

GreenLight™: Same-Day Analysis of Aerobic Plate Counts (APCs, TVCs) in raw meat samples

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Introduction

An increasing appreciation for the importance of food and microbiological safety has resulted in a demand for a more rapid, high-throughput method for total viable count (TVC) quantification to deal with the increasing numbers of samples that require testing. The industry standard for TVC determination (ISO.4833.2003), also known as aerobic plate count, is widely used but presents users with some very significant drawbacks. The method is both materials and labour intensive, requiring the preparation and analysis of multiple agar plates per sample. More importantly, the method is slow, with 48-72 hours typically required for a definitive result [1-3].

Luxcel Biosciences has addressed these limitations with the development of the GreenLight™ 960; a microtitre-plate based assay which provides a rapid high-throughput method for the assessment of aerobic bacterial load through analysis of microbial oxygen consumption [4,5]. When applied to the determination of Total Aerobic Viable Counts in raw meat (Beef samples) results are generated in 1-12 hours depending on microbial load.

The straight forward 'mix & measure' procedure allows rapid detection of microbial oxygen consumption and equates oxygen consumption to microbial load (CFU/g), providing a simple, yet sensitive means of assessing the microbial contamination levels in a variety of food samples. As bacteria in the test sample grow and respire they deplete O₂ which is detected as an increase in the GreenLight™ probe signal above the baseline level. The time required to reach this increase in signal can be used to calculate the CFU/g of the original food sample, based on a pre-determined calibration. The higher the initial microbial load, the earlier this threshold level is reached; expressed as a characteristic onset time t₀, thus indicating the initial microbial load.

Assay Set-Up

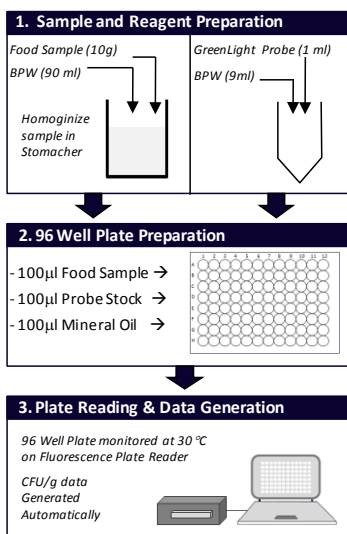


Fig . 1: Assay Schematic

- 1 **GreenLight™** probe is reconstituted in 1ml of BPW and diluted to 10ml in BPW
- 2 Add 10g of food sample + 90ml of PBW and homogenise on stomacher for 1min
- 3 Add 100µl of **GreenLight™** probe to each assay well of a sterile 96-well plate
- 4 Dispense 100µl of homogenates or bacterial samples to the 96-well plate –per well
- 5 Overlay each assay well with 100µl of pre-warmed mineral oil to seal the sample
- 6 The plate is measured kinetically on a fluorescence plate reader form 0.5-12h hours

Proof of Principle and Typical Data Output

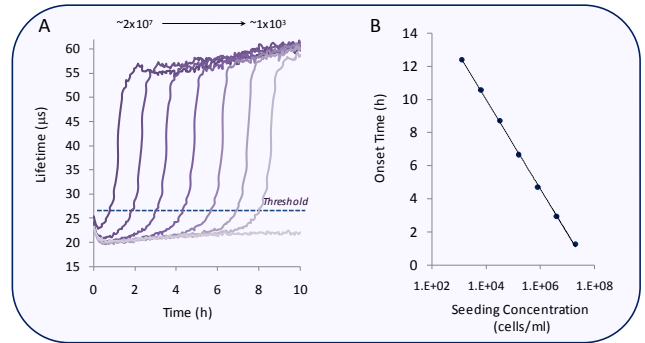


Fig . 2: Typical Data Output. A) Plate map view of individual kinetic profiles, B) Oxygen-based growth curves from serial dilution of *E. coli* (CFU/ml) C) Correlation between Onset time (t₀) and seeding concentration.

As bacteria replicate, oxygen consumption rate increases. At a critical point, oxygen consumption exceeds back diffusion. This is seen as a marked increase in probe signal exceeding the threshold limit (29µs). The time to reach this signal threshold signal reflects the seeding concentration and is dependent on the replication rate and cellular oxygen consumption rate. Instrument software allows for visual inspection of all bacterial oxygen consumption profiles alongside control samples (flat profile), in 96-well plate map view. The software automatically determines the time to threshold (Onset time t₀) for each sample and subsequent Log CFU/g values using a predetermined calibration.

TVC Calibration and Method Comparison

To assess the correlation between measured **GreenLight™** onset time and bacterial load as measured by the standard method, both parameters were measured in parallel in five raw meat types (Ground beef, lamb, pork, chicken, turkey) across the range of interest with results yielding a Pearson Correlation coefficient (r) of 0.946.

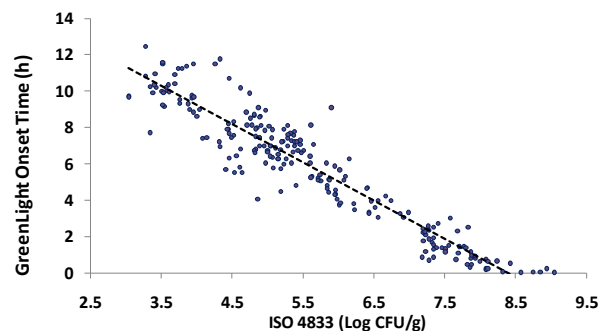


Fig. 3: GreenLight™ vs. Agar Plate TVC's (ISO 4833 Ref) Correlation Ground Beef at a range of microbial contamination levels

Sample Assessment

To assess the usability of **GreenLight™**, naturally contaminated ground beef samples were quantitatively assessed over their shelf life. In some cases, controlled temperature abuse was used to increase growth of natural microflora to achieve higher contamination levels. Calculations of Log CFU/g were performed for each beef sample (n=2) using a predetermined calibration (mixed meat). The data output of the measurement is presented in Fig. 4. and the summary output of the method is presented in table 1.

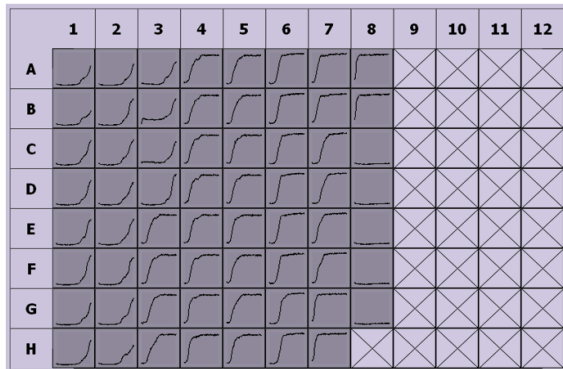


Fig. 4: Data output of a GreenLight™ 960 analysis of Ground Beef TVC's

All the necessary data processing and calculations are performed by the plate reader software thereby reducing the amount of effort required by the end-user to generate a definitive result.

The data summary generated (table 1) details the time at which the signal threshold is exceeded in each well and gives the corresponding CFU/g values. Statistical information such as average and standard deviation values are available if required and samples that exceed a specific CFU/g value can be red-flagged automatically.

Analysis can be performed in real time thereby allowing detection of contamination levels of 5×10^6 CFU/g in under 6 hours (see table 2). Such contamination levels are deemed 'unsatisfactory' as defined by EU regulation 2073, 2005 [6].

Table 1: Sample CFU/g results for samples from Fig. 1

Product	Sample ID	Profiles	Onset Time (h)	Log	CFU/g
Beef	12 A1, B1		8.605	4.50	3.2E+04
Beef	2 C1, D1		8.632	4.49	3.1E+04
Beef	10 E1, F1		8.577	4.51	3.3E+04
Beef	21 G1, H1		8.854	4.39	2.5E+04
Beef	9 A2, B2		8.712	4.45	2.9E+04
Beef	105 E3, F3		1.918	7.38	2.4E+07
Beef	115 G3, H3		2.013	7.33	2.2E+07
Beef	55 A4, B4		1.524	7.55	3.6E+07
Beef	85 C4, D4		1.454	7.58	3.8E+07
Beef	78 E4, F4		1.687	7.48	3.0E+07
Control	probe C8, D8		-	-	-

Conclusions

The **GreenLight™** assay is a simple 'mix and measure' assay: Using food homogenates, prepared as for traditional TVC testing (ISO:4833:2003), probe, sample & oil are added in sequence and resultant aerobic TVC values quantified using an appropriate calibration.

The **GreenLight™** assay can measure aerobic bacterial respiration across a wide contamination range (10^3 - 10^8 CFU/g) and can be incorporated into an easy-to-use system for the determination of aerobic TVC's.

In comparison to the conventional approach **GreenLight™** provides a much improved 'time-to-result' with $\leq 10^3$ CFU/g detectable in ~ 12 hours in comparison to the 48 hours required by the standard method ($\geq 10^8$ CFU/g are detectable within 1 hour, see table 2).

Contamination levels are generated automatically on standard instrument software. **GreenLight™** is therefore much less labour intensive and less subjective than the standard method.

A strong correlation is observed between the **GreenLight™** and ISO:4833:2003 methods for the assessment of bacterial contamination in ground beef samples, (Pearson's Correlation Coefficient = 0.961).

Table 2: Relationship between bacterial load and time-to-result

CFU/g	$\geq 10^8$	$\geq 10^7$	$\geq 10^6$	$\geq 10^5$	$\geq 10^4$	$\geq 10^3$
Onset Time (h)	0 - 0.8	0.9 - 2.9	3.0 - 5.0	5.1 - 7.1	7.2 - 9.2	9.3 - 11.3

Additional Measurement Details

Sample handling: Meat samples were assessed for naturally occurring microflora over the period of product shelf life, and controlled temperature abuse was used in some instances to achieve higher contamination levels. E. coli samples were cultured in LB broth overnight at 37 +/- 1 °C.

Oxygen Consumption Assay: GreenLight™ test performed as per manufacturer's instructions (see Assay Set-Up) in BPW (meat samples n=2)

Conventional TVC test: Standard aerobic plate count test of food samples was carried out according to standard method (ISO:4833:2003)

Analysis: Measurement was carried out on a Victor2 (PerkinElmer) plate reader under the manufacturers measurement settings, using a software analysis template containing a predetermined mixed meat calibration. Measurement was carried out in BPW. A correlation between the two methods was constructed using the calculated Log CFU/g results.

References

- [1] ISO:4833:2003. (2003). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C
- [2] Baylis, C. L. (2003). Manual microbiological methods for food and drinks industry (fourth ed). CCFRA
- [3] Collins, C. H. & Lyne, P. M. (1995) Microbiological methods (seventh ed.). Butterworth & Co. Ltd.
- [4] O'Mahony, F. C & Papkovsky, D. B. (2006). Rapid high throughput assessment of aerobic bacteria in complex samples by fluorescence based oxygen respirometry. Appl. Environ. Microbiol., 72, 1279-1287
- [5] O'Mahony, et al (2009). Analysis of total aerobic viable counts in samples of raw meat using fluorescence-based probe and oxygen consumption assay.
- [6] Commission Regulation (EC) No 2073/2005 on the microbiological criteria for foodstuffs.