MicroVal - ISO 16140 Evaluation Of A Defined Medium For Enumeration Of Thermotolerant *Campylobacter spp.*

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Overview

Purpose: Evaluation of Thermo Scientific™ Oxoid™ Brilliance™ CampyCount Agar for the enumeration of thermotolerant Campylobacter spp.

Methods: *Brilliance* CampyCount Agar was evaluated against the ISO reference method (ISO 10272-2:2006) with modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA)

Results: *Brilliance* CampyCount Agar proved to be an accurate and reliable alternative to mCCDA *for* the enumeration of *C. jejuni* and *C. coli.*

Introduction

Contamination of poultry flocks with the thermotolerant campylobacters; *C. coli and C. jejuni*, is the most common cause of bacterially mediated foodborne illness in many developed countries. Traditionally, media containing blood and/or charcoal have been utilized for the enumeration of *Campylobacter spp.* from foods. However, these media often lack specificity and individual colonies are difficult to count due to the swarming nature and morphology of Campylobacter. *Brilliance* CampyCount Agar is a novel defined medium for the direct enumeration of the thermotolerant Campylobacters, *C. jejuni* and *C. coli,* from foods. The medium has been validated according to ISO 16140:2003 and certified by MicroVal.

Methods

Brilliance CampyCount Agar was evaluated against ISO 10272-2:2006)with mCCDA according to the quantitative technical specifications detailed in ISO 16140:2003. In addition to the confirmation processes detailed in ISO 10272-2, presumptive positive colonies isolated on *Brilliance* CampyCount Agar were confirmed with two rapid confirmation tests: O.B.I.S. Campy (ID0800M) and Oxoid DrySpot Campylobacter (DR0150M).

FIGURE1: *Campylobacter jejuni* on *Brilliance* CampyCount Agar

Results

Inclusivity and Exclusivity

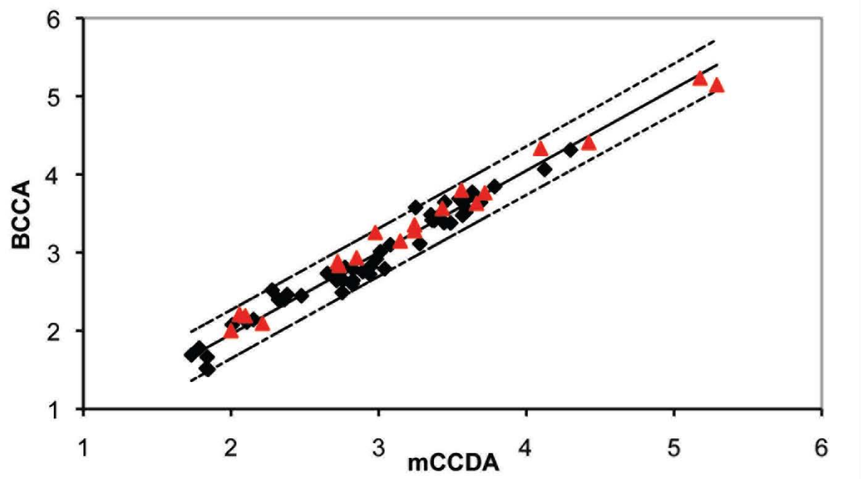
Pure cultures of *C. jejuni (19), C. coli (10), C. lari (3), C. upsaliensis (3)* and *C. hyointestinalis* (1) were used to assess the performance of *Brilliance* CampyCount Agar. All isolates of *C. jejuni, C. coli*, and two of the *C. lari* strains were recovered by both the reference and alternative media. *Campylobacter upsaliensis* and *C. hyointestinalis* failed to grow on both the reference and alternative media.

For exclusivity testing, 21 non-campylobacters relevant to poultry meat were analysed. With the exception of *Acinetobacter baumanii* and one *E. coli isolate*, no growth was recorded on either media. The *A. baumanii* and *E. coli* (an extended spectrum – lactamase (ESBL) isolates were rapidly confirmed as non-campylobacters by microscopy (ISO confirmation) or by latex agglutination and O.B.I.S Campy tests.

Detection and Quantification Limits

The detection and quantification limits of *Brilliance* CampyCount Agar were determined to be equivalent to mCCDA at 3 colonies per plate and 10 colonies per plate, respectively.

FIGURE 2: Calculated regression line with 95% confidence limits for all poultry products, except chicken thigh skin samples



Relative Accuracy, Sensitivity and Linearity

The relative accuracy and sensitivity of *Brilliance* CampyCount Agar were shown to be similar to the reference method with poultry meat, skin and liver samples (figure 2), regression line: y (alternative method)=1.05 x (reference method)= –0.14). The alternative method was also shown to have good linearity (figure 2), and there was no statistical evidence of lack of fit (p=0.25) between the alternative and reference methods.

Interlaboratory Study

An interlaboratory study was conducted according to ISO 16140 and MicroVal rules in seventeen laboratories across eight European countries, using a matrix of minced chicken meat spiked with an isolate of *C. jejuni* at low (log10 3.4 cfu/g), medium (log10 4.7 cfu/g) and high levels (log10 6.0 cfu/g). Statistical analysis demonstrated no significant bias between the two methods (D=0.08, 0.14 and 0.19 for each of the contamination levels respectively).

In addition, the average repeatability (0.21 for mCCDA and 0.18 for *Brilliance* CampyCount Agar) and reproducibility (0.35 for mCCDA and 0.23 for *Brilliance* CampyCount Agar) standard deviations across levels were demonstrated to be not significantly different (table 1).

TABLE 1: Average standard deviations for repeatability and reproducibility
S_r: repeatability, standard deviation, S_R: reproducibility, standard deviation

Spiking level	mCCDA			*Brilliance* CampyCount Agar			Bias (D)
	Median level (log10 cfu/g)	S_r	S_R	Median level (log10 cfu/g)	S_r	S_R	
Low	3.59	0.17	0.30	3.67	0.24	0.27	0.08
Medium	4.66	0.19	0.36	4.80	0.13	0.20	0.14

Conclusion

Brilliance CampyCount Agar proved to be an accurate and reliable alternative to mCCDA *for* the enumeration of *C. jejuni* and *C. coli.* Presumptive positive colonies (which are dark red) are easy to enumerate against the transparent background. Confirmation of presumptive positive isolates is simplified by using either the Oxoid DrySpot Campylobacter latex kit or O.B.I.S. Campy test. The certificate of compliance can be found at www.microval.org.

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