New developments in rapid microbial screening methods

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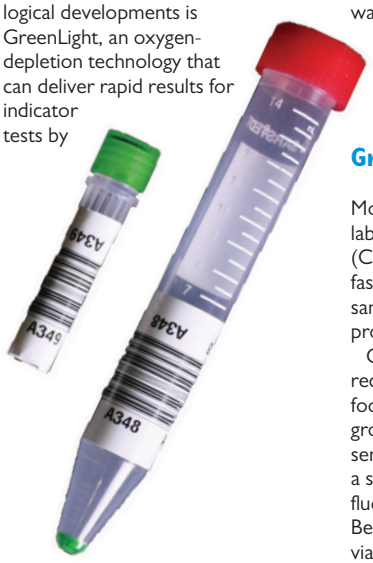
Quality control professionals in the meat industry continue to search for reliable rapid methods for screening samples at all stages of food production. Despite an increased focus on pathogenic organism detection, the current technologies are expensive and require highly qualified personnel to operate.

One of the traditional techniques for screening food samples to identify the most at-risk batches is to test for Total Viable Count (TVC), also known as Aerobic Plate Count and Total Plate Count. Additionally, it is possible to narrow the range of organisms of interest by the use of selective growth media.

Two of the key 'indicator organism' tests in use across the global food industry are total Enterobacteriaceae (EB) and total coliform counts.

These tests select for bacteria that are likely present in the human and animal digestive tracts and can represent faecal contamination. Recently, there has been an increased focus on rapid test technologies that overcome the productivity drawbacks of official methods.

At the forefront of these technological developments is GreenLight, an oxygen-depletion technology that can deliver rapid results for indicator tests by measuring the respiration of aerobic organisms in the sample.



In a recent study conducted in collaboration with the University of Minnesota, meat samples were tested for the presence of EB and coliforms. The format of the trial was to use meat purchased at retail stores in the area. Therefore, the meats were to be a variety of treatments including ground, steak, MAP-packed or untreated.

By performing a comparative study versus another popular plate counting technique, an estimate of the reproducibility of the method was possible with a view to generating a globally acceptable protocol for GreenLight testing of raw meats.

GreenLight technology

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

GreenLight is a novel sensor that records the reduction of 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 optical fluorescence response of the sensor. 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 shown high correlations to the ISO and FDA reference methods for almost any sample homogenate, liquid or beverage. GreenLight is easy to adapt for other indicator tests by the inclusion of a selective broth in the test vial.

Using this method, the GreenLight reader and the basic consumable vial remain the same for at least three types of indicator test, reducing the inventory complexity for a working laboratory and allowing all the screening tests to be run on the instrument at the same time.

Methods

All meat samples in the study came from local retail stores in the USA and were selected for their range of treatments, such as ground beef, steak, MAP-packaged or untreated. 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 usually require serial dilutions for plate readability and resolution 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 laboratory practices.

In this study, comparative testing was conducted used a Violet Red Bile Broth with Glucose in the GreenLight system versus 3M Enterobacteriaceae Petrifilm.

Coliforms testing standards are very similar to those for EB.

Method summary

- Weigh out 10g of sample into a sterile filter bag.
- Pipette 90mL of BPW into the filter bag.
- Stomach the filter bag for two minutes.
- Pipette 9mL of VRBG broth into the GreenLight APCheck vial.
- Pipette 1mL of the sample preparation into the same vial.
- Invert two times and vortex the vial for 30 seconds.
- Place the vial into the GreenLight 930 reader and run test.

It should be noted in the above summary that the preparation of the plating method is significantly longer than the GreenLight method, with more steps and with higher probabilities for error.

Much of the preparations for traditional plate count methods are focused on serial dilution of the original sample to meet the criteria for countable plates after incubation.

If the target value for the food sample is unknown, this can result in several dilutions with several plates and a large quantity of media and labware that is consumed in the preparation.

In comparison, GreenLight requires only a single dilution while stomaching. In the case of EB and

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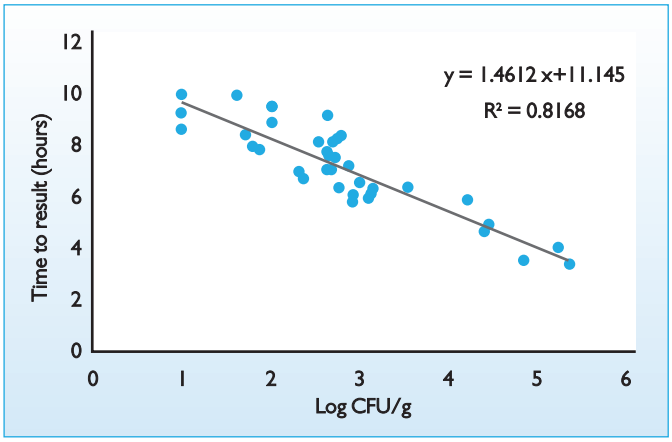

Fig. 1. GreenLight EB counts versus petrifilm.

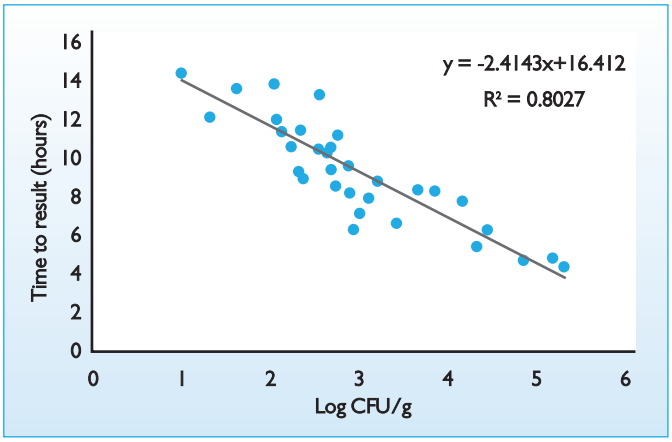

Fig. 1. GreenLight coliform counts versus petrifilm.

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coliform, GreenLight does require the addition of the selective broth to the vial, but this is relatively easy compared to the time and labour employed in achieving plate counts.

One of the advantages of using oxygen depletion technology in assessing viable counts is the sensor's ability to cover a measurable range of only a few cells, up to many millions of cells. Oxygen use is inversely proportional to the level of microbial load in the sample; therefore the result of the GreenLight test is faster the higher the load.

The method employed for total coliform testing is nearly identical to that employed for EB testing, except a different broth is used.

The standard broth in this case is Violet Red Bile Broth with Lactose (VRBL, ISO4832:2006).

The official plate count method usually used to compare to GreenLight in this case is FDA Bacteriological Analytical Methods (BAM) Chapter 4. However, the study used the 3M product, Coliform Petrifilm.

Results

Both the EB and the coliform trials were generated from 30 samples of meat obtained from eight local sources in Minnesota, USA. There were 30 data pairs produced for each type of indicator organism, defined as GreenLight time-to-result paired with Petrifilm result. It was therefore possible to produce a correlation curve for each indicator test. The EB comparative results are shown in Fig. 1.

There is a strong correlation between the GreenLight time-to-result and the plating method for singular test pairs.

Further to these results, a maximum test time to infer presence/absence can be extracted. For the GreenLight EB test, this test time would be approximately 18 hours. Therefore, a GreenLight EB test can be expected to return a result in 60% of the time taken for a plate count test.

Further, the results will be gained with less sample preparation and no extra work counting plates, since the data is measured automatically from the reader and stored in the computer database.

In the case of the coliforms test, the comparative results are shown in Fig. 2. As can be seen from the linear curve fit, the maximum test time for Coliforms can be estimated to be 12 hours.

All GreenLight oxygen depletion studies exhibit a correlation to plate count in the form of a linear relationship with negative slope, time(t)= -($\log_{10}$ plate count)*slope + TIME(0). For any food matrix, the slope and TIME(0) will remain consistent from batch to batch or sample to sample, allowing the correlation to be used as a calibration on the GreenLight system.

Thus, going forward, the user would simply apply this calibration in software and get results reported in CFU/g, or CFU/mL in the case of a liquid or beverage.

It is to be expected that a different food matrix would supply a different calibration curve, yet experimental data has shown that similar food matrices return similar curves.

Hence, a single calibration curve can serve a group of foods, such as dairy or meats.

Conclusions

The GreenLight microbial detection system can produce results for total coliform and EB counts at least 60% faster than plate counting technologies. For a typical acceptance criteria of 1000 CFU/g in a food sample, the system is expected to give results in less than 10 hours

The GreenLight system uses selective media that are commonly available, no special media are required.

The GreenLight reader and the oxygen sensing APCHeck vial can be used for indicator organism tests and total plate count (TVC) tests in any combination in an automated instrument, thereby making QC assessment of foods more efficient.

The Green Light system reduces costs due to elimination of serial dilutions that are routinely needed in plate counting methods. By automating the tests, labour and material costs are reduced.

Errors are reduced in the automated GreenLight system due to the elimination of manual process and preparation steps.

This study returned a correlation to another popular indicator organism test method of over 80% (R^2). As more data is gained on each food matrix tested, the GreenLight system can update its correlation efficiency using the new data and higher factors are expected.

The method used here is easily adaptable to other food matrices.

As noted, other food matrices are easily tested with only minor adaptations to the method. The use of replicate data sets will improve the correlation factor by improving the precision of both the plate count and GreenLight data. ■

References are available from the authors on request