Evaluation of Three Carcass Surface Microbial Sampling Techniques

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ABSTRACT

Three carcass surface microbial sampling techniques were evaluated: a double swab, an excision and an agar sausage technique. In each instance, a sampling area of 6.42 cm² was used. For the double swab technique, two sterile dry swabs were used. A sterile meat borer was used to cut out the area of 6.42 cm² for the excision technique. For the agar sausage technique, 50-cm³ medical syringes were used to take impression plate samples. All the samples obtained with the different techniques were subjected to serial dilutions, whereafter they were spread-plated in duplicate on prepoured plates. Results of the study indicated that there was a significant difference (P<0.05) between the three techniques. The excision technique was the most reliable while the agar sausage technique had a higher coefficient of determination (r²) value with the excision technique than did the swab technique.

Microbiologists have been attempting to detect and quantify microorganisms on surfaces for more than 60 years. However, it was not until the 1930s that any consideration was given to developing and evaluating optimal techniques for sampling surfaces. As new problems arose, it became evident that modifications of the original techniques would have to be made for investigating specific problems. The principle factors that influenced selection of a particular technique were type and chemical composition of the surfaces, expected levels and types of microbial contaminants and objective of the test. Some tests were designed merely to provide an index of sanitation, whereas others were concerned with precise quantification of microorganisms on surfaces (1). Ingram and Roberts (3) found that an excision technique gave the most reliable count, while Gilbert (2) showed that the agar sausage technique gave a mirror image of the distribution of bacteria on surfaces, no matter whether they appeared singly or in colonies. The swab rinse methods were found more likely to give a single cell count, because the colonies are broken up during sampling (2).

The agar sausage and swab techniques are specifically used under commercial conditions, merely to provide an index of sanitation in industry. The object of this study was to evaluate these two techniques in comparison with the excision method to find a generally acceptable technique for sampling carcass surfaces.

MATERIALS AND METHODS

Three techniques, namely an excision, a double swab and an agar sausage technique, were compared. The three techniques were evaluated by monitoring an area of equal size (6.42 cm²) on the same slice of meat. These slices of meat (100 x 100 x 5 mm) were excised at eight different positions on the left sides of carcasses (n = 7). The meat pieces were vacuum-packaged and sterilized by γ-irradiation at a dose of 11 K.Gy. After packaging and irradiation, each sample was contaminated by immersion into a separate broth containing pure cultures of Pseudomonas fluorescens with a known cell count (ca. 10⁵/cm³). Although the method of inoculation by immersion may have some effect on recovery of organisms, this procedure was standardized for all the techniques evaluated and therefore the results were regarded as unbiased. The counts on these samples were then monitored by means of the three techniques.

The excision technique

Samples with a surface area of 6.42 cm² were excised from the contaminated slice by means of a sterile meat borer, scalpel and tweezer. The samples were placed aseptically in sterile plastic bags with 10 cm³ of sterile 1/4 strength Ringer solution (Merek). The samples were then homogenized with a Colworth Stomacher 400. These homogenized meat samples were used as a standard for comparison with the agar sausage and double swab techniques and represented a count per 6.42 cm².

The double swab technique

Two dry swabs were used to swab an area of 6.42 cm². Templates were made by cutting an area of 6.42 cm² out of a piece of cardboard (5 x 5 cm), which was sterilized in aluminium foil. After swabbing the area, swabs were broken off into a test tube containing 10 cm³ of sterile 1/4 strength Ringer solution solution.

The modified agar sausage technique

The agar sausages were prepared as described by Ten Cate (8) and Ølgod (6) except that instead of artificial sausage casings, 50-cm³ syringes were used, yielding agar sausage slices with surface areas of 6.42 cm² (6.8). Furthermore, dilutions were introduced to the technique by placing the agar slice with which the impression sample was taken

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into a sterile plastic bag with 10 cm$^3$ of 1/4 strength Ringer solution. The agar slices were homogenized with a Stomacher. In each instance, serial dilutions were made from the homogenized samples and then plated on Standard 1 agar (Merck) by means of the spread plate method (5). The results obtained with each technique were statistically analysed by fitting linear regressions and analyses of variance (7). For linear regressions the excision technique was used as the independent variable and the other techniques as dependent variables. In each instance, the data were transformed to the ln-form to get a more even distribution around the regression line.

RESULTS AND DISCUSSION

The data in Table 1 clearly demonstrate that the modified agar sausage technique was more closely correlated with the excision technique than was the swab technique. The swab technique was considered to be the least desirable because of the high coefficient of variation as well as the high standard deviation. The modified agar sausage technique therefore was regarded as the more accurate technique. To illustrate the relationship among the three different techniques, linear regression values are given in Table 2. The range of the limits given in this table illustrates that the excision count could be estimated more accurately with the modified agar sausage count than with the swab count. According to data in Table 2 it is therefore possible to predict the excision count with the modified agar sausage count by means of the following equation:

$$\ln y = a + b \ln x$$

therefore, $\ln y$ (excision count) = 0.8374 + 0.5758 $\ln$ (modified agar sausage count).

To estimate whether the differences among techniques were significant, a two-way analysis of variance was carried out with three techniques and eight positions as variables with four measurements per cell. The analysis was carried out on ln-transformed data. The results of this analysis are given in Table 3. The eight different positions on the carcass had no significant influence on the reclamation of bacteria by means of the three techniques. Counts obtained with the different techniques were, however, significantly different from each other and indicated that the reclamation ability of the three techniques also differed significantly ($P<0.01$). Least significant differences indicated that all the techniques differed significantly ($P<0.01$).

CONCLUSION

This study confirms the findings of Ingram and Roberts (3) that the excision technique is the most reliable among those tested. Results of the modified agar sausage technique correlated more closely with those of the excision technique.