the enumeration of Campylobacter spp.

Methods: Brilliance CampyCount Agar was evaluated against the ISO reference method (ISO 10272-2:2006) with modified Chemical Charcoal 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 flock 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 proved to be an accurate and reliable alternative to mCCDA for the enumeration of C. jejuni and C. coli.

Methods

Brilliance CampyCount Agar was evaluated against mCCDA according to the quantitative technical specifications detailed in ISO 16140:2003. In addition, 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).

Results

Inclusivity and Exclusivity

Pure cultures of C. jejuni (19), C. coli (10), 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. jejuni strains were recovered by both the reference and alternative media. Campylobacter apsakellis 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 (F=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 (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 0.25 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, repeatability, standard deviation, Sr, reproducibility, standard deviation

<table>
<thead>
<tr>
<th>Spiking level</th>
<th>mCCDA</th>
<th>Brilliance CampyCount Agar</th>
<th>Bias (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median level (log10 cfu/g)</td>
<td>Sr</td>
<td>S</td>
</tr>
<tr>
<td>Low</td>
<td>3.59</td>
<td>0.17</td>
<td>0.30</td>
</tr>
<tr>
<td>Medium</td>
<td>4.66</td>
<td>0.19</td>
<td>0.36</td>
</tr>
</tbody>
</table>

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 test or O.B.I.S. Campy test. The certificate of compliance can be found at www.microval.org.

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