Campden BRI group:
Campden BRI (registered no. 510618)
Campden BRI (Chipping Campden) Limited (registered no. 3836922)
Campden BRI (Nutfield) (registered no. 2690377)

Registered office:
Station Road ◆ Chipping Campden ◆ Gloucestershire ◆ GL55 6LD ◆ UK

Confidential report for:

Bactest

FAO: Annie Brooking/Derek Price
St. Johns Innovation Centre
Cowley Road
Cambridge
CB4 OWS

Report on:

Application of Speedy Breedy to determine the microbiological quality of raw meat

Work performed by Campden BRI (Chipping Campden) Limited
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Contact details:

Gail Betts ◆ Microbiology ◆ Campden BRI (Chipping Campden) Limited
gail.betts@campdenbri.co.uk ◆ Tel: +44(0)1386 842071 ◆ Fax: +44(0)1386 842100
We value your opinion: **http://www.campdenbri.co.uk/campdenbri/fdbck.php**

Report issued and authorised by:

Campden BRI (Chipping Campden) Limited
Dr Gail Betts ◆ Manager, Microbiological Safety and Spoilage Section

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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 and more specifically the levels of coliforms present in raw meat 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 meats. 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

Raw rump steak was used for these trials. The first lot of steak was purchased on 19/8/13 with a use-by date of 23/8/13.

Samples of steak were taken for the first set of trials on 19/8/13 and the steak was placed back in the refrigerator at 4-6°C to allow the levels of natural organisms present to increase in numbers before testing sets 2 and 3. The steak was frozen to prevent excessive microbial growth and was thawed before use for testing set 4. A second piece of steak was purchased on 2/9/13 for the final set of samples. The testing was done over the period 19/8/13 to 2/9/13.

2.2 Organisms

E. coli CRA 16224 was used for the coliform tests. 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 inoculums onto the meat samples.

2.3 Experimental matrix

Five sets of samples were analysed at different contamination levels. For the first set of samples the levels of naturally present APC and coliforms were analysed. For the remaining 4 sets of meat, the levels of coliforms were increased using *E. coli* CRA 16224.

For each set of samples, 5 x 10g samples of meat were taken and inoculated with 0.1ml dilution of coliforms to achieve a final level of between 10 and 10^5 cfu/g coliforms. The meat samples were then analysed for levels of APC and coliforms using conventional ISO methods and the Speedy Breedy system.

2.4 Microbiological analysis

Meat samples were analysed for TVC and coliforms using the conventional standard tests methods and the Speedy Breedy system using vessels containing either TSB or MacConkey broth.

10g samples of meat were taken and added to 90ml Maximum Recovery Diluent. For the conventional tests, serial dilutions were made in MRD and 1ml samples of each dilution were transferred to 90ml Petri dishes. One set of the dilutions was analysed for coliforms and one for aerobic plate count as shown below

Organism	Test method	Method Summary*
Coliform enumeration	TES-MB-005	Pour plate plus over layer with VRBA. Incubation at 37±1°C for 24±2h
Aerobic Plate count	TES-MB-002	Pour plate with PCA. Incubation at 30±1°C for 48±4h

For the Speedy Breedy, 1ml samples of the initial dilution were added to vessels containing dehydrated capsules of TSB or MacConkey broth which had been rehydrated with 50ml sterile distilled water. The chambers were set to run at 30°C for the TSB vessels and at 37°C for the MacConkey Broth vessels. The Speedy Breedy was set to run for 5 days but was stopped once a significant event was recorded.

2.5 Analysis of results

For the conventional test, the numbers of cells per gram 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/g were plotted against the $\log_{10}$ DT in hours and the correlation was calculated.

An example of the Speedy Breedy output for APC in meat is shown in Figure 1 and for coliforms in meat in Figure 2. Figure 1 is characteristic of all the graphs for APC where an initial drop in pressure is followed by an increase in pressure later as microbial growth increases. Figure 2 is characteristic of all the graphs obtained for coliforms in meat where the change is pressure was negative throughout incubation, although experience from the manufacturer has shown that there is usually a rise in pressure towards the end of the growth curve when the incubation time is extended.

Figure 1: Speedy Breedy Graph for APC in meat

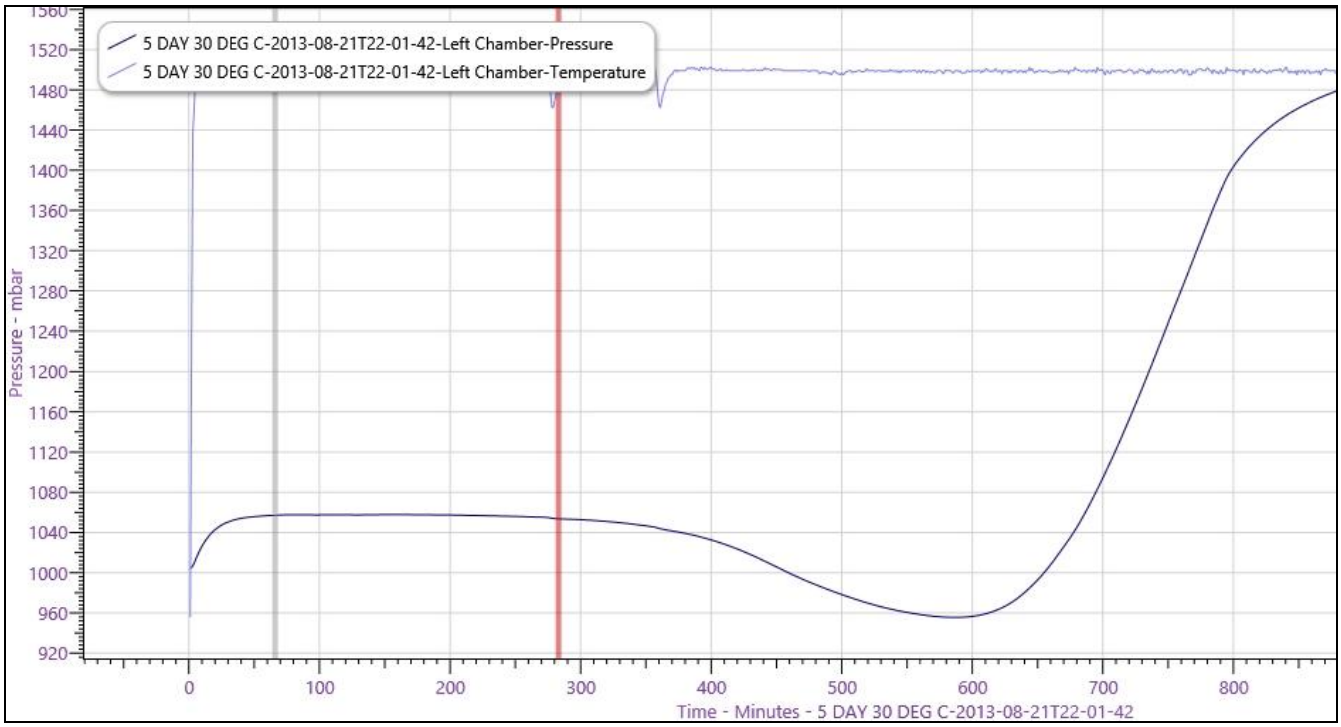
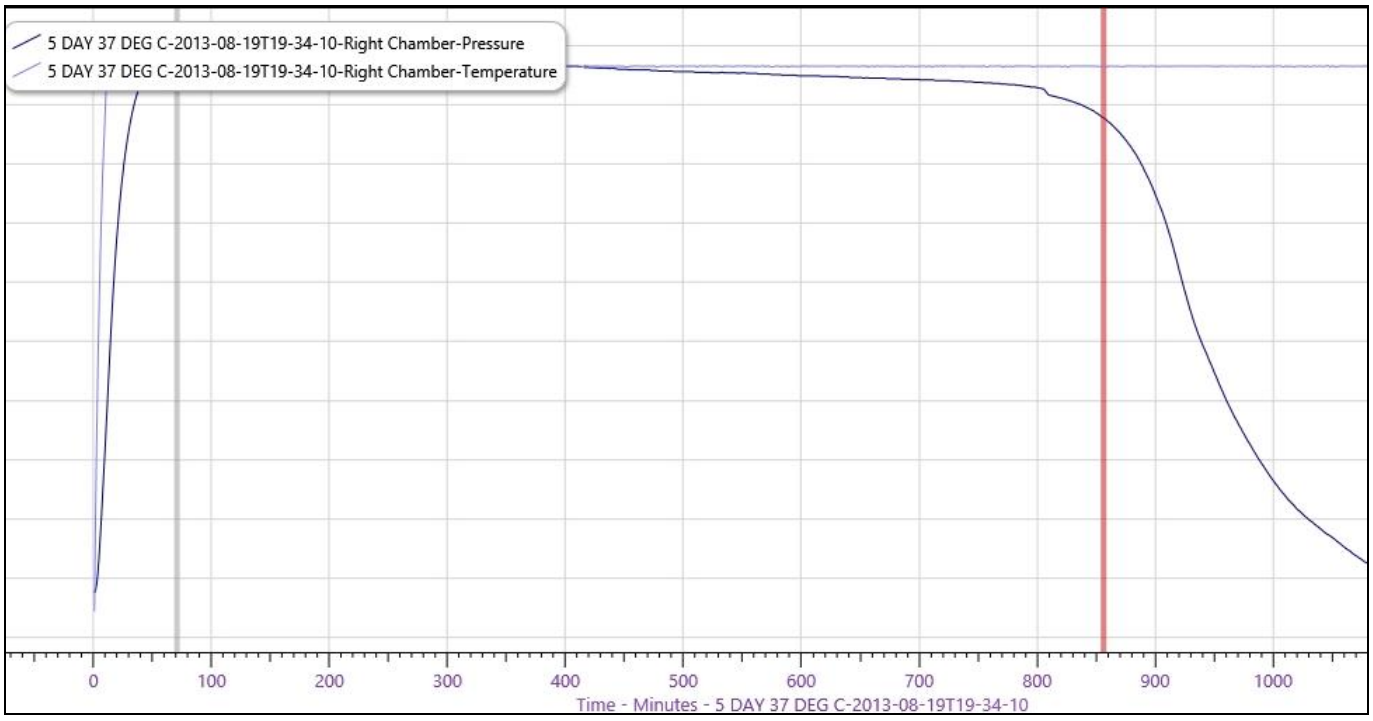


Figure 2: Speedy Breedy Graph for coliforms in meat



3 RESULTS AND DISCUSSION:

Table 1 contains the data for the meat samples as cfu/g, detection time in minutes and detection time in hours. This is also shown in Figure 3 as $\log_{10}$ cfu/g coliforms versus $\log_{10}$ detection time in hours and in Figure 4 as $\log_{10}$ cfu/g APC versus $\log_{10}$ detection time in hours.

There was a good correlation between $\log_{10}$ coliforms and $\log_{10}$ DT in hour (R-sq = 0.91). The use of a selective broth to selectively enrich the coliforms present in the meat samples seems to produce a consistent detection time based on the strain of *E. coli* used in these studies.

The agreement between $\log_{10}$ APC and detection time was not so consistent and appeared to be affected by the mix of organisms in the population. For the first three sets of samples there was an excellent agreement between the cfu/g and detection time. The correlation based on these samples alone would be R-sq =0.99. These samples were tested on consecutive days and had the same mix of microorganisms in them, albeit at increasing levels. The fourth set of samples was from the same batch of meat but had been frozen before testing. This may have selectively reduced the levels of some groups of organisms within the total population which seems to have reduced the overall respiration rate. The fifth set of samples was from a different piece of steak and the growth rate of the organisms present seemed slower than the organisms present on the first piece of steak.

Users of the system would need to build up a calibration graph based on the types of organisms present in their samples.

Figure 3: Log cfu/g coliforms in meat versus log DT (hr)

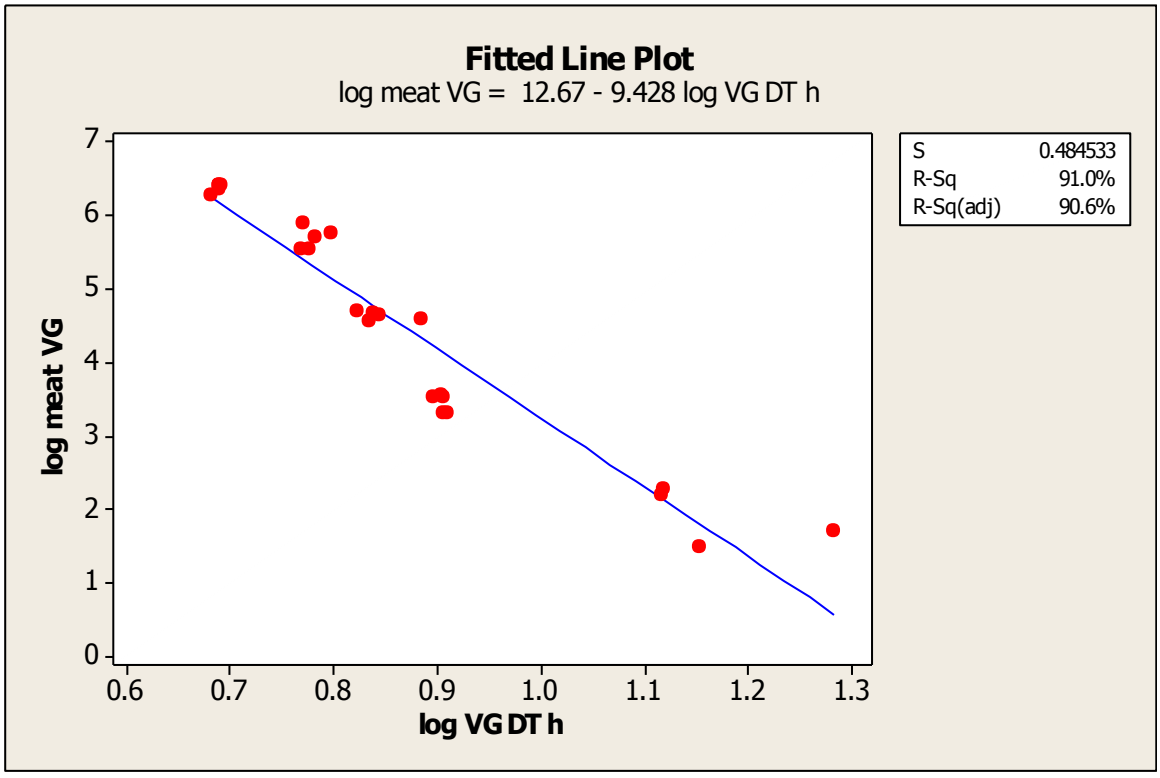
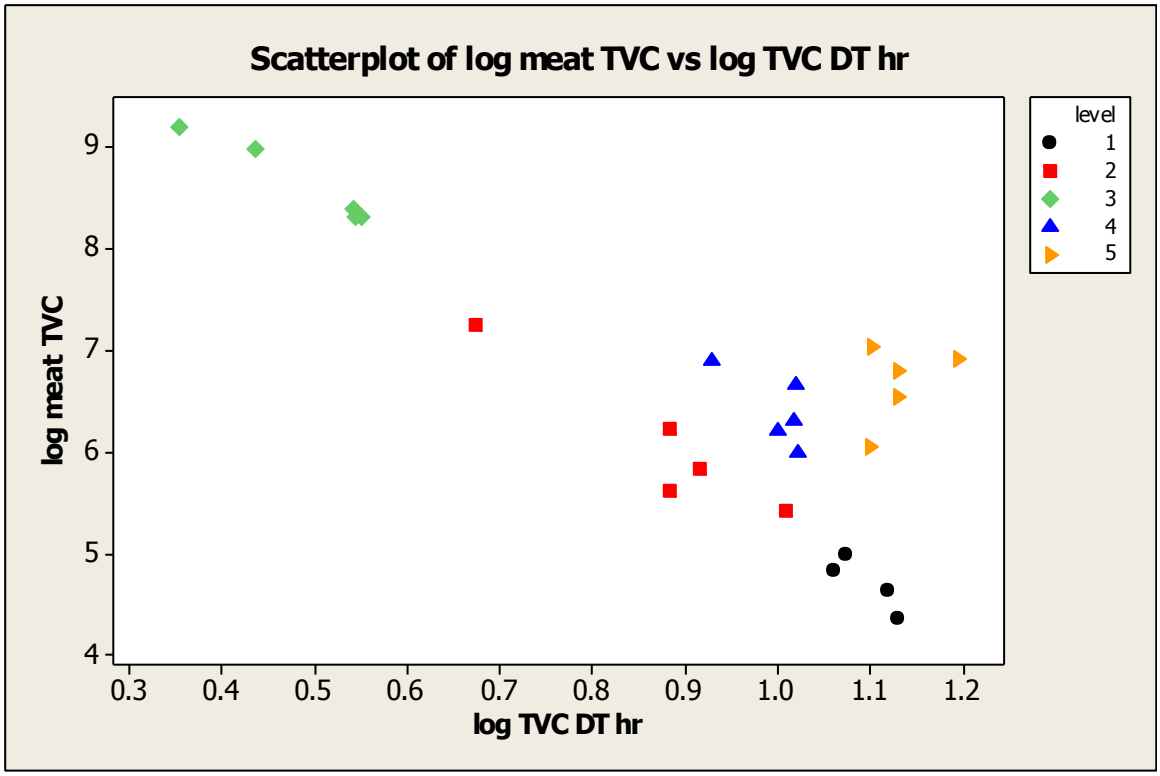


Table 1: Data for naturally occurring APC and inoculated coliforms in meat

Samples	TVC cfu/g	DT (min)	DT (hr)		Coliforms cfu/g	DT (min)	DT (hr)
1a	9.5×10^4	710	11.8		160	786	13.1
1b	6.8×10^4	689	11.5		190	789	13.2
1c	2.3×10^4	808	13.5		30	856	14.3
1d	*	858	14.3		<10	>1200	>20
1e	4.3×10^4	789	13.2		50	1153	19.2
2a	2.6×10^5	611	10.2		3.9×10^4	460	7.7
2b	4.2×10^5	459	7.7		4.5×10^4	419	7.0
2c	1.7×10^6	458	7.6		5.0×10^4	399	6.7
2d	6.8×10^5	494	8.2		4.7×10^4	414	6.9
2e	1.8×10^7	283	4.7		3.6×10^4	410	6.8
3a	1.6×10^9	136	2.3		8.0×10^5	354	5.9
3b	2.1×10^8	210	3.5		3.5×10^5	353	5.9
3c	2.5×10^8	209	3.5		3.5×10^5	360	6.0
3d	2.1×10^8	213	3.6		5.8×10^5	378	6.3
3e	9.4×10^5	164	2.7		5.2×10^5	364	6.1
4a	1.0×10^6	630	10.50		2.10×10^3	483	8.05
4b	2.0×10^6	626	10.43		2.0×10^3	488	8.13
4c	1.6×10^6	600	10.00		3.4×10^3	483	8.05
4d	7.7×10^6	510	8.50		3.3×10^3	474	7.90
4e	4.5×10^6	628	10.47		3.6×10^3	481	8.02
5a	1.2×10^7	758	12.63		2.6×10^6	294	4.90
5b	6.2×10^6	811	13.52		2.5×10^6	294	4.90
5c	8.2×10^6	938	15.63		1.9×10^6	289	4.82
5d	3.5×10^6	810	13.50		2.3×10^6	294	4.90
5e	1.1×10^6	754	12.57		2.5×10^6	296	4.93

Figure 4: Log cfu/g APC in meat versus log DT (hr)



4 CONCLUSIONS

- The Speedy Breedy has shown excellent correlation between log cfu/ml and detection time for coliforms in meat juice (r =0.91).
- The data shows promise for use of the Speedy Breedy as a quantitative tool for determination of the levels of coliforms in meat.
- The correlation between APC in meat and Speedy Breedy detection time appeared to be affected by differences in the microbial populations on the samples of meat examined. Where the same population was examined there was an excellent correlation between APC and detection time. However, when different pieces of meat containing different populations of organisms was examined the detection times were affected. Where the microbial populations in a meat processing facility were stable then it would be possible to use the Speedy Breedy as a semi quantitative tool after building up a suitable calibration graph.
- Speedy Breedy was fast compared with current techniques, taking only a few hours to detect positive contamination compared with two days for plate counts.
- Speedy Breedy can be used at the site of meat processing, removing the need for samples to be shipped to a laboratory, further reducing the time to achieve a result. Based on the results, Speedy Breedy would therefore represent a suitable screening tool for rapid on-site testing.

In summary, all micro-organisms tested in this project were detectable 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 low cell concentrations.

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

Campden BRI (Chipping Campden) Limited – part of the Campden BRI group
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