Validation of a Compact Dry Plate Method for enumeration of *Staphylococcus aureus* in foods

During a joint Microval, AOAC Research Institute EN ISO 16140 validation

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**Introduction**

Compact Dry (Nissui Pharmaceutical Co. Ltd; supplied by Hyserve GmbH & Co. KG) are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent. The Compact Dry X-SA medium is a ready-to-use plating method for enumeration of *S. aureus*. This validation was done according to the requirements of ISO 16140 and the rules of MicroVal and AOAC Research Institute.

**Methods**

The method validation consists of a methods comparison study (MCS) done by an expert laboratory and an inter-laboratory ring trial done by a number of independent laboratories (ILS). For the AOAC Research Institute validation, only the MCS is required.

In the MCS, the Compact Dry X-SA method and the reference method (ISO 6888-1:1999) were used to enumerate *S. aureus* cells, either inoculated into, or naturally present in a range of foods. Foods tested were cooked ham, prawns, milk, cream cakes and pasta.

The specificity of the Compact Dry X-SA (i.e. the ability of the method to detect target organisms) was determined using 32 *S. aureus* strains for inclusivity and 23 non-target strains for exclusivity. The ILS involved 10 laboratories in 6 countries testing inoculated milk samples for levels of *S. aureus*.

The test methods used in the MCS and ILS are summarised in Figure 1.

**Results**

The data was analysed in accordance with the requirements of ISO 16140. The results of the MCS showed good correlation between Compact Dry YM and the ISO reference method (Table 1).

<table>
<thead>
<tr>
<th>Food type (category)</th>
<th>Correlation Coefficient ($r^2$)</th>
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</thead>
<tbody>
<tr>
<td>Cooked ham (meat products)</td>
<td>0.993</td>
</tr>
<tr>
<td>Prawns (fish and seafood)</td>
<td>0.987</td>
</tr>
<tr>
<td>Milk (dairy products)</td>
<td>0.993</td>
</tr>
<tr>
<td>Cake (bakery products)</td>
<td>0.994</td>
</tr>
<tr>
<td>Pasta (other)</td>
<td>0.986</td>
</tr>
<tr>
<td>All Foods (global assessment)</td>
<td>0.992</td>
</tr>
</tbody>
</table>

One way analysis of variance (or paired t test) by food type and inoculation level was performed (AOAC Guidelines; 2002, Section 6.3.3.1). There was no evidence of any statistical differences between the two methods ($P>0.05$).

Results from the ILS (Figure 2) showed good correlation between the two methods when tested by 10 external laboratories and the expert laboratory.

All 32 strains of *S. aureus* grew and produced typical colonies on Compact Dry X-SA and the reference method. Four of the non-target strains grew on the reference medium but did not grow on Compact Dry X-SA, confirming the specificity of this method for *S. aureus*.

**Conclusions**

There were no substantial differences in the performance of the two test methods in the MCS or the ILS. The results of the validation study therefore showed the Compact Dry X-SA method to be equivalent to the reference method for the enumeration of *S. aureus* in a range of foods.

**Figure 1: Test methods**

Add 10g sample to 90 ml MRD: homogenise for 1 min

Serial dilute in MRD

**Compact Dry X-SA method**

Plate 1 ml aliquot of each dilution onto Compact Dry X-SA plate

Invert plates and incubate at 37 ± 2°C for 24 ± 2h

Count typical *S. aureus* colonies (light blue/blue)

Calculate cfu/g

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**Figure 2: Data from the ILS showing counts (Log cfu/ml) of S.aureus in milk**