A Quantitative Method for Determining the Bacterial Contamination of Dishes

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The three methods currently used for determining the extent of bacterial contamination on eating and drinking utensils, dairy equipment, and so forth, are thoroughly described in Standard Methods for the Examination of Dairy Products (American Public Health Association, 1948). They are known as the Rinse, Plate Contact, and Swab Contact Methods.

In studies on dishwashing, the authors desired to obtain more accurate results than are possible by the existing methods. It has been found that all bacteria on a plate are not subject to removal by swabbing, and thereby some escape detection. The surface of a plate is not perfect, and bacteria can be present on the plate in such a way that swabbing will fail to remove them. The authors have subsequently shown that even scouring may fail to remove all bacteria from restaurant plates.

In comparing the relative merits of various detergents for washing dishes, an empirical method was not considered sufficiently accurate, and hence the present method was developed. This method is briefly described in a previous publication (Flett and Guiteras, 1952) and is designed primarily for research work in the laboratory and for routine regulatory health control purposes.

METHOD

A dish or dinner plate whose bacterial contamination is to be measured is placed as a cover over a Petri dish containing melted tryptone glucose extract agar at about 45 C. The two dishes are held firmly together and then quickly inverted, so that the Petri dish is on top and the dinner plate on the bottom. If done properly, there is practically no leakage or spillage of the agar and practically no slipping of the Petri dish across the plate. After the agar has hardened at room temperature in this position, the set-up of the dinner plate containing the hardened agar with the Petri dish acting as a cover is incubated for 48 hours at 37 C. All the bacteria originally present on the surface of the dinner plate within the area circumscribed by the Petri dish have had a chance to multiply in the agar and form colonies. The dinner plate is removed from contact with the agar by inserting a wide spatula between the plate and the Petri dish. The plate can then be removed easily, leaving the agar in the Petri dish, where the colonies are counted with the aid of a Quebec Colony Counter. This method has the disadvantage that the set-up of Petri dishes and dinner plates occupies considerably more space in the incubator than an equal number of Petri dishes with their covers. It has the advantage, however, that all the bacteria on the portion of the plate covered by the Petri dish are trapped and held in the agar during the entire incubation period, thus affording them the maximum opportunity to grow and form colonies.

EXPERIMENTAL PROCEDURE

To establish the validity of the method and to compare it with the Swab Contact Method, the following experiment was set up. Ten dinner plates were washed thoroughly with soap and water, rinsed, and sterilized with dry heat. An area the size of a Petri dish was circumscribed on the center of the eating surface of each plate with red crayon, using a Petri dish as a guide. A culture of Micrococcus pyogenes var. aureus was diluted 1:1,000,000 to give 2,850 bacteria per ml, and 0.1 ml of

<table>
<thead>
<tr>
<th>Plate no.</th>
<th>Swab 1</th>
<th>Swab 2</th>
<th>Total, swab 1 and swab 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>50</td>
<td>170</td>
</tr>
<tr>
<td>2</td>
<td>140</td>
<td>60</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>130</td>
<td>50</td>
<td>180</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>30</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td>140</td>
<td>50</td>
<td>190</td>
</tr>
<tr>
<td>Average</td>
<td>130</td>
<td>48</td>
<td>178</td>
</tr>
</tbody>
</table>

* Each plate artificially contaminated with 0.1 ml bacterial suspension diluted to give 285 microorganisms per plate.
The dilution was placed in the center of each plate and thoroughly distributed with the pipette over the surface of the dish contained within the red crayoned circle. To facilitate wetting of the plates with the bacterial suspension, 0.2 per cent of Triton X-100 was added to the diluted culture. Previous tests had shown that this had no influence on the growth of the organism. The contaminated plates were then allowed to dry face-up in an incubator at 37 C. The plates were visibly dry within half an hour. Bacterial counts were then taken on five of the plates by the method described above, and on the other five plates by the Swab Contact Method. In using the latter method, the entire contaminated surface of the plate was swabbed thoroughly with a wet swab and then re-swabbed with a second swab, counts being taken on both swabs by the usual procedure. The experiment was repeated on a second series of ten plates, using as the contaminating organism a spore suspension of Bacillus subtilis diluted 1:10,000 and containing 260 organisms per 0.1 ml. The results are given in tables 1 and 2.

The total number of organisms recovered by the authors’ method is quantitative, within the limits of experimental error. The number recovered by the Swab Contact Method is significantly lower, even when two swabs are used. The first swab does not remove all the organisms from the surface of the dish, as can be seen from the fact that a substantial number are removed by the second swab. Furthermore, once the organisms are on the swab, they are not quantitatively transferred from the swab to the solution which is used to determine the bacterial count.

**SUMMARY**

A quantitative evaluation has been made of a previously described method for determining the bacterial contamination of dishes. A quantitative recovery of organisms is obtained from dishes artificially contaminated with Micrococcus pyogenes var. aureus and with Bacillus subtilis. The method has been shown to be a valuable quantitative procedure for evaluating bacterial contamination.

**REFERENCES**
