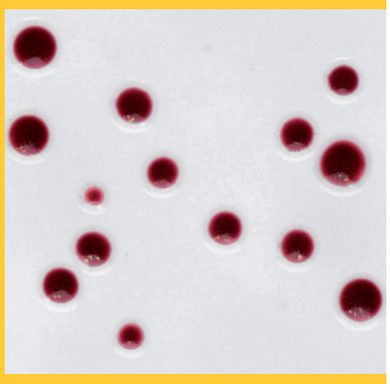
OXOID

Culture Media

Brilliance CampyCount



***Brilliance*™ CampyCount Agar - a chromogenic selective medium for the enumeration of *C. jejuni* and *C. coli* from poultry and related samples.**

OBSERVATION MADE SIMPLE

- Dark red colonies on a clear background

QUANTITATIVE

- Novel selectivity enables accurate, quantitative recovery of target organisms

ACCURATE CALCULATION

- Transparent medium allows enumeration on plate readers

EASY IDENTIFICATION

- Reduced *Campylobacter* swarming for improved isolation of individual colonies

VALIDATED

- ISO 16140 validated by MicroVal

MICROVAL

MicroVal certificate no. MV2008LR12

Oxoid *Brilliance* CampyCount Agar

Brilliance CampyCount Agar is a new medium specifically designed for accurate, specific and easy enumeration of *C. jejuni* and *C. coli*, as opposed to presence/absence testing. It is a highly selective, easy-to-read agar medium for the presumptive identification and enumeration of *C. jejuni* and *C. coli* from poultry and related samples.

Brilliance CampyCount Agar is a transparent medium which makes identification of *C. jejuni* and *C. coli* significantly easier than on traditional charcoal or blood containing agars. It contains an indicator that, when metabolised by the target organisms, changes colour. As it builds up in the cells it turns colonies dark red, making all *C. jejuni* and *C. coli* colonies readily identifiable.

The components of *Brilliance* CampyCount Agar have been carefully designed to maximise growth of *C. jejuni* and *C. coli* while inhibiting non-target organisms. This defined formulation means the medium can be used to accurately enumerate the loading of *C. jejuni* and *C. coli* on poultry carcasses and related samples.

Protocol for enumeration of *C. jejuni* and *C. coli* using *Brilliance* CampyCount Agar

Day 0: Plating

Dilute sample in appropriate diluent

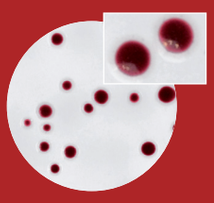
+

In duplicate, spread 0.1mL of appropriate dilution onto 2 x *Brilliance* CampyCount Agar plates

Incubate for 48h ± 1h at 41.5°C in a microaerobic atmosphere

Day 2: Results

If present, select at least 5 well isolated, dark red colonies



Confirm using O.B.I.S. Campy

Alternatively, confirm colonies using standard ISO methods

Campylobacter Food Poisoning

Campylobacter is a leading cause of enteric disease in most developed countries. The organism is endemic in many poultry populations; 98% of food-borne infections are caused by *C. jejuni* and *C. coli*. In recent years, there have been numerous improvements in animal husbandry and carcass processing that have reduced the prevalence of *Campylobacter* in poultry. However, it is unfeasible that the complete elimination of *Campylobacter* can be brought about in the near future. However, to reduce human infection, it is generally accepted that further reduction in the levels of *Campylobacter* on the fowl is a more feasible goal. In order to bring this about, a shift in industry standards from a presence/absence testing to enumeration needs to occur. *Brilliance* CampyCount Agar makes this transition easy.

ISO 16140 Validation

Brilliance CampyCount Agar has been validated and approved by MicroVal according to ISO 16140: 2003 standard against the reference method ISO/TS 10272-2: 2006 for the selective enumeration of thermotolerant *Campylobacter* spp., in particular *C. jejuni* and *C. coli*, in poultry products. For flexibility, this study included both the O.B.I.S. Campy kit and Oxoid Dryspot Campylobacter test as alternative confirmation methods to those described in the reference method ISO/TS 10272-2: 2006. MicroVal certificate no. MV2008LR12 is available in PDF format from www.microval.org.

Sensitivity was tested using a total of 81 *Campylobacter* strains isolated from poultry and associated environments and specificity was tested using 139 non-target strains.

Media	Specificity (n=139)	Sensitivity (n=81)
mCCDA	91%	100%
Brilliance CampyCount Agar	99%	100%

***Brilliance* CampyCount Agar**		SIZE/FORMAT	ORDER CODE
Brilliance CampyCount Agar (ready-to-use plates) - UK		10x90mm	PO1185A
Brilliance CampyCount Agar (ready-to-use plates) - Rest of Europe		10x90mm	PO5305A

The Oxoid product range offers the complete solution for all your *Campylobacter* testing needs

Broth media			
Bolton Broth Base		500g	CM0983B
Bolton Broth Selective Supplement	(for 500mL medium)	10 vials	SR0183E
Bolton Broth Selective Supplement (modified)	(for 500mL medium)	10 vials	SR0208E
Campylobacter Growth Supplement	(for 500mL medium)	10 vials	SR0232E
Plate media			
Campylobacter Blood-Free Selective Agar Base		500g	CM0739B
Campylobacter Agar Base (Karmali)		500g	CM0935B
Campylobacter Selective Supplement (Karmali)	(for 500mL medium)	10 vials	SR0167E
Karmali Selective Supplement (modified)	(for 500mL medium)	10 vials	SR0205E
CCDA Selective Supplement	(for 500mL medium) (for 2.0 litres medium)	10 vials 10 vials	SR0155E SR0155H
Confirmatory tests			
DrySpot Campylobacter Test Kit		50 tests	DR0150M
O.B.I.S. campy		60 tests	ID0800M
Atmosphere generation			
AnaeroJar™		1 jar	AG0025A
CampyGen™	(for use in 2.5 litre jar) (for use in 3.5 litre jar)	2.5 litre 3.5 litre	CN0025A CN0035A
CampyGen Compact		20 sachets	CN0020C
Campylobacter Gas Generating Kits	(for jars over 3 litres) (for jars under 3 litres)	10 sachets 10 sachets	BR0056A BR0060A
Quality Control organisms – Culti-Loops™			
Campylobacter coli ATCC® 33559™†		5 loops	CL9039
Campylobacter jejuni ATCC® 33291™†		5 loops	CL1400
Escherichia coli ATCC® 25922™†		5 loops	CL7050
Staphylococcus aureus ATCC® 25923™†		5 loops	CL7010
Candida albicans ATCC® 10231™†		5 loops	CL1503

† ATCC Licensed Derivative®

For more information about the Oxoid *Brilliance* range of chromogenic media and other products, please visit www.oxoid.com or talk to your local Oxoid representative.

Limitations Oxoid *Brilliance* CampyCount Agar is for laboratory use only, by experienced microbiologists. It must not be used beyond the stated expiry date, or if the product shows any sign of deterioration. Media should be validated by the end-user, under local conditions. Identifications on *Brilliance* CampyCount Agar are presumptive and should be confirmed. The MicroVal study revealed that, in chicken thighs, *Brilliance* CampyCount Agar gave a lower yield than mCCDA.

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STUDY REPORT

Thermo Scientific *Brilliance* CampyCount Agar: MicroVal EN ISO 16140-2:2016 Validation

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Summary

The Thermo Scientific™ *Brilliance*™ CampyCount Agar (alternative method) has been validated in accordance with EN ISO 16140-2:2016 for the selective enumeration of thermo-tolerant *Campylobacter* species in raw and ready to cook poultry products. This report summarizes the relative trueness study, accuracy profile study, inter-laboratory study, and inclusivity and exclusivity studies completed as part of the MicroVal™ EN ISO 16140-2:2016 validation certificate renewal.

Methodology

The alternative method was originally validated according to the superseded EN ISO 16140:2003 for enumeration of *Campylobacter* species in poultry products. The original study was carried out by RIKILT Institute of Food Safety, The Netherlands. An EN ISO 16140-2:2016 renewal study of the alternative method was carried out by Campden BRI, UK. The performance of the alternative method was compared to the EN ISO/TS 10272-2:2017 reference method 'Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique'.

The protocols for the alternative method and reference method are detailed in Appendix 1.

Figure 1. Thermo-tolerant *Campylobacter* species growing on Thermo Scientific *Brilliance* CampyCount Agar

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Relative trueness study

Five levels of contamination were used (covering low, intermediate and high levels plus two other intermediary levels). Duplicate test portions were examined for each sample tested. In the original relative trueness study there were 48 naturally contaminated and 14 artificially contaminated samples. For the renewal study, one additional artificially contaminated relative trueness data point was required to complete the raw and ready to cook poultry category (minimum of 15).

The minimum incubation times were applied for the alternative method, and five typical colonies from two plates were confirmed using the O.B.I.S. Campy Test Kit (Thermo Fisher Scientific).

Figure 2 shows extremely good agreement between the reference and alternative method with almost no positive or negative bias.

The data was also analyzed via the Bland-Altman method which showed a total of four out of 77 data points were outside of the accepted limits; two with a slight positive bias and two with a slight negative bias. The four data points were all from the same category which also had the highest number of samples tested (54 out of 77 data points). The RT data is within the acceptability limits in EN ISO 16140-2:2016 and there is no overall bias to the data.

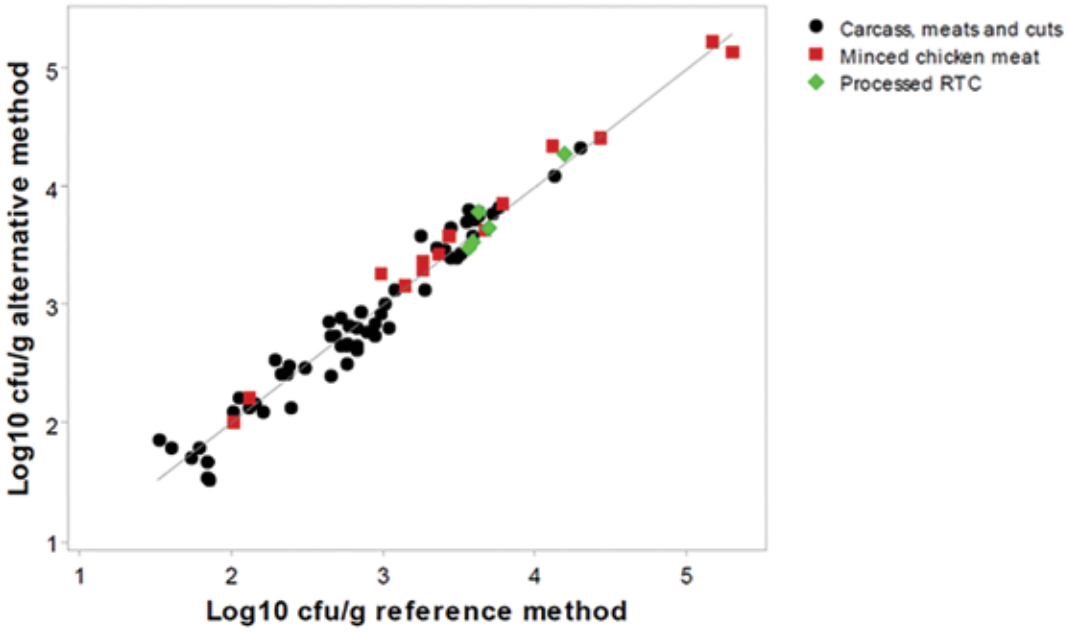

Figure 2. Scatter plot of the reference method versus the alternative method for raw and ready to cook poultry products[a]

[a] Figure 2 represents data excluding air-packed chicken thighs. During the original study, a significant bias was observed between methods for naturally contaminated air-packed chicken thighs. Therefore, data was analyzed without these samples and a limitation noted on the certificate.

Accuracy profile study

One food category was tested with two separate batches of food type, using six samples per type. The two batches were contaminated at low, intermediate and high inoculum levels. For each sample five different portions were tested (total of 30 analyses per food type). The calculations were performed using the AP Calculation Tool MCS (Clause 6-1-3-3 calculation and interpretation of accuracy profile study) as described in EN ISO 16140-2:2016.

If any of the upper or lower limits exceeded the 0.5 log AP limits and the standard deviation of the reference method was >0.125, additional evaluation procedures are required, as described in ISO 16140-2:2016 and the new acceptability limits are calculated.

In this study one level (of 0.619 log) did not meet the acceptability limit of 0.5 log, and the standard deviation of the reference method was >0.125. The additional calculations were carried out and the reference method met the newly calculated acceptability limit of ± 0.736.

The accuracy of the alternative method is acceptable as all samples met both the 0.5 log and the re-calculated acceptability limits.

Inter-laboratory study

An inter-laboratory study (ILS) was conducted with 14 laboratories across Europe using a matrix of minced-chicken spiked with *Camplyobacter jejuni* at low, medium and high inoculum levels.

The data collected during the ILS study showed there were no statistically significant differences between the alternative method and the reference method.

Inclusivity and exclusivity study

The original validation tested a total of 55 *Campylobacter* species and 30 non-*Campylobacter* species. During the renewal study an additional 11 *Campylobacter* species (66 in total) and 12 non-*Campylobacter* species (44 in total) were tested.

Overnight cultures were prepared in Mueller-Hinton Broth for the inclusivity isolates and non-selective broth for the exclusivity isolates. Cultures were diluted in Peptone Saline to achieve a concentration of >100 times the limit of detection (>100 CFU/mL). The cultures were tested in duplicate by inoculating 100 µL of the appropriate dilution onto *Brilliance* CampyCount Agar plates.

Of the 66 inclusivity strains tested, all *C. jejuni* subsp. *jejuni* and all *C. coli* strains gave a positive result with the alternative and reference methods. The *C. jejuni* subsp. *Doylei* strain did not grow with either method which was expected due to the incubation temperature of 41.5°C. Two *C. upsaliensis* strains and one *C. hyointestinalis* strain tested, were not detected by either method. One *C. lari* strain failed to grow via the alternative method but did grow via the reference method; the remaining two *C. lari* strains tested successfully recovered via all methods.

Of the 44 exclusivity isolates, 39 gave a negative result with the alternative and reference methods. *Acinetobacter baumannii, Acinetobacter calcoaceticus,* two *Pseudomonas aeruginosa* and one of four *Eschericia coli* strains tested did show growth via the alternative and reference methods. For the reference method growth on Charcoal Cefoperazone Deoxycholate Agar as atypical (white colonies versus grey colonies), whilst for the alternative method typical growth was observed on *Brilliance* CampyCount Agar plates (red colonies). Confirmatory tests confirmed these strains as non-*Campylobacter* species.

Conclusion

The studies conducted as part of the EN ISO 16140:2016 certificate renewal study show that the *Brilliance* CampyCount Agar is an accurate and reliable method for the selective enumeration of thermo-tolerant *Campylobacter* species from poultry meat products. MicroVal Certificate number 2008LR12, available from http://microval.org/.

www.thermofisher.com

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USA +1 800 255 6730
International +44 (0) 1256 84114

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April 2019

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Appendix 1. Protocol for the *Brilliance* CampyCount Agar enumeration method and the ISO 10272-2:2017 reference method.

Day 0

Add 10 g of poultry food sample to 90 mL of diluent
(e.g. Buffered Peptone Water or Maximum Recovery Diluent) and homogenize thoroughly

Prepare the required number of decimal dilutions in a suitable diluent (as described in EN ISO 6887)

EN ISO 10272-2:2017 Reference Method

In duplicate spread 100 µL, or in triplicate spread 1 mL of the dilutions onto modified Charcoal Cefoperazone Deoxycholate Agar

Alternative Method

In duplicate spread 100 µL, or in triplicate spread 1 mL of the dilutions onto *Brilliance* CampyCount Agar

Incubate the inverted plates at 41.5±1 °C for 40-48 hours in microaerophilic conditions

Day 2

EN ISO 10272-2:2017 Reference Method

If present count colonies and select up to 5 well-isolated typical grey colonies and confirm using an oxidase test or aerobic growth at 25°C

Alternative Method

If present count colonies and select up to 5 well-isolated typical dark red colonies and confirm using either the Thermo Scientific™ O.B.I.S Campy Test or the Thermo Scientific™ Campylobacter Latex Kit

MicroVal Validation of the Thermo Scientific *Brilliance* CampyCount Enumeration Method in Comparison to EN ISO 10272-2:2017 in Accordance with ISO 16140-2:2016

David Crabtree[1], Jessica Williams[1], Ana-Maria Leonte[1], Gail Betts[2]
[1]Thermo Fisher Scientific, Basingstoke, UK, [2]Campden BRI Group, Chipping Campden, UK

INTRODUCTION

The Thermo Scientific™ *Brilliance*™ CampyCount Agar method enables the selective enumeration of thermo-tolerant *Campylobacter* species in raw and ready to cook poultry products.

The aim of this study is to renew the MicroVal™ validation of the *Brilliance* CampyCount Agar (alternative method) in comparison to EN ISO/TS 10272-2:2017[1] in accordance with EN ISO 16140-2:2016[2].

MATERIALS AND METHODS

The protocols for the alternative and reference methods are summarised below in figure 1.

Figure 1. Protocol for the Thermo Scientific *Brilliance* CampyCount Agar method and the EN ISO 10272-2:2017 reference method

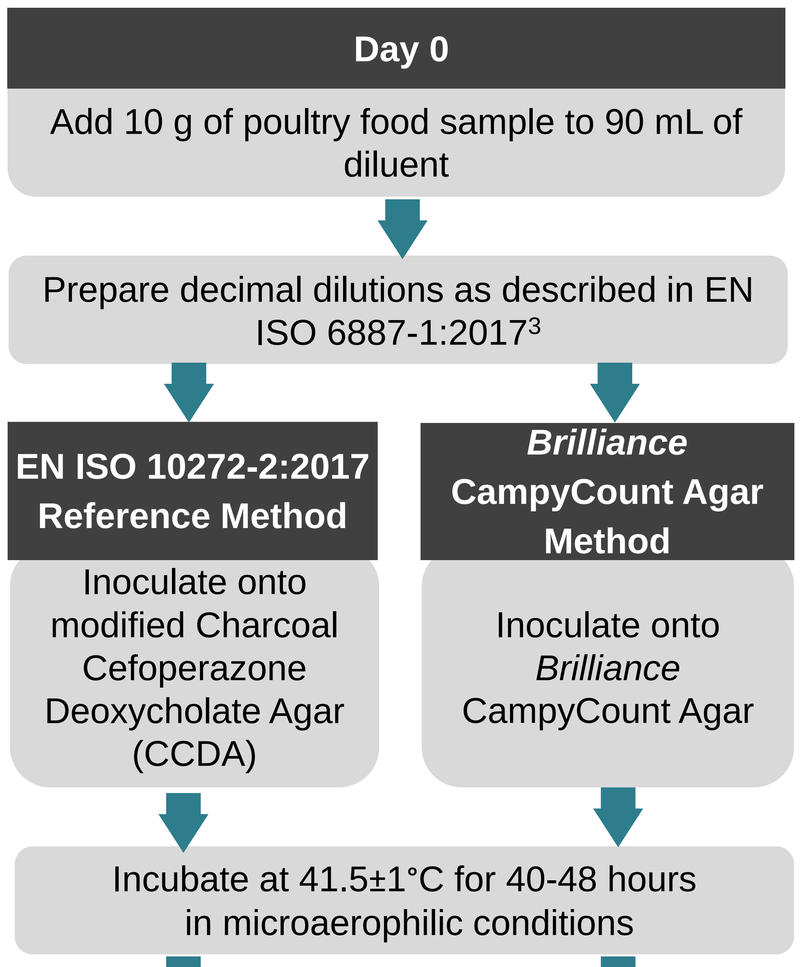

Figure 2. Thermo Scientific *Brilliance* CampyCount Agar with typical thermotolerant *Campylobacter* species colonies

RESULTS

Relative Trueness

Five levels of contamination were used and samples were tested in duplicate. A total of 48 naturally and 15 artificially contaminated samples were tested.

Figure 3. Scatter plot of the reference method vs the *Brilliance* CampyCount Agar method

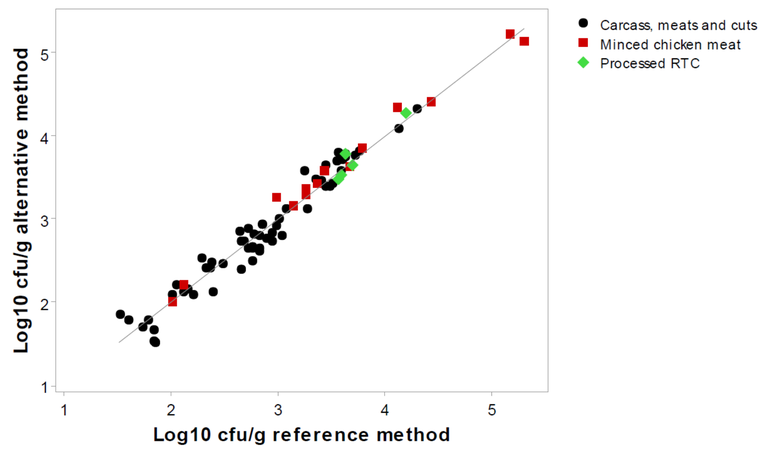

Figure 3 shows extremely good agreement between the reference and alternative method with almost no positive or negative bias.

Accuracy Profile

The poultry food category was tested with two separate batches of food type, using six samples per type. For each sample five different portions were tested (total of 30 analyses per food type). The calculations were performed as described in EN ISO 16140-2:2016.

In this study, one level (of 0.619 log) did not meet the acceptability limit of 0.5 log or the reference method standard deviation limit of >0.125. Therefore the required additional calculations were carried out as described in ISO 16140-2:2016 and the reference method met the newly calculated acceptability limit of ±0.736.

The accuracy of the alternative method is acceptable as all samples met both the original 0.5 log and the re-calculated acceptability limits.

Inter-laboratory study

The inter-laboratory study performed as part of the original validation showed no statistically significant differences between the alternative method and the reference method.

Inclusivity

Of the 66 inclusivity strains tested, 61 were successfully detected. One *C. jejuni* subsp. *Doylei* strain, Two *C. upsaliensis* strains and one *C. hyointestinalis* strain were not detected by either method. One *C. lari* strain failed to grow via the alternative method but did grow via the reference method; the remaining two *C. lari* strains tested successfully recovered via both methods.

Exclusivity

Of the 44 exclusivity isolates tested, 39 were correctly excluded by both methods. Confirmatory tests confirmed the five detected strains as non-*Campylobacter* species for both methods.

CONCLUSION

The Thermo Scientific *Brilliance* CampyCount Agar method is an accurate and reliable method for the selective enumeration of thermo-tolerant *Campylobacter* species in raw and ready to cook poultry products in accordance with EN ISO 16140-2:2016.

REFERENCES

1. EN ISO 10272-2:2017 ‘Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique’
2. EN ISO 16140-2:2016 Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
3. EN ISO 6887-1:2017 Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 1: General rules for the preparation of the initial suspension and decimal dilutions

TRADEMARK STATEMENT

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April 2019