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Campden BRI
food and drink innovation

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*

Work performed by Campden BRI (Chipping Campden) Limited
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Our ref: MB-REP-131801-2 E.coli
Page count: 8

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* 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 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
Escherichia coli	16041
Escherichia coli	16189
Escherichia coli	16244

Prior to each experiment, the culture were grown in Nutrient Broth at 37°C for 18-24hours. The numbers of cells present were estimated microscopically using a haemocytometer and the cultures were 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, 10^1 cfu/g, 10^2 cfu/g, 10^3 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 16649-2:2001. Serial dilutions were made in MRD and 1ml samples of each dilution were transferred to 90ml Petri dishes and levels of *E.coli* enumerated.

Organism	Test method	Method Summary*
E.coli enumeration	ISO 16649-2:2001	Pour plate plus TBX Incubation at 44±1°C for 18-24h

For the Speedy Breedy, the chambers were set to run at 36°C for 48hours or 44°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 $\log_{10}$ number of cfu/ml were plotted against the $\log_{10}$ DT in hours. 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 at 36°C. This is also shown in Figure 1 as $\log_{10}$ cfu/ml versus $\log_{10}$ detection time for both ice-cream types 36°C, in Figure 2 for Vanilla ice-cream 36°C and in Figure 3 for Chocolate ice-cream at 36°C

Table 2 and Figures 4, 5 and 6 contain similar data for Speedy Breedy MacConkey vessels incubated at 44°C.

The data in Tables 1 and 2 show 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 10^4 cfu/ml were detected within 4.87 to10.38 hours at 36°C and within 9.77hrs at 44°C.

Table 1: Data for *E.coli* in ice-cream at 36°C

Set up date	Product	cfu/ml E.coli	DT min	DT hour
11-Dec 2013	Vanilla	<10	470	7.83
11-Dec 2013	Vanilla	<10	471	7.85
11-Dec 2013	Vanilla	100	292	4.87
11-Dec 2013	Vanilla	440	484	8.07
11-Dec 2013	Chocolate	<10	603	10.05
11-Dec 2013	Chocolate	<10	623	10.38
11-Dec 2013	Chocolate	40	541	9.02
11-Dec 2013	Chocolate	100	530	8.83
12-Dec 2013	Vanilla	60	476	7.93
12-Dec 2013	Vanilla	800	450	7.50
12-Dec 2013	Vanilla	12000	389	6.48
12-Dec 2013	Vanilla	9800	403	6.72
12-Dec 2013	Chocolate	70	545	9.08
12-Dec 2013	Chocolate	1100	476	7.93
12-Dec 2013	Chocolate	13000	402	6.70
12-Dec 2013	Chocolate	9800	418	6.97

Figure 1 shows that there was a good correlation between detection time and bacterial numbers for *E.coli* at 36°C. It is apparent from these that the agreement was better for chocolate ice-cream than vanilla. The data for vanilla ice-cream only is shown in Figure 2 where it can be seen that there is some scatter in the data. The data for chocolate ice-cream showed excellent agreement between detection time and bacterial counts (Figure 3)

Figure 1: Fitted line plot of log_{10} cfu/ml *E.coli* versus log_{10} detection time in hours at 36°C

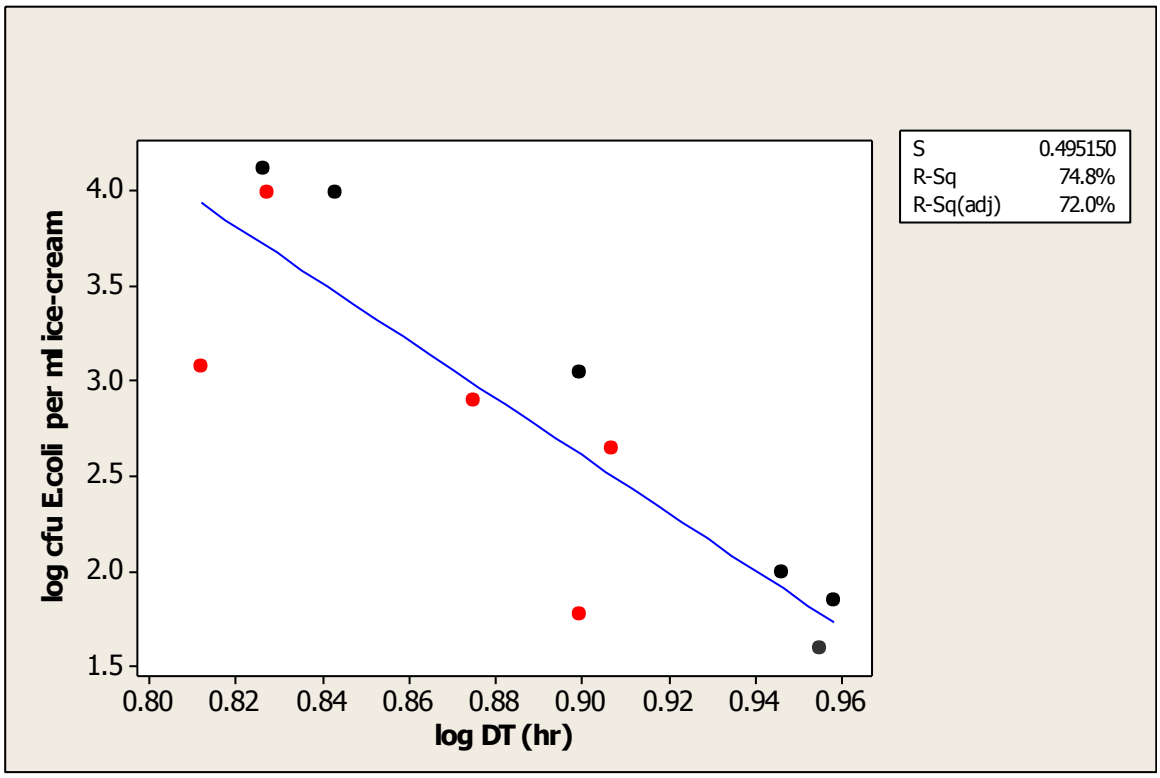


Figure 2. Fitted line plot of log_{10} cfu/ml *E.coli* in Vanilla ice-cream versus log_{10} detection time in hours at 36°C.

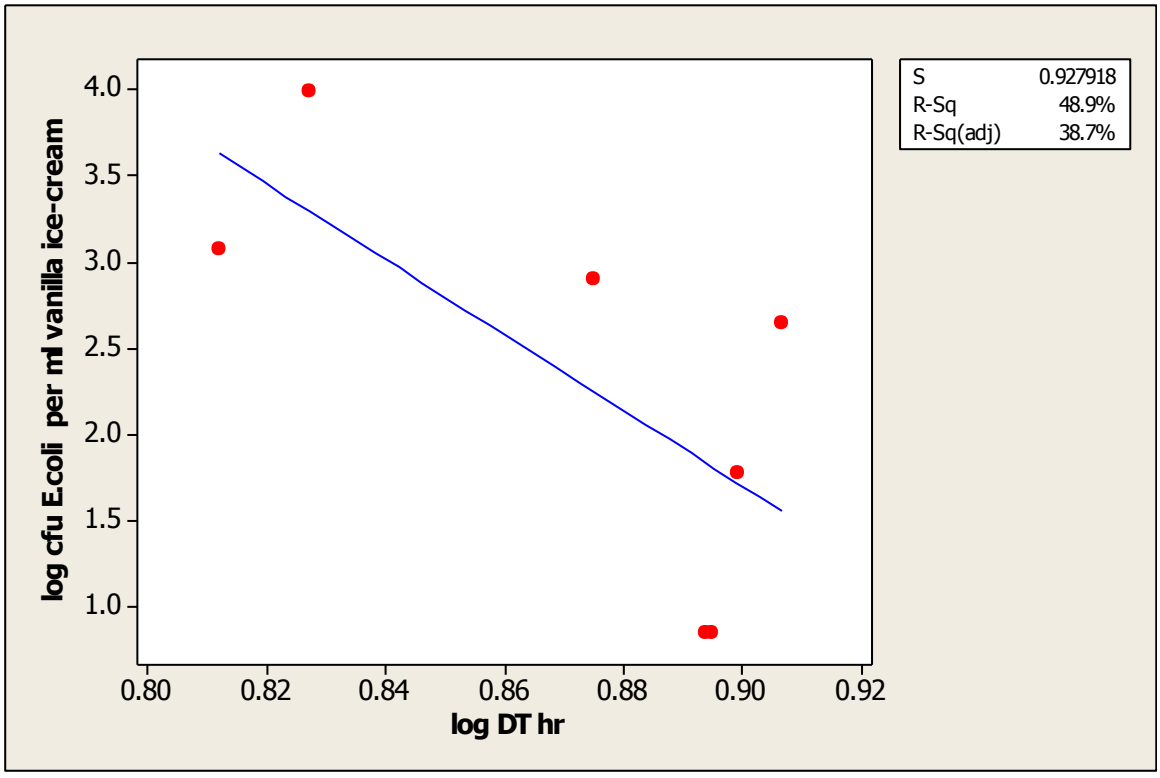


Figure 3. Fitted line plot of log_{10} cfu/ml *E.coli* in Chocolate ice-cream versus log_{10} detection time in hours at 36°C.

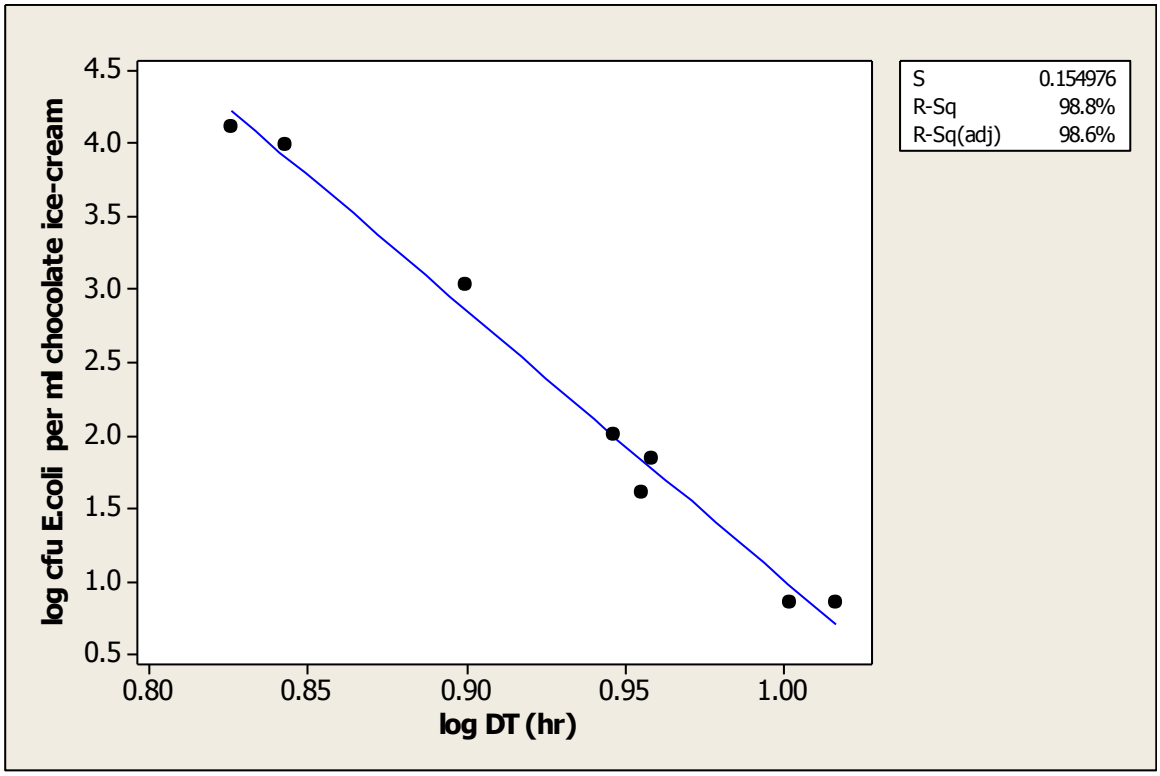


Table 2: Data for *E.coli* in ice-cream at 44°C

Set up date	Product	cfu/ml E.coli	DT min	DT hour
08-Jan 2014	Vanilla	<10	550	9.17
08-Jan 2014	Vanilla	10	539	8.98
08-Jan 2014	Vanilla	130	520	8.67
08-Jan 2014	Vanilla	1400	471	7.85
08-Jan 2014	Chocolate	<10	586	9.77
08-Jan 2014	Chocolate	<10	541	9.02
08-Jan 2014	Chocolate	110	552	9.20
08-Jan 2014	Chocolate	850	552	9.20
09-Jan 2014	Vanilla	<10	557	9.28
09-Jan 2014	Vanilla	90	508	8.47
09-Jan 2014	Vanilla	490	98	1.63
09-Jan 2014	Vanilla	5200	75	1.25
09-Jan 2014	Chocolate	<10	586	9.77
09-Jan 2014	Chocolate	<10	556	9.27
09-Jan 2014	Chocolate	427	72	1.20
09-Jan 2014	Chocolate	3500	79	1.32

The data for tests done at 44°C are shown in Figures 4 to 6. Figure 4 shows the data for both ice-cream types. There are some fast detection which appeared unusually fast compared to the general

trend in the data. If these data are taken out of the data set then it can be seen that there is good agreement between detection time and bacterial count for vanilla ice-cream (Figure 5) but not for chocolate (Figure 6) where there are fewer data points.

Figure 4: Scatter plot of log_{10} cfu/ml *E.coli* versus log_{10} detection time in hours at 44°C

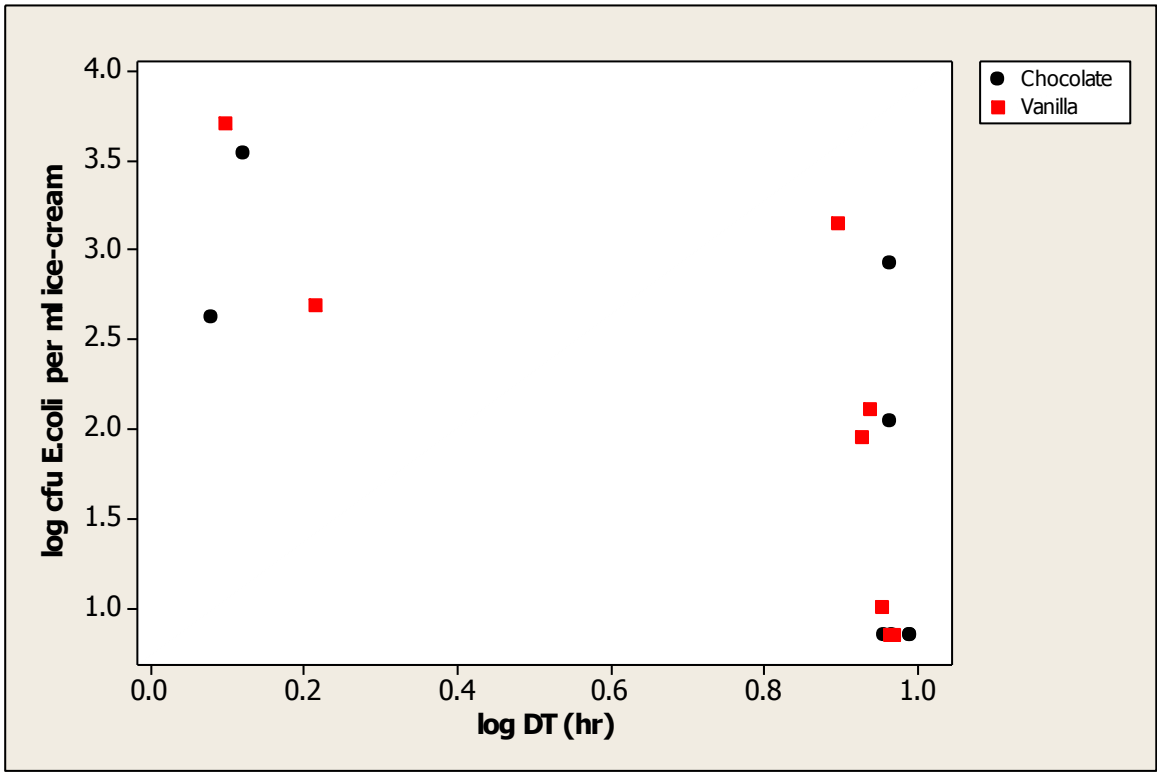


Figure 5. Fitted line plot of log_{10} cfu/ml *E.coli* in Vanilla ice-cream versus log_{10} detection time in hours at 44°C.

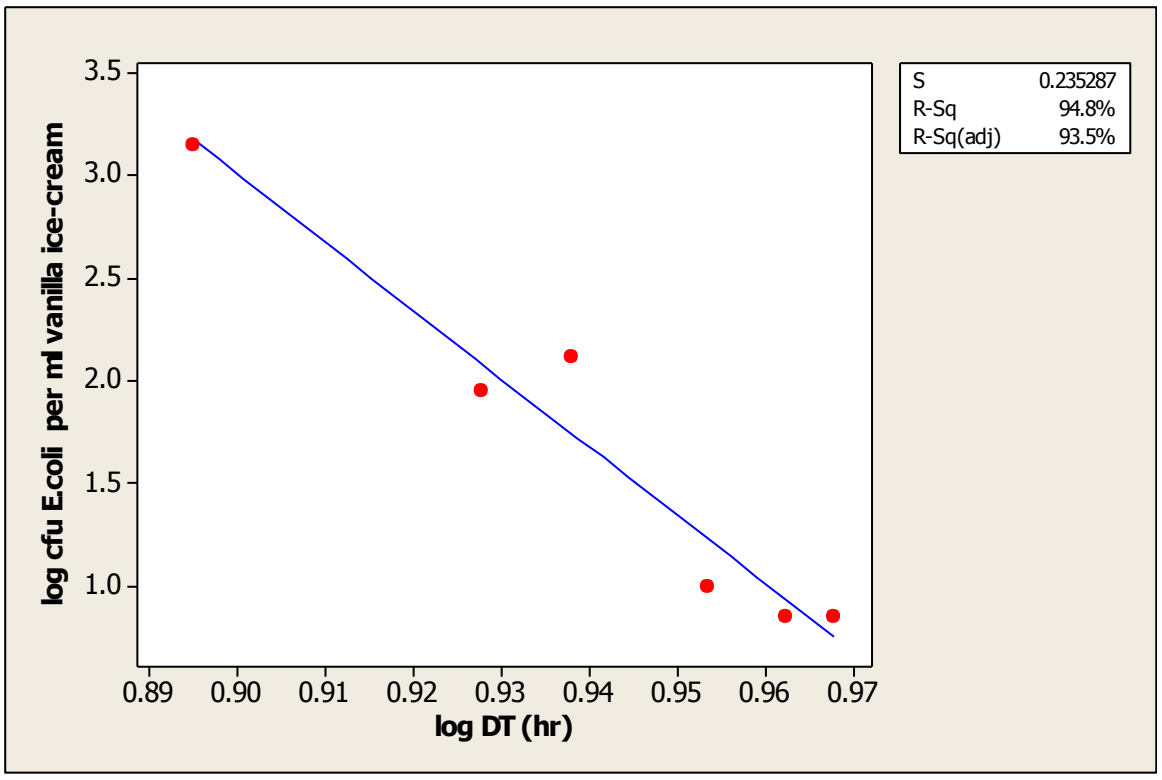
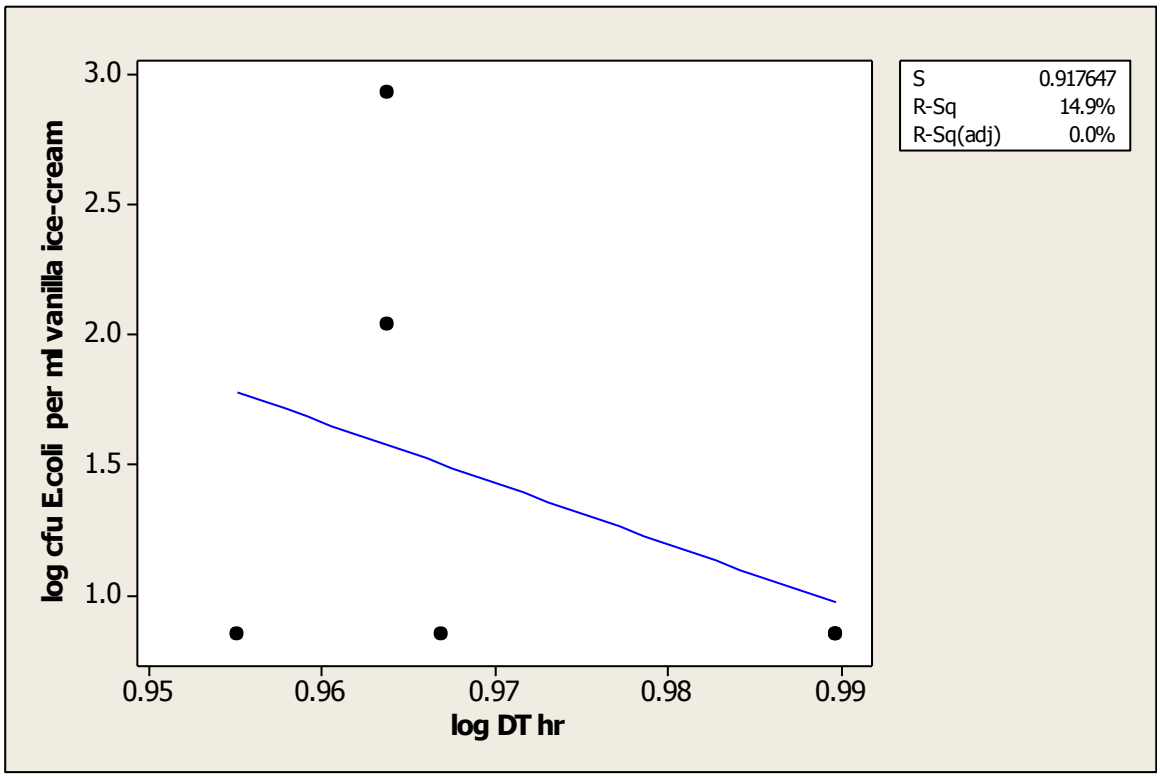


Figure 6. Fitted line plot of log_{10} cfu/ml *E.coli* in Chocolate ice-cream versus log_{10} detection time in hours at 44°C.



4. CONCLUSION

The data from this study has shown

- The Speedy Breedy shows the potential to be used as a screening tool where samples of ice-cream containing *E.coli 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 and incubation temperature used. 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 6.8 hr or greater would equate to an *E.coli* level of between 10 and 10^4 cfu/g ice-cream for test done at 36°C. Faster detection times would indicate a higher level of contamination than 10^4 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.