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# Bacterial counts on fabrics: a comparative study of three methods

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## SUMMARY

One contact plate and two homogenization methods have been compared for efficiency in assessing the bacterial contamination of fabrics with high or low, natural or artificial contamination. The contact plate method resulted in considerably lower counts than any of the homogenization methods, which closely resembled one another. One of these, utilizing a Stomacher 400®, was found to be more practical, and is therefore recommended for counting bacteria on fabrics.

## INTRODUCTION

Micro-organism counting on fabrics is useful for many purposes, for example to determine the bioburden before sterilization, assess the reduction in bacterial counts in connection with various laundry processes, or trace transfer routes in infection control investigations. Therefore a validated, reproducible and rational method is needed.

Many methods have been used: contact plate (Hall & Hartnett, 1964), sweep plate (Blowers & Wallace, 1955), washing (Hambraeus, 1973), homogenization methods (Nyström, 1981), etc. Comparisons between these methods are scarce but have demonstrated large differences (Nicoles, 1970; Hambraeus, 1973).

For food sample processing, a stomaching technique has been described by Sharpe & Jackson (1972). The purpose of the present study has been to compare the efficiency of one contact plate and two homogenization methods, one of the latter using a Stomacher 400®, in assessing the contamination on fabrics with high and low bacterial counts.

## MATERIALS AND METHODS

### *Fabrics*

Cotton and cotton polyester fabrics were used in the study. The cotton fabric was either in the form of 162 g/m², as used in operating towels, or 20-thread gauze. The cotton polyester fabric used in protective gowns had a surface weight of 348 g/m².

## *Contamination*

### *Artificial contamination of cotton drape fabric*

One of the methods described by Jerram (1958) for standardized contamination of textile materials with *Streptococcus faecalis* was used. The growth from an 18 h culture of *Str. faecalis* (NCTC 10927) on blood agar was washed off with inactivated horse serum. Pieces of fabric (∅ 12·5 cm) were immersed in the suspension, withdrawn, and dried at room temperature.

### *Natural contamination of raw gauze*

Pieces of raw gauze with the natural contamination from cotton, from spinning and weaving and from transportation were investigated prior to washing or bleaching.

### *Natural contamination of cotton–polyester protective gowns*

Clean protective gowns, after normal laundry, were analyzed after having been used for one day by the nursing staff at the intensive care unit of Huddinge University Hospital, Stockholm.

## *Bacterial count methods*

### *Contact plate counts*

Rodac plates (19·6 $cm^2$) with blood agar were used. They were pressed on to the fabric test sample for 15 s and then incubated for approximately 40 h at 37 °C. The number of colonies on the agar plate surface was then counted.

### *Omnimixer counts*

A piece of fabric (1 $cm^2$) was cut out using a pair of sterile scissors and cut into small pieces. These were put into the 'micro chamber' of a Sorvall Omnimixer® (Du Pont Co., Newtown, U.S.A.) containing 5 ml 0·9% NaCl with 5% nutrient broth. The homogenizer was run for 1 min at approximately 4400 rev./min.

The homogenized slurry was serially diluted and spread on to the surface of blood agar or TGE agar (Oxoid Ltd, Basingstoke, England) plates which were incubated for approximately 40 h at 37 °C. Colony counts were then carried out.

### *Stomacher counts*

A fabric test sample, 100–125 $cm^2$, was placed in a sterile plastic bag together with 100 ml 0·9% NaCl. The bag was sealed and run for 3 min in a Stomacher 400® (Seward Medical, London, England). The fluid was poured into a sterile bottle with sterile glass beads and shaken on a HETO® (Heto, Birkerød, Denmark) shaker for 10 min. The fluid was serially diluted and spread on to the surface of TGE agar plates which were incubated at 37 °C for approximately 40 h. Colony counts were performed.

## *Experiments*

### *Series 1*

Bacterial counts were performed with Omnimixer and Stomacher techniques on artificially contaminated cotton fabric test samples. Four sets of experiments were performed.

*Series 2*

Bacterial counts were performed with contact plate, Omnimixer and Stomacher techniques on polyester/cotton fabric test samples cut out from the front of protective gowns used for one day in the intensive care unit. Three sets of experiments were performed.

*Series 3*

Bacterial counts were performed with Omnimixer and Stomacher techniques on naturally contaminated raw gauze, which had not been subjected to any cleaning process after weaving. One set of experiments were performed.

The experiments in series 1 and 2 were carried out with samples from the same batch in parallel in the two laboratories available to the authors.

### *Statistical methods*

In comparing the results obtained with the different methods Student's $t$ statistics were used (Snedecor & Cochran, 1973).

## RESULTS

The results of the experiments are presented in Table 1.

Measured with the homogenization methods, the artificially contaminated cotton test samples had a contamination level of $10^7$ c.f.u./cm$^2$ of enterococci. The cotton–polyester samples of used protective gowns had a lower contamination level of $10^2$ c.f.u./cm$^2$, as was the case with the naturally contaminated raw gauze.

In comparisons between the two homogenization methods no significant count difference was found in 5 of the 8 sets of experiments (exp. nos. 1, 2, 3, 5 and 6). In 3 sets of experiments (exp. nos. 4a, 7a and 8), there were statistically significant count differences between techniques. In 2 sets of experiments (exp. nos. 4a and b, 7a and b), results were compared from the two laboratories with the same homogenization technique (Stomacher). A statistically significant difference was found in experiment no. 4.

The standard deviations using the homogenization methods were generally low in all experiments except for one of the two laboratories in experiment no. 5.

The geometric average of contact plate counts in three sets of experiments (exp. nos. 5, 6, 7a) were tenfold to a thousandfold lower than those obtained with the homogenization methods. The variation range in contact plate counts was from 0·05 to a few c.f.u./cm$^2$.

## DISCUSSION

The magnitude of the total bacterial counts in the present study found with contact plates is quite different from that found with any of the homogenization methods. The low counts reported in this investigation with the contact plate technique are in accordance with other similar studies (Babb, Davies & Ayliffe, 1983).

There can be many explanations for the difference in counts between techniques. The contact plate counts colony-forming units on the surface of the fabric. The

Table 1. *Bacterial counts on fabrics with various laboratory methods*

| Series | Material | Experiment | Number of samples | c.f.u./cm² Omnimixer Geometric mean | Lab* | Log s.d. | Number of samples | c.f.u./cm² Stomacher Geometric mean | Lab* | Log s.d. | Control plate Geometric mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Cotton. Artificially contaminated with *Str. faecalis* | 1 | 12 | $0{\cdot}5 \times 10^{7}$ | H | 0·39 | 12 | $1{\cdot}4 \times 10^{7}$ | G | 0·29 | — |
| | | 2 | 10 | $2{\cdot}4 \times 10^{7}$ | H | 0·29 | 8 | $4{\cdot}6 \times 10^{7}$ | G | 0·18 | — |
| | | 3 | 10 | $2{\cdot}1 \times 10^{7}$ | H | 0·21 | — | — | — | — | — |
| | | 4a | 6 | $3{\cdot}2 \times 10^{7}$[a] | H | 0·17 | 6 | $6{\cdot}3 \times 10^{6}$ | H | 0·15 | — |
| | | 4b | — | — | — | — | — | $5{\cdot}9 \times 10^{7}$ | G | 0·21 | — |
| 2 | Cotton-polyester. Naturally contaminated in hospital use | 5 | 10 | $2{\cdot}6 \times 10^{2}$ | H | 1·16 | 10 | $21 \times 10^{2}$ | G | 0·25 | 0·7 H, G |
| | | 6 | 10 | $2{\cdot}7 \times 10^{1}$ | H | 0·57 | 10 | $2{\cdot}0 \times 10^{1}$ | G | 0·36 | 1·7 H |
| | | 7a | 6 | $3{\cdot}7 \times 10^{2}$[b] | H | 0·36 | 10 | $1{\cdot}7 \times 10^{2}$ | H | 0·90 | 3·8 H, G |
| | | 7b | — | — | — | — | — | $1{\cdot}9 \times 10^{2}$ | G | 0·60 | — |
| 3 | Raw gauze. Naturally contaminated | 8 | 5 | $0{\cdot}8 \times 10^{2}$[a] | G | 0·11 | 5 | $2{\cdot}3 \times 10^{2}$ | G | 0·11 | — |

* Laboratory: H = Huddinge, G = Göteborg.

[a] Exp. 4a and 8: Omnimixer – Stomacher, s.d. $P < 0{\cdot}001$.

[b] Exp. 7a: Omnimixer – Stomacher, s.d. $0{\cdot}05 > P > 0{\cdot}01$.

[c] Exp. 4a and b: Stomacher Huddinge – Göteborg, s.d. $0{\cdot}05 > P > 0{\cdot}01$.

homogenization procedure disperses larger colony-forming units into smaller ones, possibly into single cells. The efficiency of the contact plate method depends on the evenness of the surface tested (Maunz & Kanz, 1969). The structure of a textile, due to the mechanical combination of warp and weft, offers a relatively small contact area. Thus a contact plate method should here be relatively inefficient. Micro-organisms that have penetrated into the deeper structure of the material will not be detected by contact plate methods. However, it may well be that the contact plate technique is the most relevant in studies of contact transfer from fabrics.

The results obtained with the two homogenization methods were quite similar. Even if statistical differences were found in a few of the sets of experiments, these were of little practical significance, all being less than tenfold.

The low standard deviation values for both methods demonstrate their good reproducibility. The variations in counts between the two participating laboratories with the Stomacher technique were small, not more than fivefold, even though one of the laboratories had much less experience with the method than the other. It should be emphasized that the shaking of the Stomacher homogenizing fluid with glass beads was essential for the low variations in counts.

Of the two homogenization techniques tested, the Stomacher is the more handy. This facilitates laboratory work and reduces the processing time. Large pieces of fabric are easier to handle in the Stomacher. Contrary to the Omnimixer technique, the Stomacher does not destroy the test piece. It is reasonable to assume that, due to the less complicated preparation and quicker handling of the samples, laboratory contamination should be less of a hazard with the Stomacher than with the Omnimixer. For these practical reasons, the Stomacher technique should be preferred when counting bacteria on fabrics.

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