



Food Safety Webinar: Same-day Aerobic Plate Count Analysis for Meat & Food Products

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WHAT IS FOOD SAFETY?

MOCON's commitment to Food Safety emphasizes a ***preventative vs. reactionary*** approach. We strive to help companies prevent losses through faster/cheaper testing for APC's. This allows for more screening to be done, increasing the statistical confidence level of the food being tested.



What is APC?

Microorganisms capable of growing aerobically at 30°C. This is equivalent to a mesophilic aerobic plate count, sometimes referred to as aerobic plate count (APC) or total viable count (TVC) or total plate count (TPC) or standard plate count (SPC).



Why is determination of APC levels important?

- It's required by law
 - EU: Reg. (EC) 2073/2005 and CAC/GL 21-1997
 - 5×10^6 CFU/g is acceptable limit for raw meats
- Indicator of potential pathogenic contamination
- Test environmental hygiene of the facility for bacteria



Standard Method (ISO 4833:2003)

- Prepare maximum recovery diluent (MRD) and Plate Count Agar (PCA)
- Homogenize the food sample
- Prepare serial dilutions (minimum of 5)
- Transfer 1ml of appropriate dilution to 2 sterile petri dishes (min 2 plates/dilution)
- Mix inoculum in medium by rotating the petri dish, allow to solidify, overlay with 4mls of PCA
- Incubate @ 30 °C ± 1 °C for 72 h ± 3 h
- After specified incubation period count colonies and calculate CFU/g



Drawbacks of standard method

- Time-to-result (48 – 72 hours)
- Labor costs – laborious and tedious
- Media costs
- Minimum number of plates/sample = 20
- Incubator space (must not stack plates more than 6 high)
- Difficult and tedious to count. Particles of un-dissolved /particulate matter in dishes may be mistaken as pin point colonies (giving an inaccurate result)



Why is *fast* determination of APC levels important?

- Get results in hours – not days
- Screening tool used in conjunction with PCR
- Grade incoming raw materials, in-process & finished goods
- Rapid environmental hygiene analysis for facilities
- Research tool for spices, antimicrobials and new products
- Increase brand awareness with better quality control
- Have suppliers provide better APC results to your company

Safer foods for consumers



New Sensor Technology

- Based on optical oxygen sensing
- Measures bacterial O₂ consumption and equates to viable microbial load
- Proprietary sensors emit light (phosphoresce) and this light emission is quenched by molecular O₂
- As microbes grow, they consume O₂
- Less O₂ = stronger signal - reversible



GreenLight

900 SERIES

- Time-to-result (1-12 hours)
- Lower Labor costs
- Lower Media costs
- Required Samples = 1
- Less Counter Space
- No Counting Necessary



How does it work?

- Measures bacterial O_2 consumption and equates it to microbial load
- As bacteria grow and consume dissolved O_2 , the reagent's phosphorescence signal increases
- The time taken to reach a threshold signal is used to calculate initial microbial load.
- The greater the initial microbial load the faster the time to result.

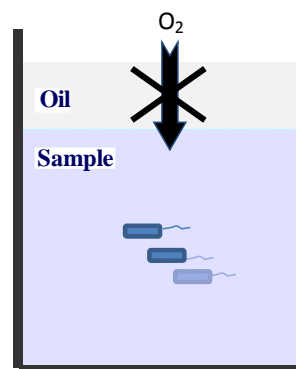


Fig. 1
Bacteria in sample consume O_2

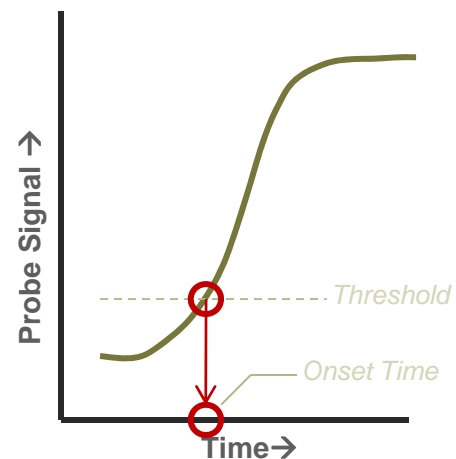
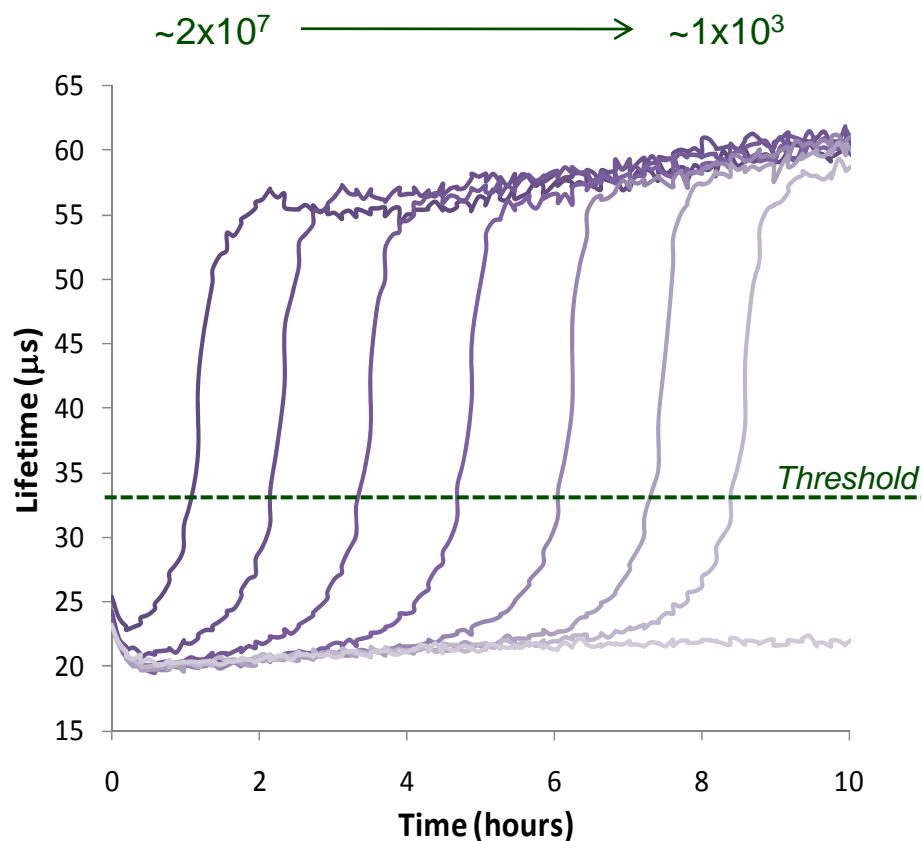


Fig. 2
Respiration Profile



It's functional ! Detects *viable* bacteria

The greater the microbial load the faster the onset of respiration profiles

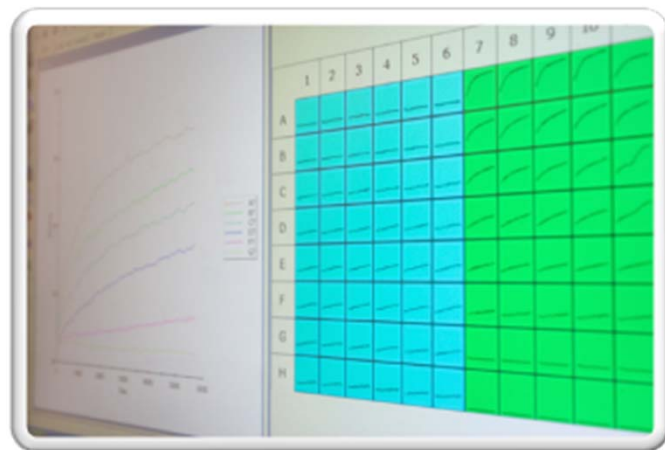


Oxygen-based growth curves from serial dilution of *E.coli* (CFU/ml)



It's fast ! *Preventive*

Time to Threshold (hr)	CFU/g
0 – 0.8	$\geq 10^8$
0.9 – 2.9	$\geq 10^7$
3.0 – 5.0	$\geq 10^6$
5.1 – 7.1	$\geq 10^5$
7.2 – 9.2	$\geq 10^4$
9.3 – 11.3	$\geq 10^3$
Flat Profile	$\geq 10^2$

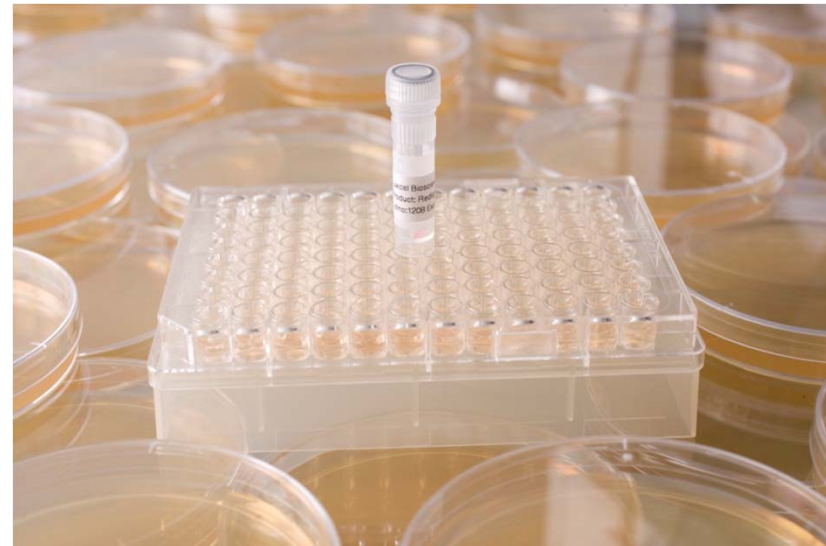




Key Features

- **Fast results/ Same-day turnaround –**
Preventive Approach
 - APC level of $\sim 1 \times 10^8$ CFU/g in under 45mins
 - APC level of $\sim 1 \times 10^6$ CFU/g in under 5 hrs.

- **Cost-Effective**





Key Features

- Simple protocol / easy to perform
- Low & High Throughput capability
- Easy Interpretation of Results





Key Features

- High quality / certified assay
- Comparable to reference method
- Preventative approach to food safety





Types of Data Analysis?

- Three levels of data analysis are available:
 - Quantitative (CFU/g)
 - Pass/Fail
 - Visual



The Model 960



GreenLight™
Reagent Vials



96- well plates



Fluorescence
plate-reader



It's accurate !

Comparison to Ref method (ISO 4833; 2003)

- Table below shows the relative accuracy relationship between GreenLight™ 960 method and reference method.

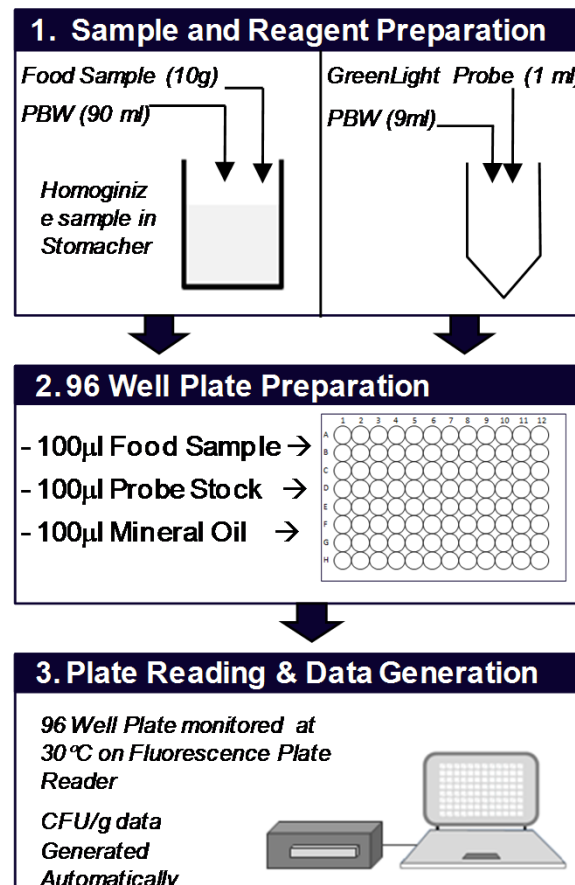
Meat Types	Correlation Coefficient (r)
Beef	0.96
Lamb	0.97
Pork	0.95
Poultry Products	
Chicken	0.92
Turkey	0.95
All Foods (global assessment)	0.95





It's simple to use! Model 960

1. Probe is reconstituted in 1ml of PBW and diluted to 10ml in PBW
2. Add 10g of food sample + 90ml of PBW and homogenize on stomacher for 1min.
3. Add 100µl of probe
4. Add 100µl of food homogenate
5. Add 100µl of pre-warmed mineral oil
6. The plate is measured kinetically on a fluorescence plate reader for 1-12hrs





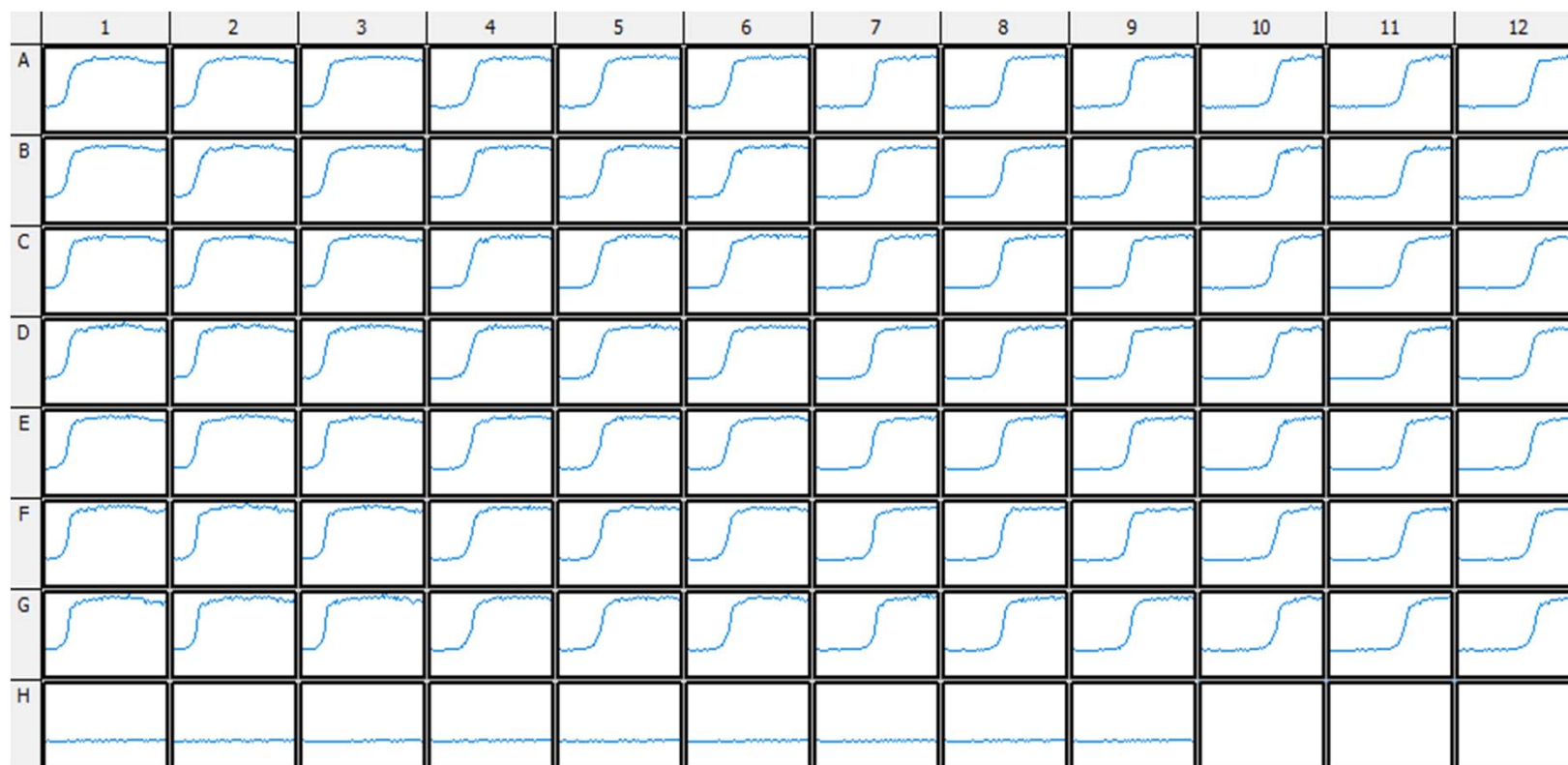
It is easy to read!

	1	2	3	4	5	6	7	8	9	10	11	12
A	7.25	7.25	7.18	6.54	6.46	6.54	5.82	5.82	5.89	5.24	5.24	5.17
	Unacceptable	Unacceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Satisfactory	Satisfactory	Satisfactory
B	7.25	7.25	7.25	6.54	6.54	6.54	5.89	5.89	5.89	5.24	5.24	5.24
	Unacceptable	Unacceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Satisfactory	Satisfactory	Satisfactory
C	7.25	7.25	7.25	6.61	6.61	6.61	5.96	5.96	5.96	5.32	5.32	5.24
	Unacceptable	Unacceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Satisfactory	Satisfactory	Satisfactory
D	7.25	7.25	7.25	6.61	6.61	6.61	5.96	5.89	5.96	5.24	5.24	5.24
	Unacceptable	Unacceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Satisfactory	Satisfactory	Satisfactory
E	7.25	7.25	7.25	6.54	6.54	6.54	5.96	5.89	5.89	5.24	5.24	5.24
	Unacceptable	Unacceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Satisfactory	Satisfactory	Satisfactory
F	7.25	7.25	7.25	6.54	6.54	6.54	5.89	5.89	5.96	5.24	5.24	5.24
	Unacceptable	Unacceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Satisfactory	Satisfactory	Satisfactory
G	7.25	7.25	7.25	6.54	6.54	6.54	5.82	5.82	5.89	5.17	5.17	5.17
	Unacceptable	Unacceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Satisfactory	Satisfactory	Satisfactory
H	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Sample Data: GreenLight™ Ground beef analysis - pass/fail and quantitative Log CFU/g values automatically calculated by instrument software



It's visual - predictive



See respiration profiles in real time



Why is it simpler?

- Sample preparation (1:10 dilution)
- Direct application to 96 well plate
- Automatic assay with results in hours
- Significantly reduced labor and associated costs



GreenLight 910 - March 15, 2011



Test Completed

Pass/Fail Test

Test ID: 123456789012345678901234567890123456 Rack ID: 12345678 Last Signal Reading: 9.6

The test is now complete. You can Export to CSV, Print Reports or Move to History.

Caution A 1234	Pass A 1234	Fail A 1234		Caution B 1234	
Caution a 1234	Pass a 1234	Pass a 1234		Fail a 1234	
	Caution a 1234	Pass a 1234			Fail a 1234
Pass a 1234	Caution a 1234	Fail a 1234	Pass a 1234		

Test Complete

- Export to CSV
- Print Reports
- Move to History

Pass/Fail Criteria:

[View Details](#)

Key

- Pass <10^A
- Caution 10^A
- Fail >10^A
- Inactive
- Active
- Selected Vial
- Scanned Vial

Close

Lower throughput – 2ml and 15ml APCheck™ sample vial formats



Model 910 Features

- Test 1 – 50 + Samples
- Sensor material in bottom of Vial
- Use 2ml or 15ml APCheck™ Vial
- Each APCheck™ vial is bar coded
- Sensitivity 2 log 10 for 2 ml vial
- Sensitivity 1 log 10 for 15 ml vial
- Test Single or Multiple Samples
- Run multiple samples using external incubator or heater block

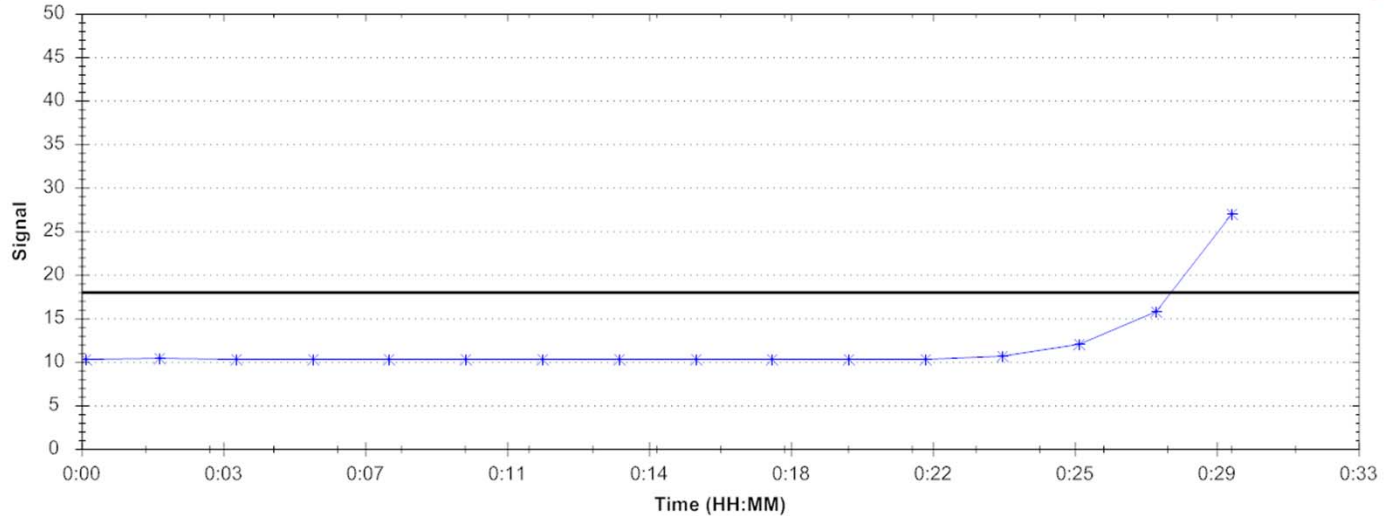




Test Complete

Continuous TVC Test Vial ID: D016

TVC = 5.88E7 log₁₀(CFU/g)



Time	Signal
00:00:30	26.92
00:00:28	15.72
00:00:26	11.96
00:00:24	10.66
00:00:22	10.3
00:00:20	10.28
00:00:18	10.31
00:00:16	10.32
00:00:14	10.29
00:00:12	10.3
00:00:10	10.25
00:00:08	10.24
00:00:06	10.22
00:00:04	10.25
00:00:02	10.42
00:00:00	10.24

Export Data

Print Reports

Test Criteria: View Details Move To History Close



What can you test?

- Meat
- Poultry
- Fish
- Beverages
- Dairy
- Produce





GreenLight™ 960:
AOAC RI PTM approved

GreenLight™ 910 - Pending



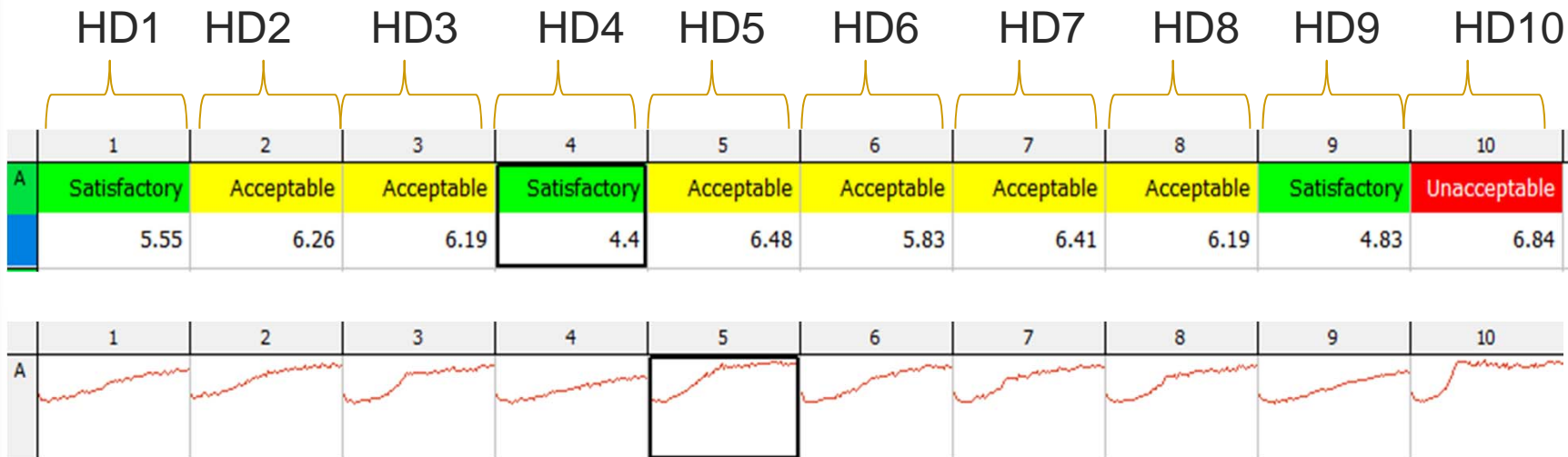
What is it certified for?

- 2 food categories: Meat, Poultry
- 5 specific food types
 - ✓ *Raw ground beef*
 - ✓ *Raw lamb*
 - ✓ *Raw pork*
 - ✓ *Raw chicken*
 - ✓ *Raw turkey*
- Additional food categories and types pending



Example of other foods

- Hot dogs – Batch test from Irish retail store





GreenLight™ Model 960: Sample Publications

1. O'Mahony F, Green RA, Baylis C, Fernandes R, and Papkovsky DB. Analysis of total aerobic viable counts in samples of raw meat using fluorescence-based probe and oxygen consumption assay. Food Control 20: 129-135, 2009.
2. O'Mahony F and Papkovsky DB. Rapid high-throughput assessment of aerobic bacteria in complex samples by fluorescence-based oxygen respirometry. Appl Environ Microbiol 72: 1279-1287, 2006.

mocon[®]



GreenLight[®]

900 SERIES

Fast, *preventative* results with reduced costs

- Labor savings vs. traditional methods
- Same day results
- Test aerobic bacteria
- High throughput screening method
- Allows for more tests to be completed for better food safety
- Complementary to PCR testing





Who is MOCON?

- Overview:
 - Instrument manufacturer for permeation, head space analysis, burst testing, leak detection, oxygen analyzer and bacteria detection.
 - Mocon offers consulting and testing services
 - Also offers instruments for gas analysis for aromas/odors & VOC analyzers for hydrocarbons and other impurities
- Core Business Areas:
 - Laboratory Instruments & Services
 - Field Instruments & Services
 - Food and Microbial Safety



Who is Luxcel Biosciences?

- Overview:
 - Irish Biotechnology Company – Based in Cork Ireland
 - 10+ years experience in Oxygen sensing & respirometry technologies
 - Focus on Phosphorescence based Probes, Bioassays
- Core Business Areas:
 - Food and Microbial Safety
 - Packaging
 - Pharmaceutical R&D: safety assessment, Toxicity





GreenLight™ Food Safety Video



www.mocon.com/foodsafety.php



Thank You!

Questions?

Email mtheno@mocon.com if you have any further questions or comments.