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ORIGINAL ARTICLE

# Microbiological verification of the control of the processes of dressing, cooling and processing of beef carcasses at a high line-speed abattoir

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*Methods were described, and are offered to inspection agencies, to verify the microbiological adequacy of the processes of dressing, chilling and fabricating beef carcasses. The isolation rate of specific pathogens from the fabrication floor was also determined for pre-bagged subprimal beef cuts at the end of fabrication. The microbiological adequacy of the process on the slaughter floor was verified by inspection staff of Agriculture and Agri-Food Canada over an 11-month period at one high line-speed abattoir. Similar methods using hydrophobic grid membrane filter (HGMF) technology were applied by research projects at the same abattoir to verify the microbiological adequacy of the processes of chilling and fabricating beef carcasses. Ten excision samples (5×5 cm) were taken from each of 15–20 carcasses per evaluation of the slaughter and chill process. The range of the estimated mean $log_{10}$ of the most probable number of growth units per centimeter squared (LMPN) of 11 monthly evaluations (and a second evaluation in June 1993) of the slaughter floor was 1·64±0·06–2·00±0·07. The quality control programs, which were created by the management for the slaughter process at this abattoir, were verified to be working effectively for the 11 month period under study. For a single evaluation of the chill floor the LMPN was 1·22. On the fabrication floor 140 subprimal cuts of carcasses were examined after bagging (pre-vacuum) at the end of five conveyer lines. Seventy surfaces freshly 'cut' and 70 surfaces 'not-cut' on the fabrication floor were sampled as above. Most probable numbers of aerobic bacteria, coliforms and* Escherichia coli *were determined per centimeter squared and log transformed (LMPN, LCOL and LEC, respectively). LMPN for bagged subprimal cuts (2·72± 0·07) were more than 1 $log_{10}$ $cm^{-2}$ higher than for the single evaluation at the end of the chill floor. For LMPN and LCOL there was a significant effect of sampling date (*$P<0·05$*) but no effect of conveyer line. LMPN were significantly lower (*$P<0·001$*) from 'cut' than from 'not-cut' surfaces of bagged beef cuts. This difference was less than one $log_{10}$ $cm^{-2}$, and therefore, of questionable practical significance. There was no effect (*$P>0·10$*) of 'cut' or 'not-cut' surfaces on LEC which had an overall mean level of 0·39. The associations among these variables was strongest for LCOL and LEC (*$r=0·81$, $P<0·001$*) and weak for the others.* Listeria monocytogenes *was isolated in four of 31 pooled samples (90 samples of bagged beef cuts, each pool two to three samples, 50–75 $cm^2$) and verotoxigenic* E. coli *in five of these pools. No* E. coli O157:H7 *was isolated.*

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## Introduction

Agriculture and Agri-Food Canada (AAFC) has created a Food Safety Enhancement Program which was designed to encourage plant inspection systems based on hazard analysis and critical control point (HACCP) principles (Anon. 1991). HACCP systems require the objective measurement of hygienic risks for the definition and monitoring of critical control points (CCP), and the verification of the workings of these systems. For these purposes quantitative data are needed to directly or indirectly measure the microbiological risks associated with the process and to evaluate intervention strategies (Gill 1995). For beef carcasses or their subprimal cuts, estimates of aerobic bacterial counts are accepted as a useful objective measure of cleanliness (Gill 1995, Mackey and Roberts 1993), although it is acknowledged that there is no clear relationship between aerobic bacterial counts and the numbers of pathogenic organisms nor the likelihood of human illness from consumption of the product. Therefore, aerobic bacterial counts are herein referred to as a measure of cleanliness, and pathogen results, as a measure of hygiene (defined as the science of health and its preservation). However, the interpretation of the consequences of the presence of pathogens must take into account that the association between pathogens and human illness is subject to numerous specific and nonspecific conditions (Duncan and Edberg 1995).

It is acknowledged that at specific product sites the correlation of aerobic bacterial counts with specific pathogens is weak. However, corrective procedures at CCP of process lines control not only specific pathogens but also other bacteria. Therefore, the workings of HACCP systems may be measured by specific pathogens (if present in sufficient numbers to accommodate a practical sample size), indicator organisms of faecal contamination such as *E. coli* (Gill et al. 1995a,b) or aerobic bacterial counts (Mackey and Roberts 1993).

Our method of evaluating the cleanliness of the slaughter floor by estimating the aerobic bacterial counts on groups of beef carcasses has also been used to compare heifer and steer carcasses, measure the efficacy of the carcass-wash control point and conduct one-time evaluations of the slaughter process of six abattoirs in Alberta (Jericho et al. 1993, 1994a,b, 1995). In this study, we applied the same methods to conduct 12 evaluations (11 monthly, two in June 1993) of the cleanliness of the slaughter process and a single evaluation of the chill floor process of one abattoir. We also applied our procedure of sampling and enumeration of aerobic bacteria to develop a method for verifying the control of cleanliness of the fabrication floor of the same abattoir.

For the purpose of monitoring control points of production processes quick results are desirable; however, for verification of HACCP systems by AAFC the development of relevant and reliable data takes precedence over speed of verification.

The objectives of this work at this abattoir were: (1) to measure the level of control and variability of cleanliness of the slaughter floor over an 11 month period, (2) to enrich the existing datafile which will be used to statistically define the minimum sample size needed to evaluate groups of carcasses at the end of the slaughter floor in Alberta, (3) to give a one time direct measure of the cleanliness of carcasses at the end of the cooling process, (4) to develop a method for verifying the cleanliness of carcass cuts at the end of the fabrication floor, and (5) to measure the hygienic adequacy of final bagged (prevacuum) product and cotton gloves of workers who handled the final product by enumerating the faecal contaminants of coliforms and *E. coli*, and determining the presence or absence of the foodborne pathogens *L. monocytogenes*, verotoxigenic *E. coli* (VTEC) and *E. coli* O157:H7.

## Materials and Methods

### *Abattoir*

A brief description of the slaughter facilities of this abattoir may be found in Jericho et al. (1993). At the time of the present study this abattoir operated without a HACCP system but had an industry-developed quality con-

trol program. Visual demerits were minimized by asking all staff to immediately trim any matter appearing on carcass surfaces at their work station, and by placing trimmers on the slaughter floor and at the end of the chilling process. The line speed ranged from 255–293 carcasses $h^{-1}$. Of the 221 carcasses sampled four had been on the 'held' rail and 75 had been examined for visual demerits as part of the quality control program just before sampling. More than 90% of carcasses were sold as vacuum packaged boxed beef.

### *Group carcass evaluations at end of the slaughter floor*

Monthly evaluations were conducted from May 1993 to March 1994 (two for the month of June) by methods previously described (Jericho et al. 1993, 1994a). Briefly, the first halves of split carcasses were moved by plant staff, at their discretion, from the moving line to a secondary line for sampling at the end of the slaughter floor, after the carcass-wash and just before entering the 'spray-cooler'. Excision samples ($5\times5$ $cm^2$) were taken at 10 designated sites (see Table 1) from 15–20 carcasses per evaluation. Aerobic mesophilic bacteria were cultured using hydrophobic grid membrane filter (HGMF, ISO-Grid, QA Life Sciences Inc., San Diego, CA, USA) technology and most probable number of growth units (MPNGU) were assessed by an automated HGMF interpreter (MI100 HGMF Interpreter System, Richard Brancker Research Ltd., Ottawa, Ontario, Canada). Estimates of the $\log_{10}$ MPNGU $cm^{-2}$ variability at sites of carcasses from six abattoirs in Alberta indicated that 15 carcasses were more than adequate to estimate the mean within the desired precision level of 0·5 log units (Jericho et al. 1993). For each evaluation samples were taken from four to eight carcasses per day on 3 consecutive days. HGMF were inoculated within 1 h of sampling at the abattoir and incubated (35°C) for 42 h before interpretation (Jericho et al. 1993). Data were collected on MPNGU, carcass number, site, date, line speed, and a code indicating if carcasses had been on the 'held' rail or were subjected to visual demerit assessment for the quality control program.

Sample collection and processing, and data collection and storage were done by inspection staff at this abattoir. Materials (reagents, petri dishes etc) for the study were supplied by the research project.

### *Group carcass evaluation at end of the chill floor*

The first halves of 20 carcasses were railed out onto a secondary line about 20 min before the main carcass line reached the end of the chill floor. Carcasses were moved by plant staff, at their discretion, from the moving line to a secondary line and samples collected and data processed as above. At this point carcasses had passed through one of four water 'spray-coolers' (chlorine content of water was 0·0 ppm) for at least 18 h and had resided for at least 6 h on the chill floor, which is without sprayers. Total cooling time for carcasses studied had been 1 day (eight carcasses), 3 days (five carcasses) or 4 days (seven carcasses). The variability of counts at sites of chilled carcasses had not been studied by this laboratory, and therefore, the minimum number of carcasses required for this group carcass evaluation was not known.

### *Evaluation of the cleanliness of the fabrication process and pathogen identification*

At this abattoir some 90% of carcasses produced are cooled, fabricated into subprimal cuts, vacuum-bagged, boxed, and cooled before sale. Carcasses move from the chill floor to the cooled (10°C) fabrication floor where some 230 carcasses $h^{-1}$ are fabricated into subprimal cuts during the day shift and fewer carcasses during the late shift. Carcass sides, whilst suspended by a hock on a moving line, are broken down with the primal cuts transferred directly to conveyer belts. Roughly 400 cotton-gloved workers handle the beef cuts on five main fabrication lines with 10 or so secondary lines. The secondary lines feed bagging and boxing stations. On each of eight daily visits (January–April 1994) to this floor, 10 (first two visits) or 20 samples were randomly sampled by asking a different employee on each visit to bring the

bagged subprimal cut (pre-vacuum) to the sampling table from secondary lines. In this manner, the five main fabrication lines were sampled in consecutive order. Samples (5× 5 $cm^2$) were excised from surfaces made on the fabrication floor ('cut', $n$=70) and surfaces made before the fabrication floor ('not-cut', $n$= 70) and MPNGU were enumerated as above (Jericho et al. 1993, 1994a). In addition, on three visits coliforms and *E. coli* were enumerated from 60 and 50 (10 samples were negative for coliforms) samples, respectively, by filtering 2 ml of sample wash through an HGMF with the use of a Spread-filter (Gelman Science, Montreal, Quebec, Canada). Each HGMF was placed on a plate of lactose monensin glucuronate agar (ISO-GRID, QA Life Science Inc., San Diego, CA, USA) and incubated at 35°C for 24 h. Coliforms were enumerated by counting squares (1600) of HGMF with blue colonies. Each HGMF was then transferred to a plate of buffered 4-methylumbeliferyl-β-D-glucuronide agar (QA Life Science Inc.) and incubated at 35°C for 2 h before being examined under long wave length UV light. *E. coli* were enumerated by counting squares containing large, blue-white, fluorescent colonies (Entis and Boleszczuk 1990).

Thirty-one pooled samples (90 samples, two to three samples per pool or 50–75 $cm^2$) were examined for the presence of *L. monocytogenes* and VTEC. *E. coli* were enumerated for 21 of these pools.

Pooled tissue samples (two and three samples per pool) were mixed in 52 or 78 ml of 0·1% peptone–1% Tween 80, respectively, for 30 s in a Stomacher (Colworth 400). One-half of the suspension volume was added to 200 ml LEB (Oxoid) and incubated for 18 h at 37°C for isolation of *L. monocytogenes* and the remainder of the suspension was added to 200 ml modified trypticase soy broth (mTSB) (Doyle and Schoeni 1987) and incubated for 24 h at 42°C with aeration for the isolation of VTEC (see below).

For isolation of *L. monocytogenes*, 100 µl of each LEB-sample mixture was plated onto LPM agar (Difco) and inoculated into 10 ml Fraser broth (Oxoid). After incubation for 18 h at 37°C, DNA was extracted from the LPM plates and used as template in polymerase chain reaction (PCR) as described by Golsteyn-Thomas et al. (1991). PCR assays were performed on bacterial DNA using the listeriolysin gene forward primer LL5 (5'-AACCTATCCAGGTGCTC-3') and reverse primer LL4 (5'-CGCCACACTTGAGATAT-3') in a GeneAmp PCR System 9600 thermal cycler (Perkin Elmer Cetus) using 35 cycles of 15 s at 94°C, 15 s at 55°C, 75 s at 72°C, and a final extension at 72°C for 5 min. PCR products were analyzed by submarine gel electrophoresis using 1·2% agarose gels containing ethidium bromide and visualized by UV transillumination. Cultures in Fraser broth, which had turned black, were plated onto LPM agar and incubated for approximately 18 h at 37°C. Suspect colonies were streaked onto 5% bovine blood plates and inoculated into 10 ml BHI broth, and grown overnight at 37°C for biochemical identification (Anon. 1990a).

For isolation of VTEC, 1 ml of mTSB-sample culture was removed at 6 and 24 h of incubation and centrifuged at 13 000 *g* for 15 min. The DNA was extracted from the bacterial pellets and PCR assays were performed as described by Gannon et al. (1992) for detection of VTEC. In addition, the supernatants of the 24 h broth cultures were tested for toxicity in the Vero cell assay (Clarke et al. 1989) and positivity confirmed by Vero toxin (VT) neutralization assays (broth culture supernatants were considered VT positive if toxicity for Vero cells was apparent after 48 h of incubation and VT could be neutralized by anti-VT1 and anti-VT2 sera). VTEC were isolated from bacterial cultures positive in the VT PCR and/or Vero cell assays using a modification of the colony DNA hybridization procedures described by Ramotar et al. (1995). Briefly, cultures were diluted 10-fold in brain–heart infusion broth (BHIB) (from $10^{-2}$ to $10^{-6}$) and plated onto sorbitol MacConkey (SMAC) agar. After 24 h of incubation at 37°C, cultures were overlain with nylon membranes (Magnagraph Nylon; 0·45 µm pore size; MSI Separations Inc., Westboro, MA, USA). Membranes were removed from the agar surface and treated as described by Hii et al. (1991) to lyze adherent bacterial cells. The membranes were then hybridized with digoxigenin (DIG)-labelled

VT1 and VT2-specific gene probes. These were synthesized as follows: purified chromosomal DNA which was extracted from the VT1+ and VT2+ *E. coli* O157:H7 strain 319 was PCR amplified with oligonucleotide primers specific for VT1 and VT2 (Gannon et al. 1992). The 614-bp VT1 and 779-bp VT2 PCR products generated were purified and labelled with DIG-dUTP (Boehringer Mannheim Canada Ltd., Laval, Quebec, Canada) according to the manufacturer's instructions. The DIG-labelled VT gene probes were then used in colony blot assays. Colonies from the original SMAC plates corresponding to DNA probe positive areas of the nylon membranes were subcultured onto SMAC agar and isolated colonies were tested with VT PCR and VT cell assays. Confirmed VT isolates were tested biochemically for species identification. VT positive isolates and non-sorbitol fermenting colonies from SMAC agar plates were tested by slide agglutination using the *E. coli* O157 Latex Test (Unipath, Nepean, Ontario, Canada).

### *Glove studies*

A total of 30 individual gloves were taken from the glove collection bin when gloves were deposited for the noon work break. Ten gloves were selected on each of three visits. The first set of 10 gloves were heavily soiled with tissue debris, the second set of 10 less so and the third least. These sets of gloves represented the beginning, middle and the end of the processes on the fabrication floor. Gloves were placed individually into sterile bags to which 100 ml of 0·1% peptone–1% Tween 80 was added. Gloves were 'washed' by squeezing the bag and glove 12 times. Twenty-five milliliters of suspension was used for each of the identifications of *L. monocytogenes* and VTEC by the methods described above.

### *Statistical analysis*

For each of the monthly slaughter floor evaluations and the chill floor evaluation, various descriptive statistics (Snedecor and Cochran 1980) were calculated for the $\log_{10}$ MPNGU $cm^{-2}$ (LMPN) counts using the SAS program (Anon. 1990b). For the LMPN at each site and the average over sites, means, standard errors of an observation and mean and 95% confidence limits for the population mean were obtained. For the data from the fabrication floor, analyses of variance (Snedecor and Cochran 1980) were carried out to compare LMPN, and $\log_{10}$ of the most probable number of coliform (LCOL) and *E. coli* (LEC) for 'cut' and 'not-cut' surfaces. Counts of coliforms and *E. coli* were converted to MPN by application of the formula:

$$\text{MPN}=N\log_e[(N/N\text{-}X)]$$

where $N$ is the total number of squares on a filter ($N$=1600), and $X$ is the count of squares containing blue (coliform), or blue-white fluorescent colonies (*E. coli*) (Entis and Boleszczuk 1990). Variation due to sampling date, fabrication line, cutting treatment and the line *x* treatment interaction was accounted for in the statistical model. The strength of associations among LMPN, LCOL and LEC was determined from correlations calculated for pairs of these variables. The statistical calculations were carried out using SAS (Anon. 1989, 1990b).

## Results

### *Group carcass evaluations at the end of the slaughter line*

Descriptive statistics for one of the 12 group carcass evaluations with the highest mean are given in Table 1. The LMPN for the sites ranged from 1·69 (thorax)–2·46 (brisket) and the range of standard deviations was 0·483 (rump lateral)–0·758 (axilla). The length of the 95% confidence interval for LMPN for the individual sites averaged about 0·6 log units.

Descriptive statistics for the site (shank) with the largest ranges for the means of LMPN [1·51 (January 1994)–2·38 (July 1993)] and standard deviation [0·465 (January 1994)–0·950 (May 1993)] are given in Table 2.

Descriptive statistics for the means of LMPN over 10 sites for the 12 group carcass evaluations are given in Table 3. The LMPN averaged over the sites and their standard

deviations were fairly constant for the slaughter process over the 11 month period.

The ratings of slaughter cleanliness are given in Table 3. These ratings are based on $\log_{10}$ colony forming units (LCFU–standard pour plate method) and the advisory scale by Mackey and Roberts (1991). LCFU were estimated with the regression equation LCFU= 1·340+1·143 LMPN where LMPN are the mean $\log_{10}$ MPNGU per $cm^2$ (Jericho et al. 1996).

### *Group carcass evaluation at the end of the chill floor*

The descriptive statistics for the mean of the single evaluation for the chilling process are given in Table 3. This mean is 0·42 $\log_{10}$

**Table 1.** Descriptive statistics for the $\log_{10}$ MPNGU $cm^{-2}$ of 10 sites of a group-carcass evaluation at the end of the slaughter floor in October 1993

| Site | *N*[b] | Mean | s.d.[c] | s.e.[d] | 95% Limits[a] | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| A[e] | 19 | 2·00 | 0·758 | 0·174 | 1·64 | 2·36 |
| B | 19 | 2·46 | 0·558 | 0·128 | 2·19 | 2·73 |
| F | 19 | 1·84 | 0·520 | 0·119 | 1·59 | 2·09 |
| H | 19 | 1·82 | 0·676 | 0·155 | 1·50 | 2·14 |
| L | 19 | 1·87 | 0·483 | 0·111 | 1·64 | 2·10 |
| M | 19 | 1·94 | 0·584 | 0·134 | 1·66 | 2·22 |
| N | 19 | 2·12 | 0·578 | 0·133 | 1·84 | 2·40 |
| R | 19 | 2·41 | 0·690 | 0·158 | 2·08 | 2·74 |
| S | 19 | 1·86 | 0·560 | 0·129 | 1·59 | 2·13 |
| T | 19 | 1·69 | 0·662 | 0·152 | 1·37 | 2·01 |
| MEAN | 19 | 2·00 | 0·312 | 0·072 | 1·85 | 2·15 |

[a] Confidence interval for population mean.
[b] Number of carcasses.
[c] Standard deviation.
[d] Standard error of a mean.
[e] A=axilla, B=brisket, F=flank, H=hock, L=lateral rump, M=medial rump, N=neck, R=rectum, S=shank, T=thorax.

**Table 2.** Descriptive statistics for the $\log_{10}$ MPNGU $cm^{-2}$ from monthly evaluations of the shank site

| Month | *N*[b] | Mean | s.d.[c] | s.e.[d] | 95% Limits[a] | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| May 1993 | 15 | 2·36 | 0·950 | 0·245 | 1·85 | 2·88 |
| June | 19 | 2·08 | 0·887 | 0·203 | 1·65 | 2·50 |
| June | 15 | 2·03 | 0·834 | 0·215 | 1·58 | 2·48 |
| July | 20 | 2·38 | 0·691 | 0·154 | 2·06 | 2·71 |
| Aug | 20 | 1·85 | 0·678 | 0·152 | 1·53 | 2·17 |
| Sept | 20 | 2·09 | 0·687 | 0·154 | 1·77 | 2·41 |
| Oct | 19 | 1·86 | 0·560 | 0·129 | 1·59 | 2·13 |
| Nov | 20 | 1·91 | 0·945 | 0·211 | 1·47 | 2·36 |
| Dec | 20 | 2·14 | 0·622 | 0·139 | 1·85 | 2·43 |
| Jan 1994 | 18 | 1·51 | 0·465 | 0·109 | 1·28 | 1·74 |
| Feb | 20 | 1·66 | 0·705 | 0·158 | 1·33 | 1·99 |
| Mar | 15 | 1·52 | 0·530 | 0·137 | 1·24 | 1·81 |

[a] Confidence interval for population mean.
[b] Number of shanks sampled.
[c] Standard deviation.
[d] Standard error of a mean.

MPNGU $cm^{-2}$ lower than the lowest mean from the slaughter floor.

### *Evaluation of the fabrication process*

For LMPN there was significant difference among sampling dates ($P<0{\cdot}001$, see discussion), and samples from 'cut' surfaces made in the fabrication process had significantly lower ($P<0{\cdot}001$) counts than samples that were taken from surfaces 'not-cut' in the fabrication room (2·44±0·07 vs 2·72±0·07, respectively, Table 3). Note the more than one log increase in aerobic bacterial counts for product surfaces on the fabrication floor over the chill floor.

For LCOL there was a significant effect of sampling date ($P<0{\cdot}01$, see discussion), and there were fewer ($P<0{\cdot}10$) LCOL in 'cut' samples than in 'not-cut' samples (0·64±0·12 vs 0·96±0·12, respectively). There was no effect ($P>0{\cdot}10$) of 'cut' vs 'not-cut' surfaces on LEC which had an overall mean level of 0·385. For each of the variables LMPN, LCOL and LEC, there was no effect of fabrication line or a line *x* cut treatment interaction.

There were significant positive associations between LMPN and LCOL ($r=0{\cdot}67$, $P<0{\cdot}001$), LMPN and LEC ($r=0{\cdot}56$, $P<0{\cdot}001$) and LCOL and LEC ($r=0{\cdot}81$, $P<0{\cdot}001$).

### *Pathogen identification from pooled samples of fabricated cuts*

*L. monocytogenes* and VTEC were isolated from different pools of samples.

*L. monocytogenes* was detected by PCR in 4/31 pooled samples. These results were confirmed by culture of the organisms in three of the four pooled samples.

Six VTEC were isolated from 5/31 pooled samples. These belonged to *E. coli* serotypes O?:H21, O2:H29, O15:H27 (two strains), O145:NM and O163:H19. All VTEC fermented sorbitol and were negative by slide

**Table 3.** Descriptive statistics for the mean $\log_{10}$ MPNGU $cm^{-2}$ of 10 sites from monthly evaluations of groups of carcasses

| Month | *N*[c] | Mean | s.d.[d] | s.e.[e] | 95% Limits[a] | | Advisory scale[b] | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower | Upper | LCFU | Rating |
| At end of slaughter floor | | | | | | | | |
| May 1993 | 15 | 1·88 | 0·223 | 0·056 | 1·75 | 1·99 | 3·49 | 'Good' |
| June | 19 | 1·87 | 0·323 | 0·072 | 1·72 | 2·02 | 3·48 | 'Good' |
| June | 15 | 1·92 | 0·397 | 0·099 | 1·71 | 2·13 | 3·54 | 'Good' |
| July | 20 | 1·92 | 0·295 | 0·066 | 1·79 | 2·06 | 3·54 | 'Good' |
| Aug | 20 | 1·83 | 0·316 | 0·071 | 1·68 | 1·98 | 3·43 | 'Good' |
| Sept | 20 | 1·91 | 0·281 | 0·063 | 1·78 | 2·04 | 3·53 | 'Good' |
| Oct | 19 | 2·00 | 0·312 | 0·072 | 1·85 | 2·15 | 3·63 | 'Good' |
| Nov | 20 | 1·78 | 0·374 | 0·084 | 1·61 | 1·96 | 3·38 | 'Good' |
| Dec | 20 | 1·99 | 0·231 | 0·052 | 1·89 | 2·11 | 3·63 | 'Good' |
| Jan 1994 | 18 | 1·72 | 0·285 | 0·067 | 1·58 | 1·86 | 3·31 | 'Good' |
| Feb | 20 | 1·86 | 0·303 | 0·067 | 1·72 | 2·01 | 3·47 | 'Good' |
| Mar | 15 | 1·64 | 0·223 | 0·057 | 1·52 | 1·76 | 3·21 | 'Good' |
| At end of chill floor | | | | | | | | |
| Mar 1994 | 20 | 1·22 | 0·317 | 0·074 | 1·07 | 1·37 | 2·74 | X[f] |
| At end of fabrication floor | | | | | | | | |
| Cut | 70 | 2·44 | | 0·069 | | | 4·61 | X[f] |
| Not cut | 70 | 2·72 | | 0·070 | | | 4·98 | X[f] |

[a] Confidence interval for population mean.
[b] Advisory scale by Mackey and Roberts (1991). $\text{Log}_{10}$ colony forming units (LCFU) per $cm^2$ estimated with regression equation LCFU=1·340+1·143X (Jericho et al. 1996).
[c] Number of carcasses or number of surfaces sampled which had been 'cut' (70) and 'not-cut' (70) on the fabrication floor on eight daily visits in Jan–April 1994.
[d] Standard deviation.
[e] Standard error of a mean.
[f] Advisory scales for products at the end of the chill floor or fabrication floor have not been formulated.

agglutination for the *E. coli* O157 antigen. VTEC were isolated from 3/17 pools of samples with HGMF *E. coli* counts. Four out of 21 pools were without *E. coli*.

### *Pathogen identification from individual gloves*

*L. monocytogenes* was detected by PCR and culture in 11/30 gloves. One additional isolation was made by culture but not confirmed by PCR. This pathogen was isolated from 8/10 gloves heavily soiled with tissue debris, 3/10 gloves less soiled and none from the least soiled set of gloves.

*E. coli* were identifiable and countable on only 10/30 HGMFs (800–400 000 per glove). Many HGMF were covered entirely by *E. coli*-like fluorescence. No VTEC were isolated from the glove samples.

## Discussion

One important purpose of this work was to measure the workings of cleanliness and hygienic control systems (a HACCP plan was not in place) for processes at one high line-speed beef abattoir.

The aerobic bacterial counts (35°C) for group-carcass evaluations verified the level of control by the plant-created quality control program (mean $\log_{10}$ MPNGU $cm^{-2}$ ranged between 1·64–2·00 for the 11 month period) over the carcass dressing processes. Aerobic bacterial counts (30–32°C) have been previously recommended for HACCP system verification of slaughter processes (Mackey and Roberts 1993). In this study samples were taken systematically from 10 predetermined sites which accommodates the heterogenous distribution of bacteria on carcasses (Hildebrandt and Weiss 1994). These sites were selected on the basis of past process-dependent deposition of visual demerits at this abattoir. However, the association of these demerits with aerobic bacterial counts was weak (Jericho et al. 1993). Regular verification of control of a process permits definition of change over time (Tables 1 and 3) and sampling specific sites of carcasses permits focusing on procedures at CCPs (Table 2).

Other microbiological methods may also be considered for HACCP system verification. The methods used in this study evolved apart from a HACCP system creation process. The process of creating such a system may suggest methods of verification other than aerobic bacterial counts. Assessments of hygienic characteristics of the beef carcass dressing process, for example, may be based on the presence of faecal contamination. *E. coli* counts are a direct measure of faecal contamination (Gill et al. 1995a,b) and may thus be used in verifying HACCP systems. Similarly aerobic bacterial counts are a direct measure of microbiological cleanliness. Both aerobic bacteria and *E. coli* counts are indirect measures of pathogens. Application of either type of count for HACCP verification makes the assumption that lower counts of each also implies fewer pathogens. It is acknowledged that at specific sites of a carcass the correlation of aerobic bacteria or *E. coli* with pathogens is weak. For example, in this study both aerobic bacteria and *E. coli* counts were poor indicators of VTEC. Only 3/17 pools of samples with detectable *E. coli* counts were also positive for VTEC. However, corrective procedures at CCPs which will control aerobic bacteria or *E. coli* will also control specific pathogens. This facilitates the bacterial selection for HACCP verification.

The method chosen for HACCP system verification would depend on purpose, convenience and cost. Speed of method is of minor consideration as results would only signal corrective action of the process but not acceptance or rejection of the product. This decision is complicated by the vague association of microbiological characteristics of the product at the abattoir stage of the food chain with ultimate effects of consumption on human health.

Aerobic bacterial counts (35°C) at the end of the chill and fabrication processes were used to maximize the enumeration of mesophiles including all pathogens. Psychrotrophs or spoilage related organisms were not of concern. Sampling before bagging of the end-product reflects the cumulative effect of all plant procedures up to that point and simi-

larly sampling at the end of the chill process reflects all the procedures before that point. Inspection services may choose to verify the HACCP systems for all three processes of slaughter, chilling and fabrication at the product bagging station. Of course the contribution made by each process to the microbiological state of the bagged product would not be known. The minimum number of samples required at the bagging stations for verification of the process would have to be determined from the variation of counts of many more samples than reported in this study. High counts for end-products at bagging stations would signal corrective action which may include verification of HACCP systems of each of the other two processes.

For the chill floor our method is a direct measure of cleanliness, as opposed to the indirect measure of hygienic adequacy of cooling carcasses by temperature function integration (Gill et al. 1991a,b, Jones 1993). The indirect method is the most practical method to measure the adequacy of the cooling process for meat products (Gill and Jones 1992).

Aerobic bacterial counts (35°C) studied for all three processes were lowest at the end of the chilling process but they were highest for fabricated beef cuts only 20 min after carcasses left the chill floor. End product monitoring for all three processes would be needed to establish such a trend. Note the fairly constant mean LMPN over sites for the slaughter process for the 11 month period (Table 3). Confirmation of trends for the other processes by the methods described may focus industry's attention on control points for the HACCP system of the fabrication floor. The ratio of psychrotrophic to mesophilic bacteria is assumed to have been changed by the chilling process (McDowell et al. 1986). A significant increase in faecal coliform count on meat cuts has been described after the boning process of dairy cow carcasses (Charlebois et al. 1991).

The first 10 counts (5 'cut', 5 'not-cut') from bagging stations were unusually high (mean=3·69 $\log_{10}$ MPNGU $cm^{-2}$ and range of 3·51–3·78 when using HGMF on TSA with TTC). For this period plant staff recognized on the basis of microbiological monitoring that a new type of disinfectant used in night cleaning operations on the fabrication floor was inferior and was then replaced. All work surfaces and equipment on the fabrication floor were water cleaned during work stoppages but not disinfected until the end of the day's operations. All sampling periods were between 10 am and noon. Although the level of LMPN and LCOL were significantly lower for surfaces 'cut' than 'not-cut', the high level of bacteria for cuts made on the fabrication floor for this commercial setting is noted. The practical importance of this difference is questionable in view of it being less than one $\log_{10} cm^{-2}$. Bacterial contamination has also been observed for beef surfaces made by the trimming process even with sterile utensils under experimental conditions (Hardin et al. 1995).

For samples from the end of the fabrication floor the association of aerobic bacterial counts (35°C) with coliforms and *E. coli* counts was weak, and very weak with the specific pathogens of *L. monocytogenes* and VTEC. Similarly, the association of *E. coli* with VTEC was also weak, suggesting that faecal contamination does not necessarily imply the presence of pathogens. Although the isolation rate of these pathogens was low (one or the other pathogen was isolated from 9/31 pooled samples) and their distribution on carcasses is heterogenous, the prevalence of these pathogens on subprimal cuts is assumed to be considerably higher than the isolation rate indicated in view of the small area (25 $cm^2$) of the subprimal cuts (1500–5000 $cm^2$) sampled. Furthermore, the subprimal cuts sampled represent less than 0·05% of the pieces bagged during the sampling period.

The procedures of sample collection and processing described for the end of the fabrication floor may be used for HACCP system verification of the system for this floor or for all systems of the abattoir up to this point of production. This verification could be based on the level of cleanliness (aerobic bacterial counts) or faecal contamination (*E. coli*). Definition of minimum sample size would require further definition of the variability in counts for an extended database. The low prevalence of *L. monocytogenes* and VTEC does not pre-

clude other pathogens and would therefore necessitate the inclusion of other pathogens in any pathogen-based verification of HACCP systems at abattoirs. Comparative cell counts, be they aerobic or *E. coli*, from end-products of processes would indicate the level of control of the HACCP systems for each process over time or at sites (Tables 1 and 3). Monitoring by inspection and plant staff of procedures and microbiological records from CCPs would confirm this control.

While six VTEC were isolated, no *E. coli* O157:H7 were isolated. VTEC of O145:NM (Beutin et al. 1994) have been associated with haemorrhagic colitis and VTEC O163:H19 have also been associated with human disease (Karmali 1989).

The association of cattle slaughter with pathogens on hands of workers has been reported previously. *E. coli* and *Salmonella* can occur in large numbers before hand-washing and even after hand washing (De Wit and Kampelmacher 1982). The effectiveness of washing is determined by the method of washing and the type of antiseptic used (Jarvis 1994). The isolation rate of pathogens from gloves of this study may have been influenced by the amount of tissue debris, the station of the process where the gloves were used and the residual effect of the disinfectants used to wash the gloves after each 3–4 h of use. These variables make gloves an unlikely sample to verify HACCP systems for the fabrication floor at this abattoir.

This work focused on methods to microbiologically measure the control of three processes in the system of beef production. The usefulness of aerobic bacterial counts to measure the control of carcass production, cooling and fabrication was demonstrated. The cleanliness of the slaughter process was found to be 'good' for all 12 evaluations conducted, and this was achieved without a formal HACCP system. This does not negate the value of HACCP systems, but suggests that some HACCP functions were served by the prevailing quality control program. The low number of VTEC and *L. monocytogenes* on fabricated beef cuts excludes these specific hazards for routine verification of HACCP systems for the fabrication process. Instead such systems may be verified by aerobic bacterial counts as a direct measure of cleanliness or *E. coli* counts as an indirect measure faecal contamination.

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