

## *A Research Note*

# Use of Petrifilm™ 3M to Assess Coliform Numbers on Lamb Carcasses

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### ABSTRACT

Petrifilm™ (6410) was used directly on lamb carcasses to enumerate coliforms. Ten sampling locations (sites) on 30 carcasses were sampled at each of four separate meat processing establishments (works). The coliform counts obtained by this technique were statistically analyzed using analysis of variance (ANOVA) to select the optimum sampling sites on the carcass and to assess the contamination of the carcass by gut flora at a particular establishment. There was a large variation between sites and between works. In general, works 3 and 4 produced cleaner carcasses than works 1, 2, which in turn was cleaner than works 1. Since works 1, 2 and 4 used conventional dressing techniques and works 3 used the inverted dressing method, the coliform counts found at works 3 and 4 are achievable regardless of dressing technique. The coliform bacteria were most concentrated around the posterior pelvic rim and least prevalent at the carcass extremities. The posterior pelvic rim (sites 3 and 4) had higher ( $P < 0.05$ ) coliform counts than the exterior ventral flank area (sites 5, 6, 7 and 8), which in turn had higher ( $P < 0.05$ ) counts than the proximal hind and proximal fore limbs (sites 1, 2, 9 and 10) across all works. With in-line routine testing it is recommended that the majority of carcasses sampled should give coliform counts of less than 50 colony forming units (CFU)/20 cm<sup>2</sup> for sites 4 and 8.

Key Words: Petrifilm™, coliform count, carcasses.

Bacteria in meat and meat products can constitute a health hazard and affect keeping quality. The internal tissue of healthy animals is virtually sterile and bacterial contamination only occurs during slaughter, dressing, handling and storage (6). A variety of selective procedures are available for determination of the presence of pathogenic bacteria; however, pathogenic bacteria are usually only present in low numbers and are difficult to detect (7). Thus, indicator organisms, such as coliforms, generally present in larger concentrations, are enumerated. Therefore, counts of indicator organisms such as coliforms, are used to assess the level of contamination of a carcass.

Currently the most common methods for the enumeration of bacteria on carcass surfaces are the swab/plate method and excision/plate method. Both of these methods require considerable expertise, equipment and time in both

preparation and execution. Therefore, the direct application of 3M Petrifilm™ (6410) to the surface of the carcass was proposed as a means of enumerating the coliform group of bacteria. This method is simple, rapid, non-destructive and suitable for in-line quality assurance testing.

The present system of assessing hygiene in meat processing establishments uses macro (visual) inspection by government inspectors. The proposed replacement of inspection by microbiological analyses is an attempt to introduce an objective rather than a subjective test for appraising hygiene. Such a test could be an integral part of product specifications of a quality assurance program. Therefore, the project's aim was to determine the optimum site or sites for coliform enumeration and the mean count per carcass achievable with good manufacturing practices in meat processing establishments.

Initial experimentation and literature (1,2,3,4,5,8,9) suggested that 3M Petrifilm™ (6410) offered advantages over both the swab/plate method and the excision/plate method. Some advantages noted were: rapidity, simplicity, non-interference with line operations, non-destructive sampling, accuracy, good selectivity and ease of storage and preparation of materials. Therefore, 3M Petrifilm™ (6410) — violet red bile, a selective medium for the enumeration of the coliform group, was chosen for this trial.

Coliform counts cannot be directly correlated with keeping quality or public health risk. However, the presence of coliforms is regarded as a good indicator of poor hygienic practices in food industries.

### MATERIALS AND METHODS

#### *In-line sampling.*

Sampling involved four meat processing establishments (works), 30 ovine carcasses per establishment and 10 sampling locations (sites) per carcass. The ovine carcasses were sampled after the final wash, just prior to chilled storage. The location of the 10 sites on the external surfaces of the carcass (Fig. 1) were chosen for their significance in the dressing procedure after consultation with government meat inspectors. Works 1, 2 and 4 used the conventional hind-leg dressing procedure and works 3 used the inverted fore-leg dressing procedure.

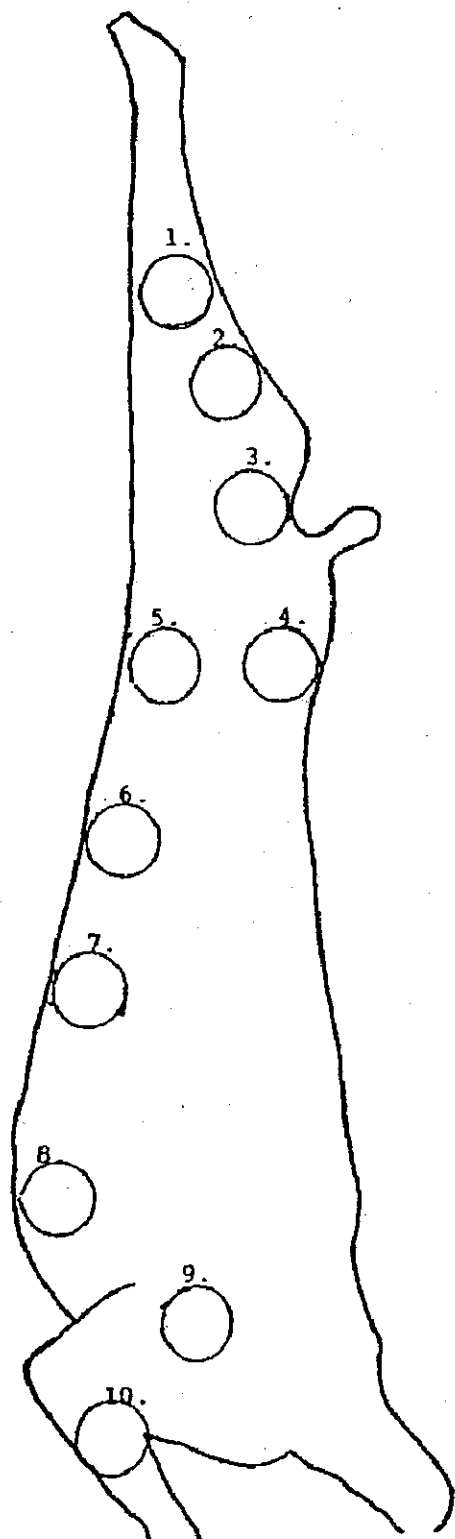


Figure 1. External sampling sites on ovine carcass.

The sampling of the ovine carcasses was carried out over the duration of 1 day's production run. All samples were collected during the winter month of July.

#### Preparation of 3M Petrifilm™ (6410).

The 3M Petrifilm™ (6410) was pre-hydrated approximately 12 h prior to use with 1 ml of sterile 0.1% peptone water per plate. The hydrated plates were subsequently refrigerated at 4°C until required.

#### Sampling procedure.

The top film was lifted and the gel surface carefully pressed against the external ovine carcass surface at the designated site. The external gel surface was rubbed gently to ensure good contact between the carcass surface and the gel. The top film was then replaced ensuring the exclusion of air bubbles.

#### Incubation and enumeration procedures.

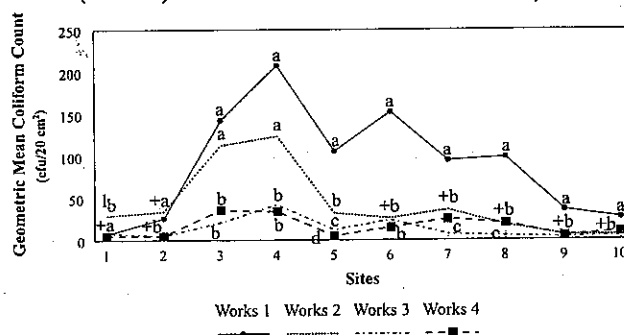
The 3M Petrifilm™ plates were incubated at 37°C for 24 h. Coliform counts were recorded as the number of CFUs per 3M Petrifilm™ plate. The circular growth area is approximately 20 cm<sup>2</sup>. Estimates were made on plates containing greater than 150 colonies by counting a representative number of squares and multiplying by the appropriate number to obtain an estimated count for the total 20 cm<sup>2</sup> growth area.

#### Statistical analysis.

Coliform counts were analyzed by ANOVA after a log transformation. Initially a split plot model was used in which the site on a carcass was the sub-plot factor. In view of the significant interaction between works and site and the non-homogenous error variation across sites, it was decided to also analyze each site separately; however, geometric means were underestimated for some works using ANOVA techniques as counts larger than 300 were recorded as 301 for analysis. To overcome this difficulty, graphs were prepared in which the number of carcasses were grouped into the following categories: counts ≤10, >10, ≤50, >50, ≤100 and >100 (Fig. 3). Chi-square statistics were calculated on the number of carcasses with counts >50 to test whether the differences between works were statistically significant for each site. No apparent trends in counts, due to operator variability, were reflected in the microbial results.

## RESULTS

Coliform counts were higher ( $P < 0.05$ ) at sites 3 and 4, with site 4 recording the highest mean count (Fig. 2). The sites on the carcass trunk (sites 5, 6, 7 and 8) recorded lower ( $P < 0.05$ ) coliform counts than sites 3 and 4, which in



<sup>1</sup> For each site, works given the same letter are not different ( $P > 0.05$ ).  
<sup>+</sup> Where the symbols are closely grouped, the letter refers to all symbols within the group.

Figure 2. Comparison of coliform counts (geometric means) across works for each site. Works 1, ●; Works 2, □; Works 3, ○; and Works 4 ■.

<sup>1</sup> For each site, works given the same letter are not different ( $P > 0.05$ ).

<sup>+</sup> Where the symbols are closely grouped, the letter refers to all symbols within the group.

turn were higher ( $P < 0.05$ ) than the sites on the carcass extremities (sites 1, 2, 9 and 10).

A significant site by works interaction occurred. It can be substantially explained by grouping sites showing similar patterns across works. Sites 2, 3 and 4 recorded no statistical difference ( $P > 0.05$ ) between works 1 and 2 nor between works 3 and 4. However, works 1 and 2 recorded higher ( $P < 0.05$ ) mean counts than works 3 and 4 (Fig. 2).

For sites 5 to 10, works 2 recorded lower ( $P < 0.05$ ) mean counts than works 1 and similar mean counts to works 4 except for site 5. Works 3 recorded lower ( $P < 0.05$ ) mean counts than all other works at sites 7 and 8.

The mean counts for each works were similar at sites 1 and 2. However, works 1 recorded lower ( $P < 0.05$ ) counts than works 2 at site 1.

A similar pattern of results was observed when the number of carcasses with a count larger than 50 was graphed for each works at each site (Fig. 3). Each works recorded its highest number of carcasses with a count greater than 50 at site 4.

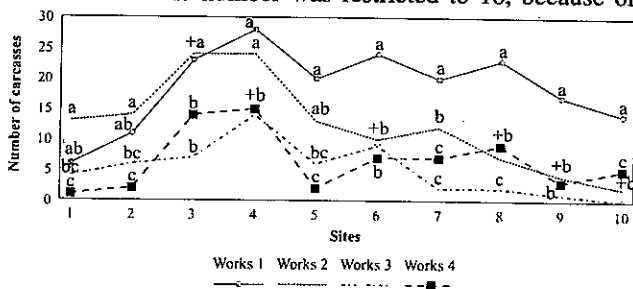
The majority of carcasses that recorded sites with counts greater than 300 occurred at works 1. At this works, 93 of the 300 counts recorded were greater than 300 CFU/20 cm<sup>2</sup>, compared to 29 sites at works 2, 7 sites at works 3 and 0 sites at works 4. This converts to 31, 10, 2 and 0% of the total number of sites at works 1, 2, 3 and 4, respectively.

In terms of sites rather than works, the highest incidence of counts greater than 300 occurred at site 4 (27% of counts). More generally, the majority of counts greater than 300 occurred at sites 3, 4, 5, 6, 7 and 8 which correspond to the trunk of the animal (Fig. 1).

Samples were collected at equal time intervals during the day's production and there was no correlation between time of day and level of contamination.

## DISCUSSION

Ten sites per carcass were examined to determine the distribution of coliform bacteria over the external surface of the carcass. The positions of the 10 sites (Fig. 1) were chosen because of their significance to procedures, such as evisceration. The number was restricted to 10, because of



<sup>1</sup> For each site, works given the same letter are not different ( $P > 0.05$ )  
 \* Where the symbols are closely grouped, the letter refers to all symbols within the group.

Figure 3. Number of carcasses with counts greater than 50 CFU/20 cm<sup>2</sup> at each site. Works 1, ○; Works 2, □; Works 3, △; and Works 4, ◆.

<sup>1</sup> For each site, works given the same letter are not different ( $P > 0.05$ ).

\* Where the symbols are closely grouped, the letter refers to all symbols within the group.

time and cost factors. However, this number would be excessive in an in-line production situation at a meat processing establishment. Therefore, to minimize disruption to the production line, the number of sites per carcass should be kept to four or less. Preferably, sites chosen would be indicative of evisceration procedures and have counts below 300 colonies per plate.

The difference between works was more pronounced at sites with higher counts (Fig. 2). In general, at sites with higher counts, works 3 and 4 had lower counts than works 2 which in turn were lower than works 1 (Fig. 2). The coliform counts at works 3 were similar to those found at works 4. Since works 1, 2 and 4 used conventional dressing techniques and works 3 used the inverted dressing method, the coliform counts found at works 3 and 4 are achievable independent of dressing technique.

The sampling of the ovine carcasses was carried out over the complete day's production run at each meat processing establishment. The coliform counts showed no significant trend with time. Therefore, under a quality assurance system, sampling could be undertaken at any time during a day's production.

Coliform counts were highest at sites 3 and 4 and lowest at the carcass extremities, that is, sites 1, 2, 9 and 10 (Fig. 1 and 2). Sites 3 and 4 had higher coliform counts than sites 5, 6, 7 and 8, which in turn had higher counts than sites 1, 2, 9 and 10. It is proposed that sites 4 and 8 be used for in-line routine testing in a production situation. Site 4 was chosen because it gave countable results at works 3 and 4 (Fig. 2), that is, the works with lower counts. Also, its position on the carcass indicates procedural problems during the "ringing" (excision of the anus) operation. With site 8, countable coliform results were achieved at works 1 and 2 (Fig. 2), that is, the works with higher counts. This site indicates procedural problems during the evisceration step of processing.

These results suggest that with in-line routine testing, such as that required for a quality assurance audit, the majority of carcasses sampled at clean works should give coliform counts of less than 50 for sites 4 and 8. This was demonstrated for sites 4 and 8 at the works having lower coliform counts, that is, works 3 and 4 (Fig. 2). The number of carcasses sampled would follow a sampling plan based on throughput of the works.

## ACKNOWLEDGMENTS

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