	345	5.75
14th Jan 2014	Vanilla	120	333	5.55
14th Jan 2014	Vanilla	1300	301	5.02
14th Jan 2014	Vanilla	14000	246	4.10
14th Jan 2014	Vanilla	220000	193	3.22
14th Jan 2014	Chocolate	30	444	7.40
14th Jan 2014	Chocolate	80	412	6.87
14th Jan 2014	Chocolate	180	366	6.10
15th Jan 2014	Vanilla	163	348	5.80
15th Jan 2014	Vanilla	200	347	5.78
15th Jan 2014	Vanilla	345	327	5.45
15th Jan 2014	Vanilla	1200	290	4.83
15th Jan 2014	Vanilla	42000	232	3.87
15th Jan 2014	Vanilla	570000	166	2.77
15th Jan 2014	Chocolate	20	421	7.02
15th Jan 2014	Chocolate	160	371	6.18
15th Jan 2014	Chocolate	380	337	5.62
16th Jan 2014	Chocolate	83000	209	3.48
16th Jan 2014	Chocolate	430000	178	2.97
16th Jan 2014	Chocolate	1300000	133	2.22
16th Jan 2014	Chocolate	90000	209	3.48
16th Jan 2014	Chocolate	310000	178	2.97
16th Jan 2014	Chocolate	2200000	136	2.27

It can be seen from Figure 1 that there is very good agreement between the TVC in ice-cream and the Detection time on the Speedy Breedy. There was a good correlation between the two methods ($R^2 = 96\%$)

Figure 1: Scatter plot of \log_{10} cfu/ml TVC versus \log_{10} detection time in hours. (red symbols = vanilla, black symbols=chocolate)



4. CONCLUSION

The data from this study has shown

- The Speedy Breedy was a useful screening tool for testing the total bacterial count in ice-cream samples.
- There was a good agreement between detection time and microbial count. End users should be able to produce a calibration graph that would allow them to determine microbial levels of their ice-cream products based on detection time
- 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.