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Confidential report for:

Bactest

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Report on:

Application of Speedy Breedy to determine the microbiological quality of pasteurised milk

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 the potential of the system 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 total microbial population present in pasteurised milk samples.

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 data provided in this report is intended to provide demonstration data that the Speedy Breedy can be used to determine the microbiological quality of milk. 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

2.1 Product

Chilled pasteurised whole milk was used for these trials. A 2 litre bottle of pasteurised milk was purchased with approximately 7 days remaining on its shelf life. Samples were taken from the milk for the first set of quintuplet samples and the remainder of the milk was stored at 4-6°C to allow the levels of natural organisms to continue to develop in the milk. Samples were then tested at regular time intervals throughout the life of the product for the 2nd, 3rd, 4th and 5th set of quintuplet samples.

The testing was done over the period 23/7/13 to 2/8/13.

2.2 Organisms

There was no artificial contamination of the milk. All organisms were naturally present in the milk as purchased.

2.3 Experimental matrix

Each of five contamination levels were analysed in quintuplet making a total of 25 analyses of Speedy Breedy versus ISO standard ISO 4833:2003.

For each set of samples, milk was analysed for levels of APC using both conventional ISO methods and the Speedy Breedy system.

2.4 Microbiological analysis

For the conventional tests, 5 x 1ml samples of milk were taken and added to 9ml Maximum Recovery Diluent (MRD). Serial dilutions were made in MRD and 1ml samples of each dilution were transferred to 90ml Petri dishes and analysed for aerobic plate count as shown below.

Organism	Test method	Method Summary*
Aerobic Plate count	TES-MB-002	Pour plate with PCA. Incubation at 30±1°C for 48±4h

For the Speedy Breedy, 5 x1ml samples of the milk were added to vessels containing dehydrated capsules of TSB which had been rehydrated with 50ml sterile distilled water. In addition 5 X 50ml samples of milk were added to empty vessels without any laboratory media. The chambers were set to run at 30°C for the TSB vessels and milk vessels. The Speedy Breedy was set to run for 48h but was stopped once a significant event was recorded.

2.5 Analysis of results

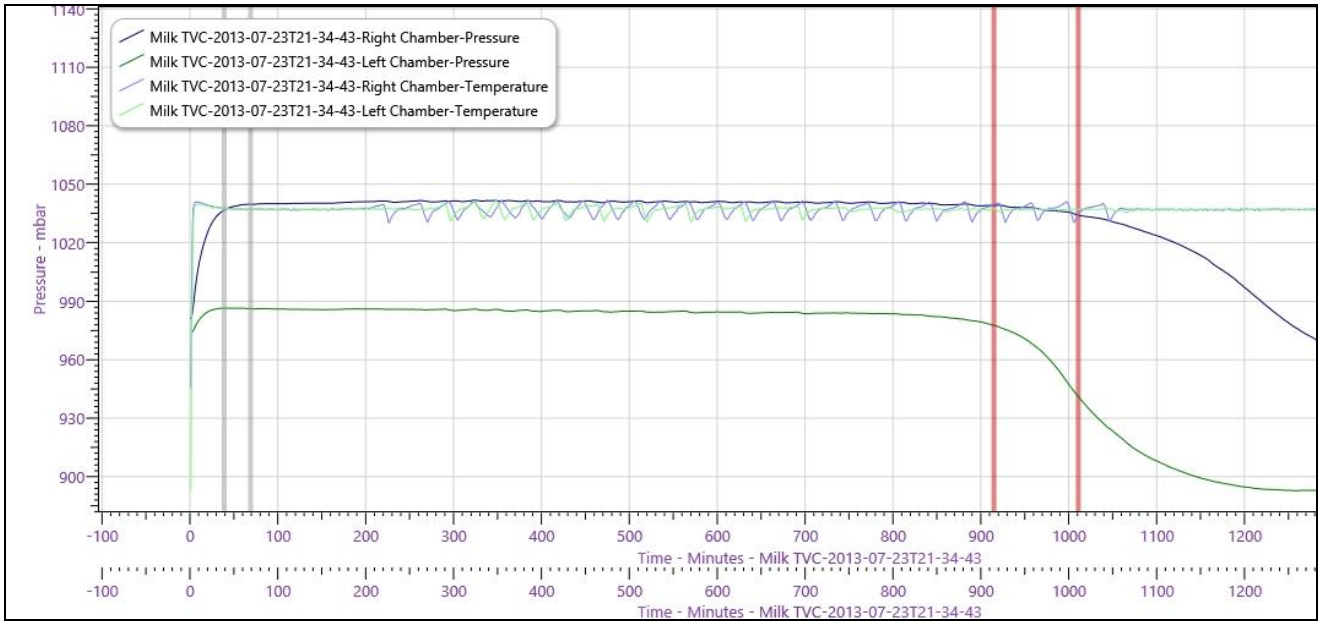
For the conventional test, the numbers of cfu per ml of product were calculated.

For the Speedy Breedy, the times 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 and the correlation was calculated.

An example of the Speedy Breedy output for milk is shown in Figure 1. This was characteristic of the graphs for milk where the change is pressure was always negative.

Figure 1: Speedy Breedy Graph for milk and milk in TSB



3 RESULTS AND DISCUSSION:

Table 1 contains the data for the milk samples as cfu/ml, detection time in minutes and detection time in hours. This is also shown in Figures 2 for milk as $\log_{10}$ cfu/ml versus $\log_{10}$ detection time in hours.

The data show that the Speedy Breedy is capable of detecting low levels of organisms in pasteurised milk or pasteurised milk incubated in TSB. For the neat milk, the level of organisms ranged from 10^2 to 10^6 cfu/ml. The correlation between the plate count and Speedy Breedy Detection times were reasonable (r= 0.79) but the data showed that whilst the times were consistent at the highest contamination level, there was a lot of variation in count and detection time at the lower levels.

Table 1: Data for naturally occurring organisms in neat milk and milk in TSB

Samples	neat milk cfu/ml	neat milk DT (min)	neat milk DT (hr)		TSB + milk cfu/ml	TSB + milk DT (min)	TSB + milk DT (hr)
1a	660	1258	20.97		13	1210	20.17
1b	550	1152	19.20		11	935	15.58
1c	680	1010	16.83		14	914	15.23
1d	510	970	16.17		10	1230	20.50
1e	370	1069	17.82		7	1182	19.70
2a	1000	974	16.23		20	1149	19.15
2b	3000	943	15.72		6	1394	23.23
2c	6000	1205	20.08		12	1301	21.68
2d	1100	970	16.17		22	1222	20.37
2e	500	860	14.33		10	1187	19.78
3a	2090	944	15.73		42	1243	20.72
3b	1145	1150	19.17		23	1201	20.02
3c	1545	862	14.37		31	1300	21.67
3d	1090	970	16.17		21	1201	20.02
3e	2545	791	13.18		51	678	11.30
4a	1527	402	6.70		31	1081	18.02
4b	3400	352	5.87		68	950	15.83
4c	4727	861	14.35		95	1190	19.83
4d	1627	352	5.87		33	1131	18.85
4e	1191	405	6.75		24	1065	17.75
5a	860000	148	2.47		17200	612	10.20
5b	1150000	140	2.33		23000	606	10.10
5c	1160000	139	2.32		23200	599	9.98
5d	720000	142	2.37		14400	606	10.10
5e	460000	142	2.37		9200	600	10.00

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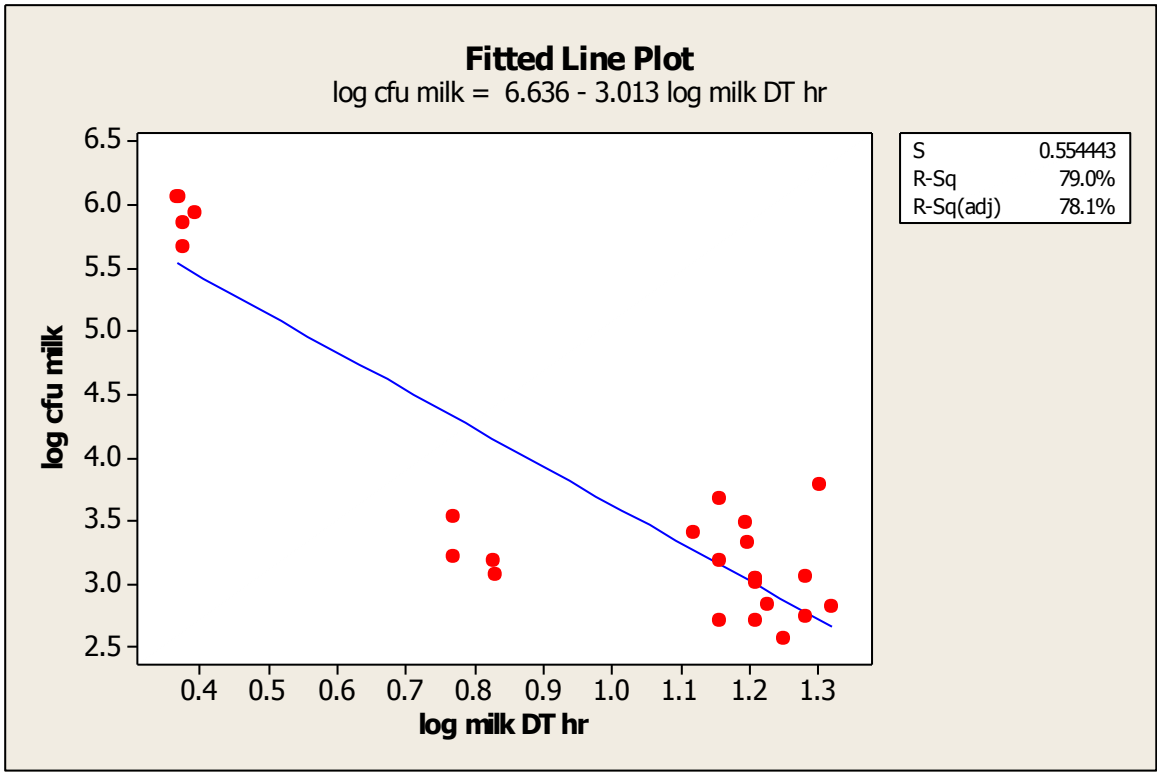
Figure 1: Log cfu/ml milk versus log DT (hr)



The data would not be suitable for a quantitative assessment of numbers based on detection time but could be used as a screening tool for batches of milk. For example, Figure 2 shows the $\log_{10}$ cfu/ml TSB versus $\log_{10}$ detection time in hours. If you draw a quadrant through the data, it can be seen that the bottom right hand corner contains all (except 1) of the samples where the $\log_{10}$ count was <2 per ml. For all these samples the detection time was equal to or greater than 15hours.

So based on this data, any sample of milk in TSB that had not detected within 15hr would have $<10^2$ cfu/ml. Any detection time faster than 15 hr would have $>10^2$cfu/ml.

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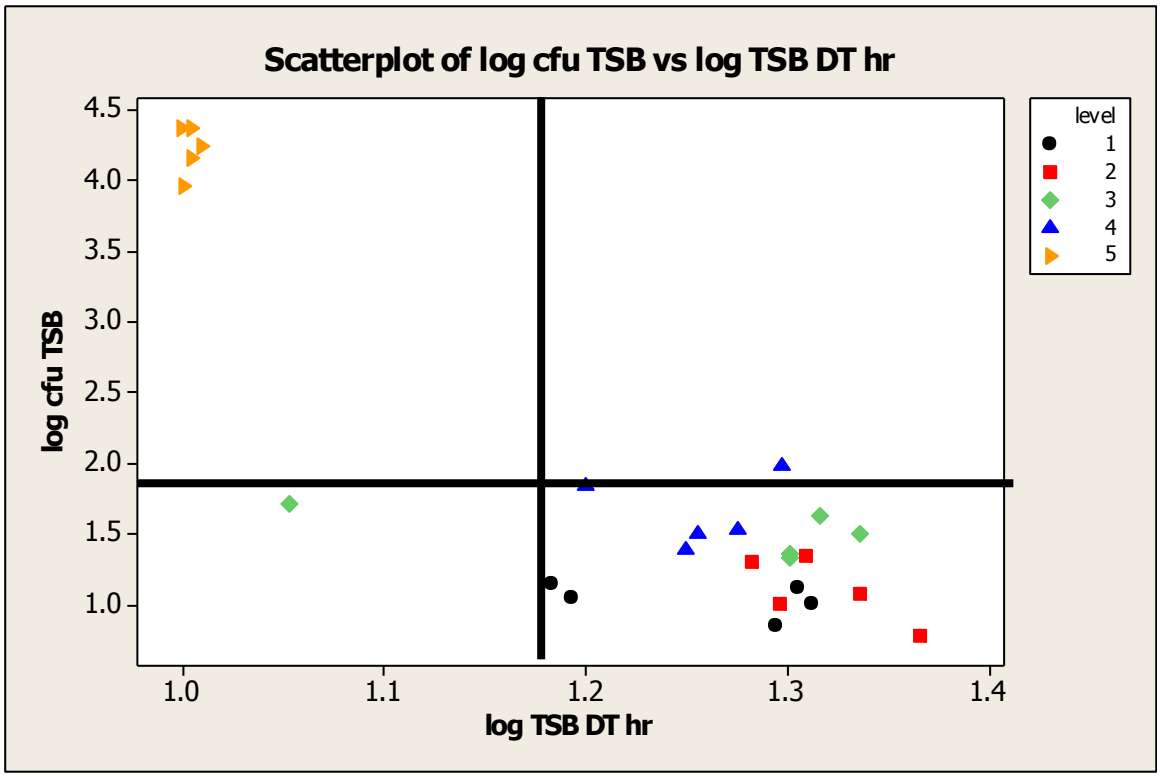
Figure 2: Log cfu/ml TSB versus log DT (hr)



4 CONCLUSIONS

- The Speedy Breedy has shown some correlation between log cfu/ml and detection time for natural microflora in milk products (r =0.79).
- There is good agreement in the detection times between replicates at high contamination levels but more variation at low levels.
- The data shows promise for use as a screening tool where samples of milk are tested to see if they detect within a threshold time. Based on the data presented here a detection time of >15hr equates to a level of $<10^2$ cfu/ml in TSB containing milk.
- 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 milk processing, 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.