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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 *E.coli*, coliforms and TVC :Summary Report**

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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 *E.coli*, coliforms and TVC 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

- 5 to 10 hrs for *E.coli* counts in ice-cream ranging from <10 to 10,000 cells per ml of ice-cream.
- 2 to 7 hrs for total bacterial counts in ice-cream ranging from <10 to over a million cells per ml of ice-cream
- 7 to 11 hrs for coliform counts in ice-cream ranging from <10 to 10,000 cells per ml of ice-cream.

Full experimental details and results are given in the individual product reports.

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. Experimental Approaches

Two types of ice-cream were used in the trial: Vanilla Ice-cream (Fat g/100ml: 2.6; Protein g/100ml: 1.3; Carbs g/100g: 9.6) and Chocolate Ice-cream (Fat g/100ml: 2.7; Protein g/100ml: 1.5; Carbs g/100g: 9.4)

The ice-cream was inoculated within different levels of the test organisms. The levels of microorganisms present per gram of product were evaluated using both conventional ISO methods and the Speedy Breedy.

The detection times in the Speedy Breedy were obtained using the following conditions.

Organism	Range of cells per ml ice-cream	Vessel type	Test conditions
TVC	<10 to 10 ⁶ cfu/ml	TSB	36°C/48hr
coliforms	<10 to 10 ³ cfu/ml	MacConkey	36°C/48hr
<i>E.coli</i>	<10 to 10 ³ cfu/ml	MacConkey	36°C/48hr
		MacConkey	44°C/48hr

For the conventional test, the numbers of cells 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. For counts of <10, the limit of detection/square root of 2 was used as the most likely count.

3. RESULTS

E.coli: Table 1 contains the data from analysis of the ice-cream samples as cfu/ml, detection time in minutes and detection time in hours at 36°C.

This is also shown in Figure 1 as log₁₀ cfu/ml versus log₁₀ detection time for ice-cream at 36°C.

The data shows that the Speedy Breedy is capable of detecting low levels of *E.coli* in ice-cream.

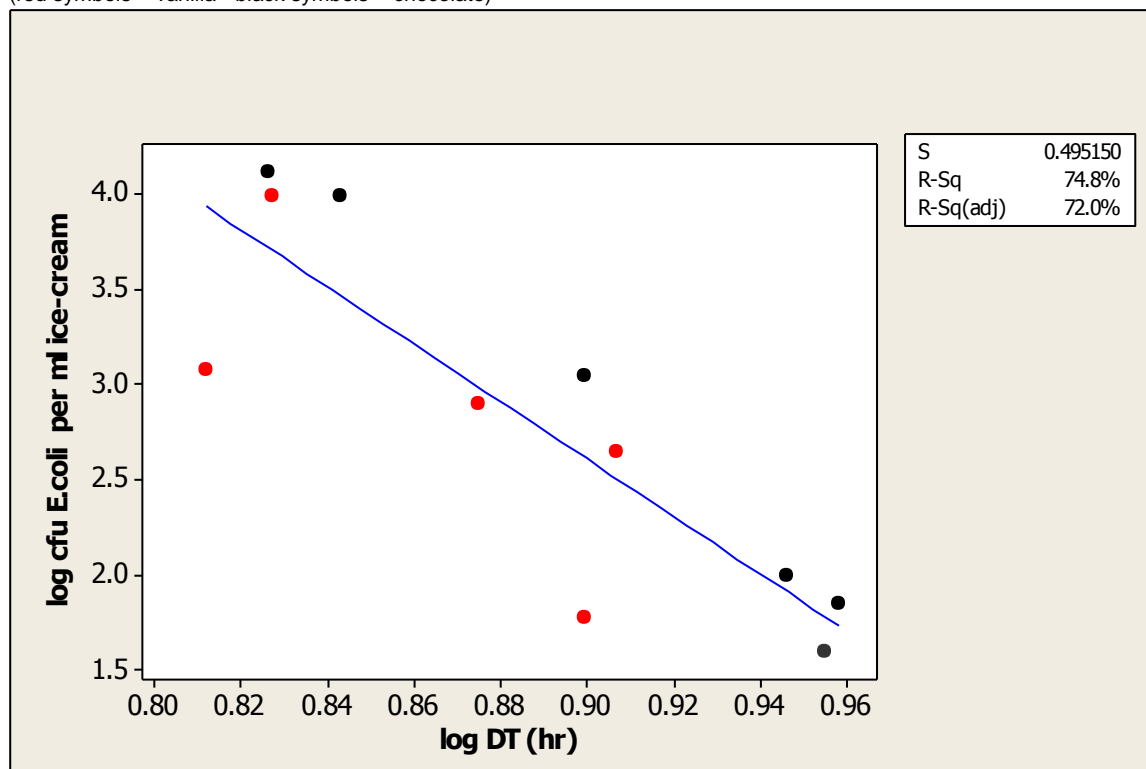
Sample of ice-cream containing *E.coli* at a level of <10 cfu/ml to 10⁴ cfu/ml were detected within 4.87 to 10.38 hours at 36°C and within 9.77hrs at 44°C.

Table 1: Data for *E.coli* in ice-cream

Studies at 36°C				Studies at 44°C			
Product	cfu/ml <i>E.coli</i>	DT min	DT hour	Product	cfu/ml <i>E.coli</i>	DT min	DT hour
Vanilla	<10	470	7.83	Vanilla	<10	470	7.83
Vanilla	<10	471	7.85	Vanilla	<10	471	7.85
Vanilla	100	292	4.87	Vanilla	100	292	4.87
Vanilla	440	484	8.07	Vanilla	440	484	8.07
Chocolate	<10	603	10.05	Chocolate	<10	603	10.05
Chocolate	<10	623	10.38	Chocolate	<10	623	10.38
Chocolate	40	541	9.02	Chocolate	40	541	9.02
Chocolate	100	530	8.83	Chocolate	100	530	8.83
Vanilla	60	476	7.93	Vanilla	60	476	7.93
Vanilla	800	450	7.50	Vanilla	800	450	7.50
Vanilla	12000	389	6.48	Vanilla	12000	389	6.48
Vanilla	9800	403	6.72	Vanilla	9800	403	6.72
Chocolate	70	545	9.08	Chocolate	70	545	9.08
Chocolate	1100	476	7.93	Chocolate	1100	476	7.93
Chocolate	13000	402	6.70	Chocolate	13000	402	6.70
Chocolate	9800	418	6.97	Chocolate	9800	418	6.97

Figure 1: Fitted line plot of log₁₀ cfu/ml *E.coli* versus log₁₀ detection time in hours at 36°C

(red symbols = vanilla - black symbols = chocolate)



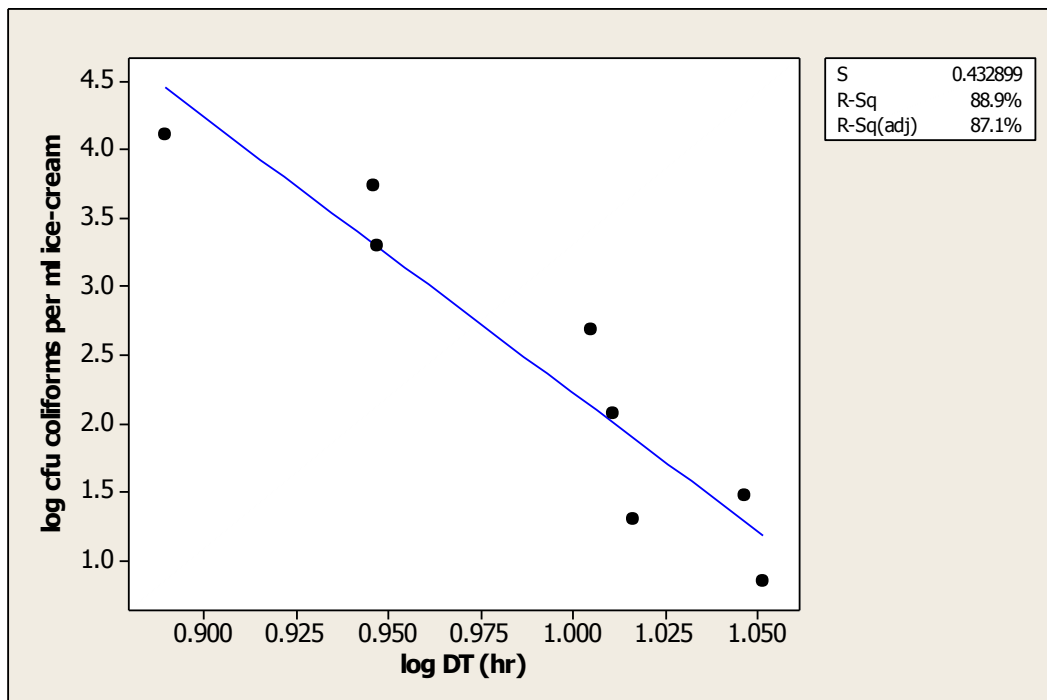
Coliforms: Table 2 contains the data from analysis of the ice-cream samples as cfu/ml, detection time in minutes and detection time in hours. This is also shown in Figure 2 as log₁₀ cfu/ml versus log₁₀ detection time in hours for chocolate ice-cream.

The data shows 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 1.2x10⁴ cfu/ml were detected within 7.67 to 11.27 hours.

Table 2: Data for coliforms in ice-cream

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

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



Total bacterial count (TVC): Table 3 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 3 as log₁₀ cfu/ml versus log₁₀ detection time in hours.

The data in Table 3 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.

Figure 3: Scatter plot of log₁₀ cfu/ml TVC versus log₁₀ detection time in hours
(red symbols = vanilla, black symbols=chocolate)

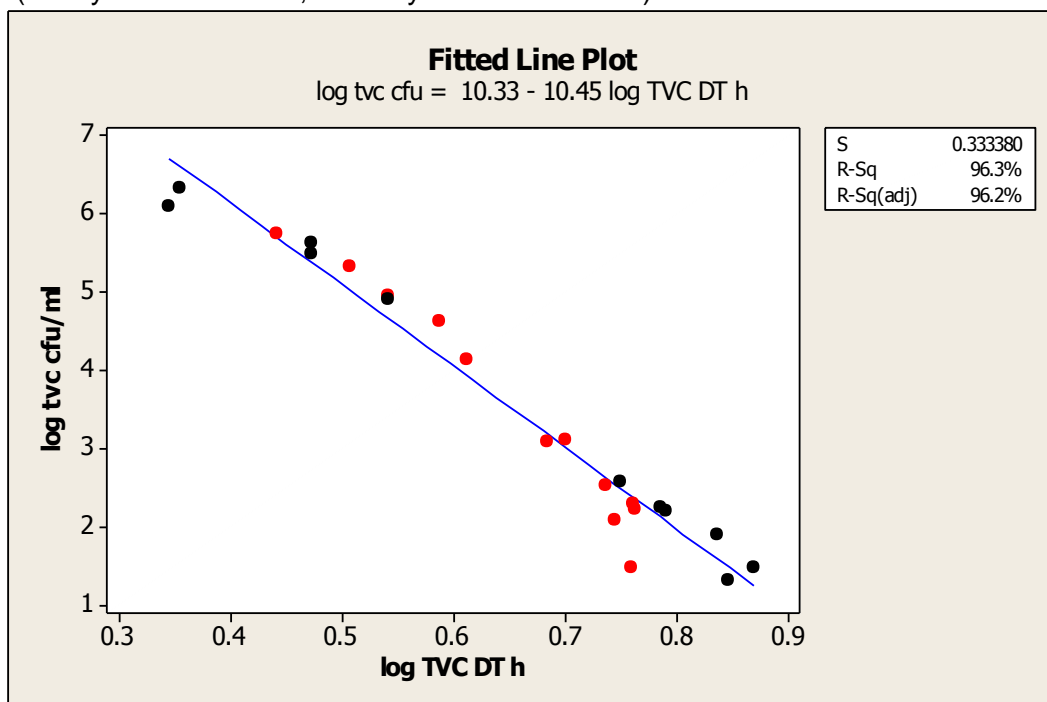


Table 3: Data for TVC in ice-cream

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

4. CONCLUSION

The data from this study has shown

- The Speedy Breedy is useful as a screening tool where samples of ice-cream containing coliform, TVC or *E.coli* bacteria can be tested to see if they give a detection event within a threshold time.
- Based on the data presented here a detection time of 2 to 11 hr would equate to microbial levels of between 10 and 10⁶ cfu/g ice-cream.
- 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. 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.

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