Rapid determination of microbial load on beef carcase hygiene swabs

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Mocon Inc recently collaborated on a study of bacterial load in beef carcase operations. A US based processor of carcases was interested in improving speed-to-result from carcase swab tests at various stages of cleaning.

The post-slaughter process contained seven quality control stages in which the carcase could be expected to have decreasing levels of bacterial load, assessed by Total Plate Count or Aerobic Plate Count (APC). The facility being analysed was at the time using an alternate agar plating method to the US BAM reference method.

Issues to consider

In most APC (TVC, TPC) protocols, the value for the reference agar plate method or alternatives take between 48 and 72 hours to enumerate, during which time the plates are incubated.

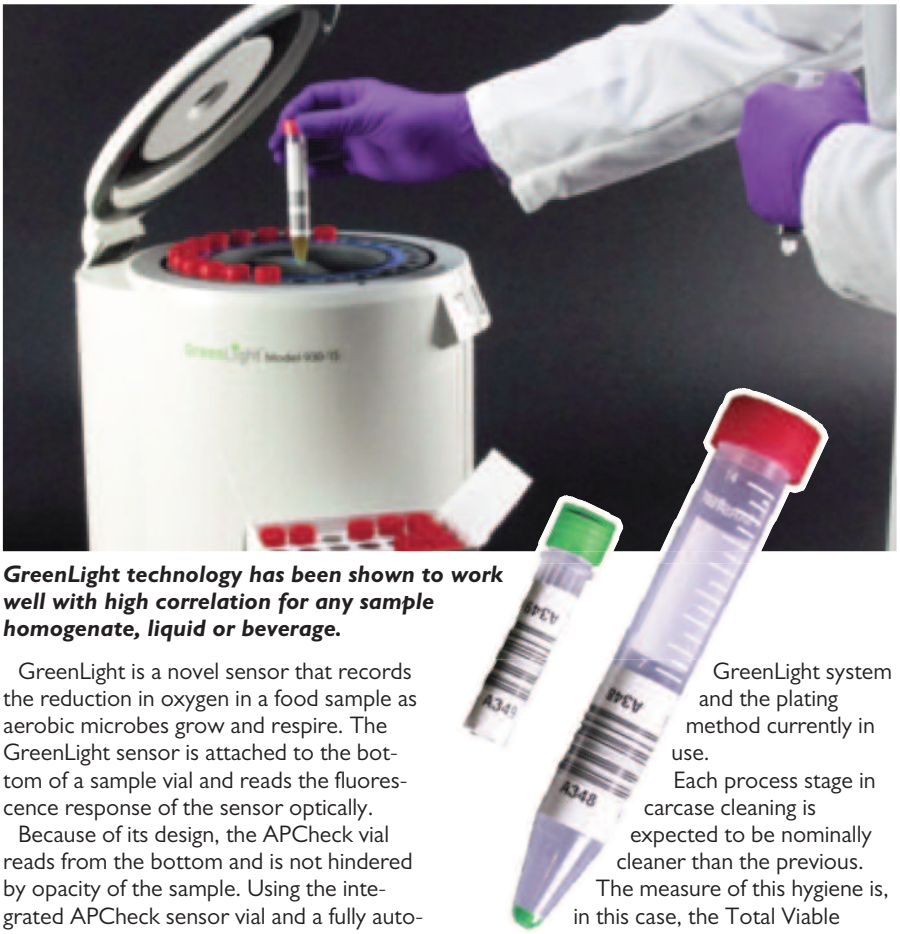
No useful information can be reliably recovered from the plates until the end of the incubation cycle. In addition, the readability of plates depends on the serial dilution of sample diluents, sometimes more than four times. This consumes large amounts of media and buffer liquids and all processes are manual and prone to error.

Another often overlooked issue is the variability of results inherent in the reference agar plate method. Errors creep into the plate readings by poor pipette technique, contamination, operator mistakes and reading errors of incubated plates.

With the continued push toward rapid methods in food microbiology, a more reliable, quantitative and less error-prone method was sought.

GreenLight technology

Mocon developed GreenLight in collaboration with Luxcel Biosciences of Cork, Ireland, in order to provide faster TVC tests with less sample preparation and better process variability.



GreenLight technology has been shown to work well with high correlation for any sample homogenate, liquid or beverage.

GreenLight is a novel sensor that records the reduction in oxygen in a food sample as aerobic microbes grow and respire. The GreenLight sensor is attached to the bottom of a sample vial and reads the fluorescence response of the sensor optically.

Because of its design, the APCheck vial reads from the bottom and is not hindered by opacity of the sample. Using the integrated APCheck sensor vial and a fully automated reader, GreenLight gives Total Viable Count results typically 10 times faster than plating.

The time-to-result from the sensor is inversely related to bacterial load. Therefore the higher the load, the faster the result. This makes for a robust and reliable screening tool. The GreenLight technology has been shown to work well with high correlation for any sample homogenate, liquid or beverage.

Method for sponge application

In the study, seven process stages, annotated PB1, PT1 and so on in Table 1, were evaluated for five different beef carcases. The aim of the study was to evaluate the reproducibility of data from both the novel GreenLight system and the plating method currently in use.

Each process stage in carcase cleaning is expected to be nominally cleaner than the previous. The measure of this hygiene is, in this case, the Total Viable Count from the carcase sponge. A commonly available pre-hydrated sponge product was used that included 10mL of Buffered Peptone Water (BPW).

Sponging was performed following the accepted ISO method and the sponges returned to their sealed containers.

In this case, the sponges were transported under refrigeration to an ISO17025 certified food testing laboratory. Under normal operating conditions, analytical tests would be performed at a local corporate laboratory facility.

One of the added benefits sought from using GreenLight was to place the actual enumeration and test equipment as close to the processing location as possible and to execute the sampling and testing without the need for a microbiology laboratory.

Continued on page 8

Continued from page 7

A comparative test was run to examine the difference in time-to-result, bias and variance between GreenLight and a commonly employed plating method (alternative to the reference method).

Once the samples were received at the expert laboratory, GreenLight vials were filled directly from the sponge diluent while samples for plating needed serial dilutions in order to meet the readability requirements under ISO 4833:2003 (alternatively the FDA BAM method).

GreenLight sample vials were then placed in the GreenLight reader and the test run via software, while the plated samples were placed in an incubator for the required period. The GreenLight results and the plate counts from five animal carcases were averaged for each of the seven process steps outlined in Table 1.

Sample description	Agar film result (logCFU/ml)		GreenLight result (logCFU/ml)	
	Mean	Range	Mean	Range
PB1	5.26	1.70	4.59	1.44
PT1	4.41	1.21	4.41	0.81
OB1	3.35	0.91	3.74	0.66
OT1	2.99	0.13	3.55	0.51
HB	<1.30	NA	<1.26	NA
HO	8.03	1.06	7.30	0.21
HIO	8.96	0.57	>7.36	NA

CFU = Colony Forming Units; Range = Highest minus lowest reading; NA = Not available

Table 1. Results from trials using the GreenLight system.

GreenLight used a pre-calculated calibration factor in order to convert time-to-result into TVC results in Colony Forming Units per mL (CFU/mL). The results are presented in Table 1.

If results were below the plating method resolution they show as less than (<). GreenLight gave instant (t=0) results in those results marked as (>).

Conclusions

In five out of seven trials run, the GreenLight system gave a lower range (highest minus lowest) result and showed acceptable bias of the mean when compared to average plate counts on agar.

Besides this promising repeatability data, it should be noted that all results were obtained using the automated GreenLight reader in less than 12 hours versus 48 hours for the US FDA BAM method for plate counts.

No serial dilutions of the swab samples were needed during the sample preparation and the system was easily adapted for use outside the microbiology laboratory, close to the carcase processing operations.

Semi-skilled staff could therefore be trained to run the assay, thereby releasing workers for other tasks. The level of automation introduced resulted in lower process variability while avoiding any increased management workload sometimes associated with complex automated equipment ■