Application Note
F13006

Assessment of Enterobacteriaceae Bacterial Load in Beef

Introduction
Enterobacteriaceae bacteria (EB) are commonly found in meat products such as beef. Levels from 10 to 1,000 colony forming units per gram (CFU/g) can be found in raw meats in the USA and Europe. Meat has a high degree of variability in how it was butchered, packaged, and how many additives may have been introduced. Because of the variables the control specification for enterobacteriaceae counts are generally 1,000 CFU/g.

Standard food testing methods for enterobacteriaceae use various agar plates or films (ISO 21528-2, 2004). Some of the most common growth media used for testing include Violet Red Bile with Glucose (VRBG, ISO 21528-2, 2004) and 3M® enterobacteriaceae Petrifilm™. The standard methods may require serial dilutions for readability and can take up to 48 hours with pre-enrichments and extended incubations. This causes higher testing costs due to expensive media and the possibility of errors due to poor lab practices dilutions. GreenLight® has now been further developed to use novel oxygen depletion technology to radically reduce laboratory preparation time and expense while making EB results available in a few hours.

Comparative testing was conducted used a Violet Red Bile Broth with Glucose[1] on GreenLight and versus 3M enterobacteriaceae Petrifilm™. All meat samples came from local stores in the USA and were selected for their range of treatment, such as ground, steak, MAP-packaged or untreated. This gave a broad understanding of GreenLight performance across a variety of beef types and sources.

Method
1. Weigh out 10g of sample into a sterile filter bag
2. Pipet 90mL of 2% Buffered Peptone Water into the filter bag
3. Stomach the filter bag for 2 minutes on high
4. Pipet 13.5mL of VRBG broth into the GreenLight APCheck™ vial
5. Pipet 1.5mL of the sample preparation into the same vial
6. Invert 2 times and vortex the vial for 30 seconds
7. Place the vial into the GreenLight model 930-15 and run
8. Run concurrent films

Results and Discussion
For the study there were 30 total samples taken from 8 different local stores and tested on 10 different days. The samples were approximately half ground beef and half some form of steak.

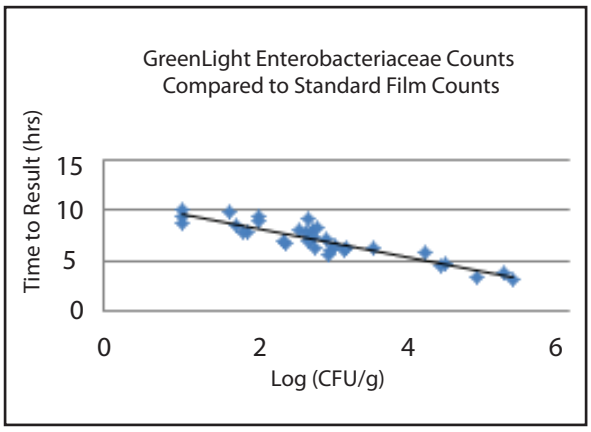

Figure 1: The GreenLight time-to-result and plate count results were compared on an x-y plot and linear regression determined with an R^2 value. A formula ($y = -1.46x + 11.15$, $R^2 = 0.82$) was generated for subsequent use in product testing, where results would be displayed in CFU/g

Figure 1 shows the correlation between GreenLight and the comparison method. For an initial experiment these values are very encouraging. The curve shows that a maximum test time should be about 12 hours for a presence/absence determination.

Conclusion
The MOCON GreenLight system can produce EB test results in much shorter times and with simpler sample preparation. GreenLight can get a result for pass/fail of 1,000 CFU/g in a matter of 7 hours with a maximum of about 12 hours for presence or absence. The standard plate counting methods would still need the full incubation time of 24 hours. Sanitary testing can be optimized and final product released faster using the GreenLight system. The GreenLight process shown can be used across many other product types and points in the production cycle.

The GreenLight system can be moved closer to slaughterhouse operations for meat butchers or used in-process, because of the reduced complexity of sample preparation and testing methods. GreenLight will give faster turn-around times, getting product to market faster for increased profits.

Reference Documents
ISO 21528-2:2004 Microbiology of Food and Animal Feeding Stuffs - Horizontal Methods for the Enumeration of Enterobacteriaceae - Part 2 Colony-Count Method
[1]Link for Violet Red Bile Broth with Glucose
http://www.neogen.com/Acumedia/pdf/ProdInfo/7425_PI.pdf
As VRBG in broth is not easily commercially available. The broth can be made from commercially available VRBG agar. Dissolve the VRBG agar in cold water, filter this solution and heat the broth that has been filtered (the agar will be left in the filter).

Acknowledgments
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