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The alternative method was originally validated according to the superseded EN ISO 16140:2003 for enumeration of *Campylobacter* species in poultry products.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.

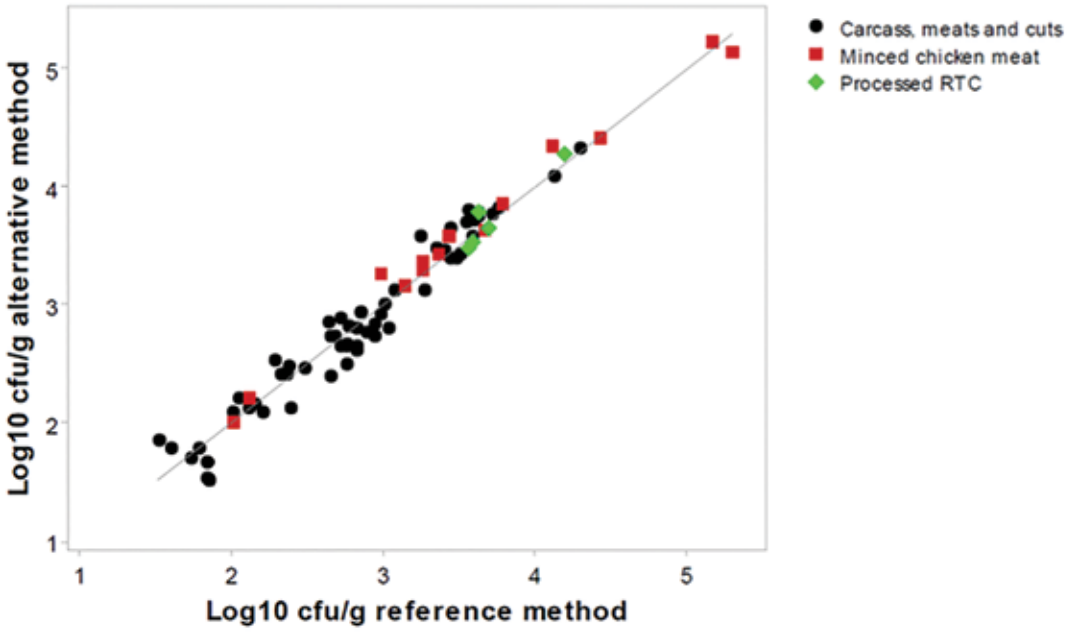

Figure 2. Scatter plot of the reference method versus the alternative method for raw and ready to cook poultry products[a]

[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/.

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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