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A minimal apparatus method for counting bacteria: comparison with reference method in surveying beef carcasses at three commercial abattoirs

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SUMMARY

In two surveys of three commercial abattoirs a minimal apparatus method for making bacterial counts, the 'loop-tile' method, detected the same trends in bacterial numbers on beef carcasses as the ISO reference method applied to the same samples. Both methods showed the carcasses from one abattoir, that with an export licence, to carry consistently higher numbers of bacteria, and one of the four sites sampled on each carcass to be consistently dirtier than the other three.

INTRODUCTION

The flesh of live healthy animals is essentially sterile, perhaps containing low numbers of bacteria from time to time (Gill, 1979; Mackey & Derrick, 1979), but the lower gut and exterior of the animal harbour enormous numbers, which, without care, may be transferred to the surface of the carcass during slaughter. Hygienic measures taken to minimize this possible transfer aim to produce meat that will store for a reasonable time under refrigeration and be free of bacteria able to cause human illness, even if stored under conditions which are less than ideal. If visible dirt is present on the carcass, it is almost certain to be mud, faeces or other gut contents, all of which contain large numbers of bacteria. Efforts to improve hygiene by eliminating the occurrence of visible dirt have, therefore, a sound rationale. In the absence of visible dirt, hygiene is more difficult to control: for example, a visually clean carcass may, indeed, carry very low numbers of bacteria – but a visually clean carcass may equally carry unacceptable numbers of spoilage or food poisoning bacteria.

Although it is generally agreed that an objective measure of hygiene is urgently needed, attempts to develop a procedure are hindered by a lack of adequate background information, and a confusion of published methods which have not been compared statistically. The judgement of abattoir hygiene therefore remains largely visual and subjective. It is assumed by regulatory agencies that visually clean premises must produce carcasses more hygienically (i.e. carrying lower numbers of bacteria) than premises which appear dirty. Hence, great emphasis has been laid on improving the appearance of abattoirs and slaughter operations, assuming that improved hygiene will follow, and that the carcasses produced will carry lower numbers of spoilage microbes and carry less frequently those associated with food-borne illness.

The bacteriological aspects of the red meat carcass and the slaughterhouse were reviewed by Ingram & Roberts (1976), and the efficacy of various methods proposed and/or implemented to control hygiene at slaughter by Roberts (1980). Both appraisals suffered from a lack of adequate systematic bacteriological data, a consequence of the difficulties in sampling carcasses and taking into account the variation in bacterial numbers due to site-site, carcass-carcass, abattoir-abattoir, and visit-visit variation.

The need for a statistically based sampling scheme led us to examine representative numbers of red meat carcasses in commercial abattoirs and to develop a scheme which would take into account the known sources of variation in bacterial numbers (Roberts, MacFie & Hudson, 1980). One of the sampling schemes suggested has been used on beef carcasses at three commercial abattoirs, and the samples taken examined for bacterial numbers by the International Standards Organization (ISO) reference method 2293 (1976) for the enumeration of aerobic bacteria on meat and meat products, which is also encoded in the British Standard method BS 5393, and by a minimal apparatus method developed at this institute referred to as the 'loop-tile' method (Hudson & Roberts, 1982).

MATERIALS AND METHODS

Samples

Samples were obtained from beef carcasses at three commercial abattoirs: Abattoir A was of medium size where carcasses were dressed on cradles and supplied to the home market only: Abattoir B, which was attached to a butcher's shop, was small and carcasses were dressed-out on the floor: Abattoir C was an EEC approved medium-sized abattoir with rail-dressing supplying meat for the home market and for export.

Each abattoir was visited three times in Survey I and a further three times 8 months later in Survey II. On each visit ten carcasses were taken at random from the end of the slaughterline, prior to chilling, and 50 cm^2 areas from the neck, brisket, forerib and round (hind leg) medial surfaces (sites 1, 2, 3 and 9 in Roberts, MacFie & Hudson, 1980) were defined by a sterile square aluminium template. Each defined area was swabbed with a sterile absorbent cotton-wool ball *ca.* 2 cm diam previously moistened with bacteriological diluent (0·85 % w/v NaCl, 0·1 % w/v peptone) from a wide-mouthed screw-capped 1 fl. oz universal bottle containing 10 ml of diluent. The swab was held in sterile forceps and the meat surface swabbed ten times from left to right and ten times from top to bottom applying a firm pressure sufficient to disturb the meat surface but not to damage it. This swabbing procedure was repeated with a dry cotton-wool ball and both swabs were placed in the bottle of diluent used to wet the first swab. The swabs and diluent were mixed by hand using a sterile aluminium rod and by shaking prior to making decimal dilutions as described below.

Culture medium and incubation

Standard Plate Count Agar (Oxoid CM 463) incubated aerobically at 30 °C for 72 h.

ISO Reference Method 2293 (BS 5393)

Decimal dilutions of the contents of the bottle containing the swabs were made with 1 ml pipettes and 9 ml of diluent in 1 fl. oz screw-capped universal bottles. Samples (0·1 ml) of each decimal dilution were spread on the surface of duplicate agar plates, utilizing the whole plate for each sample.

The loop-tile method

In principle, the diluting pipette is replaced by a standard wire loop of known capacity, and the dilution bottles (or tubes) by diluent in the depressions of a tile at nine times the volume of the loop. Mixing one loopful of sample with the diluent in a single depression therefore effects a tenfold dilution. During development of the method a glazed porcelain tile was used successfully but it became convenient to use stainless steel tiles (20 SWG) each having 12 depressions approx. 25 mm diam and 5 mm deep at the centre. The wire loop (10% Ir/90% Pt) comprises wire 0·6 mm diam welded to a loop of 6 mm internal diameter with a shank length of 60 mm (to order from Johnson Matthey Metals Ltd, 81 Hatton Garden, London EC1). Such a loop, when held horizontally, will hold approx. 0·02 ml, but each loop must be calibrated carefully by weighing its contents. The speed at which the loop is withdrawn from the diluent also influences the volume held and it must be carefully standardized in use. A device is also required to deliver $\times 9$ the volume of the loop into the wells of the tile e.g. a pipetting syringe which can withstand autoclaving such as the Socorex dispensing and self-filling syringe type A SX/2-174-05/01 (Camlab Ltd, Nuffield Road, Cambridge CB4 1TH). In abattoirs a portable burner is needed to sterilize the wire loops. Sterile bacteriological diluent (0·85% w/v NaCl + 0·1% bacteriological peptone, pH 7·0) is conveniently dispensed from a reservoir using the pipetting syringe once the volume to be delivered has been calculated from measurements on the loop.

A sterile loop is used to transfer one volume from the bottle containing the swabs from the carcass into the first depression on the tile containing diluent at nine times the volume of the loop. Mixing is effected by ten clockwise and ten anticlockwise movements of the loop, which is then drained against the side of the depression and sterilized by flaming. That dilution is then sampled with a sterile loop and a similar tenfold dilution made in the next depression. Having completed the decimal dilution series, a loop is sterilized and after cooling used to transfer a loopful of the greatest dilution to the surface of a quarter of the surface of a dried agar plate and used to spread the loopful over that quarter. A duplicate loopful is transferred similarly from the same dilution well to another plate. That loop can then be used without further sterilization to transfer samples from the whole decimal dilution series from greatest to least dilution since the dilutions sampled are each ten times more concentrated than that before it. It is important that the loop be flamed between each dilution when making the dilution series. Considerable time can be saved by using two loops which hold the same volume – the second can be used while the first is cooling after flaming to sterilize it.

Table 1. *Comparison of bacterial counts by the loop-tile and reference counting methods on beef carcasses at three commercial abattoirs*

Site...	1		2		3		9	
Counting method...	R	LT	R	LT	R	LT	R	LT
Survey I								
Abattoir A	3·35	3·38	4·02	4·22	3·41	3·44	3·46	3·50
Abattoir B	3·36	3·34	4·05	4·19	3·39	3·42	3·83	3·92
Abattoir C	3·92	4·03	4·81	5·13	4·03	4·12	3·40	3·53
Survey II								
Abattoir A	2·86	2·96	4·06	4·12	3·68	3·67	3·57	3·50
Abattoir B	3·13	3·18	4·16	4·11	3·47	3·43	3·63	3·53
Abattoir C	4·43	4·51	4·82	5·02	4·01	4·20	3·50	3·56

Each tabulated value is the mean of 30 counts (10 carcasses × 3 visits) expressed as $\log_{10}$ bacteria/cm².
R, Reference method.
LT, Loop-tile method.

Table 2. *Comparison of bacterial counts by the loop-tile and reference counting methods on beef carcasses at three commercial abattoirs each visited on three occasions*

Abattoir...	A		B		C	
Counting method...	R	LT	R	LT	R	LT
Survey I						
Visit 1	3·59	3·60	3·60	3·78	4·07	4·18
Visit 2	3·51	3·69	3·68	3·70	4·03	4·21
Visit 3	3·58	3·61	3·70	3·68	4·02	4·22
Survey II						
Visit 1	3·53	3·57	3·57	3·59	4·17	4·35
Visit 2	3·59	3·51	3·60	3·56	4·17	4·34
Visit 3	3·50	3·61	3·63	3·53	4·23	4·28

Each tabulated value is the mean of 40 counts (10 carcasses × 4 sites per carcass) expressed as $\log_{10}$ bacteria/cm².
R, Reference method.
LT, Loop-tile method.

Calculation of counts

After incubation colonies are counted at two consecutive dilution levels on each of the duplicate plates (i.e. a total of four sectors were counted for each loop-tile count and four whole plates for each count by the reference method). The number of colony forming units was calculated using the method of Farmiloe *et al.* (1954) and expressed as $\log_{10}$ number of bacteria/cm².

Statistical analyses

The counts were analysed after transformation to logarithms which made the counts normally distributed (Roberts, MacFie & Hudson, 1980). Analysis of variance was then applicable.

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Table 3. *Comparison of bacterial numbers on beef carcasses in two surveys at three commercial abattoirs*

Abattoir	Survey I	Survey II
A	$3{\cdot}60_a$	$3{\cdot}55_a$
B	$3{\cdot}69_a$	$3{\cdot}58_a$
C	$4{\cdot}12_b$	$4{\cdot}26_b$
LSD*	0·33	0·53

Tabulated values are $\log_{10}$ bacteria/cm².
Within a column, means with the same subscript are not significantly different.
* See text.

The analysis of variance carried out was of a split-plot design with the visits to each abattoir forming the whole-plots, and the ten carcasses sampled at each visit forming the sub-plots. Thus to test for significant overall abattoir differences the mean square due to the abattoir variation was compared with the whole-plot residual mean square (visit within abattoir variation). Significant overall site differences were tested for by comparing the mean square due to site variation with the sub-plot residual mean square, and if significant with the abattoir × site interaction mean square.

This analysis takes into account variation between replicate carcasses on each visit and between replicate visits, to test whether the bacterial numbers at different abattoirs differ significantly. Large variation between replicate carcasses, or replicate visits, makes differentiation between abattoirs impossible.

RESULTS

Comparison of reference and loop-tile methods

In Survey I (360 samples) the mean counts ($\log_{10}$/cm²) were 3·85 for the loop-tile and 3·75 for the reference method. Although significantly different ($P = < 0{\cdot}05$) the difference is small in bacteriological terms and of no commercial consequence. In Survey II (360 samples) the mean counts were loop-tile 3·82 and reference method 3·78, which were not significantly different.

Mean counts from carcass sites × abattoirs are shown in Table 1 and for visits × abattoirs in Table 2 which show clearly that the differences between abattoirs, sites and visits are established equally well by either the reference method of counting or by the loop-tile method.

Comparison of abattoirs

In Survey I abattoir, site, abattoir × site interaction and abattoir × site × visit interaction were significant. In Survey II abattoir, abattoir × site interaction and abattoir × site × visit interaction were significant. These effects are illustrated in Tables 3–5 where all the relevant means are given (calculated over the counting methods) together with the least significant difference (LSD) which is the amount by which the two means must differ to be significantly different.

Table 3 shows that carcasses at abattoir C, that having an export licence, carried larger numbers of bacteria in both surveys ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively),

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Table 4. *The effect of abattoir and site of sampling on bacterial numbers on beef carcasses at three commercial abattoirs*

(each abattoir was visited three times in each survey but the data are combined in this analysis.)

	Site			
Abattoir	1	2	3	9
Survey I				
A	$3{\cdot}36_a$	$4{\cdot}12_b$	$3{\cdot}42_a$	$3{\cdot}48_a$
B	$3{\cdot}35_a$	$4{\cdot}12_b$	$3{\cdot}40_a$	$3{\cdot}88_b$
C	$3{\cdot}97_b$	$4{\cdot}97_c$	$4{\cdot}08_b$	$3{\cdot}46_a$
		LSD* = 0·31		
Survey II				
A	$2{\cdot}91_a$	$4{\cdot}09_c$	$3{\cdot}67_{bc}$	$3{\cdot}54_b$
B	$3{\cdot}15_{ab}$	$4{\cdot}14_c$	$3{\cdot}45_b$	$3{\cdot}58_b$
C	$4{\cdot}47_c$	$4{\cdot}02_d$	$4{\cdot}11_c$	$3{\cdot}53_b$
		LSD* = 0·45		

Tabulated values are $\log_{10}$ bacteria/cm². Within a survey means are significantly different if they differ by more than the LSD (* see text) represented by different subscripts.

and that there was no significant difference between abattoirs A and B in either survey.

Table 4 shows the abattoir × site interaction mean counts. Although abattoir C was the dirtiest overall, in Survey II there was no significant difference between abattoirs at site 9. In both surveys site 2 carried the highest numbers of bacteria in all three abattoirs, although not significantly so at abattoir B for Survey I and abattoir A for Survey II.

Table 5 shows the variation of the abattoir × site interaction mean counts with visits by tabulating the abattoir × site × visit interaction means. In Survey I, the high bacterial counts at abattoir C on sites 1, 2 and 3, and the high counts at abattoir B on site 9, were repeated on all three visits, showing the consistency of the data. This pattern is repeated in Survey II, with the exception of abattoir B site 9 on visit 1.

Tables 3–5 indicate that although interactions between abattoirs, sites and visits existed, the abattoir differences were generally maintained regardless of site and visit. Similarly, and particularly in Survey I, site differences were generally maintained regardless of abattoir and visit.

DISCUSSION

In general, bacteriological methods are slow, cumbersome, and unsuited to use in abattoir environments e.g. many still require glass pipettes and bottles/tubes which would be hazardous in slaughterhouses. Numerous simplified methods have been developed. Those which are easiest to use, e.g. direct contact of an agar medium to the surface being sampled, are adequate for flat working surfaces but less so for the uneven surface of a carcass. Additionally, contact methods have been criticized for removing only a relatively small and irregular proportion of the total number of bacteria present. Excision of the sites to be sampled was considered at

Table 5. *Bacterial numbers at four sites on beef carcasses at three commercial abattoirs – effects due to visits*

	Site			
Abattoir	1	2	3	9
Survey I				
		Visit 1		
A	$3{\cdot}64_{ab}$	$3{\cdot}92_{b}$	$3{\cdot}42_{a}$	$3{\cdot}42_{a}$
B	$3{\cdot}41_{a}$	$4{\cdot}16_{bc}$	$3{\cdot}21_{a}$	$3{\cdot}98_{c}$
C	$3{\cdot}92_{b}$	$5{\cdot}17_{d}$	$3{\cdot}84_{b}$	$3{\cdot}55_{ab}$
		Visit 2		
A	$3{\cdot}47_{a}$	$4{\cdot}22_{b}$	$3{\cdot}28_{a}$	$3{\cdot}42_{a}$
B	$3{\cdot}24_{a}$	$4{\cdot}05_{b}$	$3{\cdot}44_{a}$	$4{\cdot}02_{b}$
C	$4{\cdot}14_{b}$	$4{\cdot}68_{c}$	$4{\cdot}24_{b}$	$3{\cdot}43_{a}$
		Visit 3		
A	$2{\cdot}98_{a}$	$4{\cdot}24_{c}$	$3{\cdot}57_{b}$	$3{\cdot}60_{b}$
B	$3{\cdot}41_{b}$	$4{\cdot}16_{c}$	$3{\cdot}56_{b}$	$3{\cdot}63_{b}$
C	$3{\cdot}86_{c}$	$5{\cdot}05_{d}$	$4{\cdot}15_{c}$	$3{\cdot}42_{b}$
		LSD* = 0·41		
Survey II				
		Visit 1		
A	$3{\cdot}04_{a}$	$3{\cdot}95_{b}$	$3{\cdot}70_{b}$	$3{\cdot}53_{ab}$
B	$3{\cdot}01_{a}$	$4{\cdot}09_{b}$	$3{\cdot}91_{b}$	$3{\cdot}31_{a}$
C	$4{\cdot}37_{b}$	$5{\cdot}05_{c}$	$4{\cdot}02_{ab}$	$3{\cdot}59_{a}$
		Visit 2		
A	$2{\cdot}86_{a}$	$4{\cdot}13_{c}$	$3{\cdot}82_{bc}$	$3{\cdot}40_{b}$
B	$3{\cdot}16_{ab}$	$4{\cdot}22_{c}$	$3{\cdot}29_{a}$	$3{\cdot}65_{ab}$
C	$4{\cdot}45_{c}$	$4{\cdot}97_{d}$	$4{\cdot}17_{c}$	$3{\cdot}45_{b}$
		Visit 3		
A	$2{\cdot}82_{a}$	$4{\cdot}21_{d}$	$3{\cdot}51_{b}$	$3{\cdot}67_{bc}$
B	$3{\cdot}29_{ac}$	$4{\cdot}09_{d}$	$3{\cdot}15_{ab}$	$3{\cdot}78_{cd}$
C	$4{\cdot}59_{de}$	$4{\cdot}75_{e}$	$4{\cdot}13_{d}$	$3{\cdot}56_{bc}$
		LSD* = 0·51		

Within a survey means are significantly different if they differ by more than the LSD (* see text) represented by different subscripts.

the beginning of this work but rejected for two reasons: (*a*) it is slow and would interfere with the normal speed of operations in abattoirs, and (*b*) it would have damaged the surface of carcasses and downgraded them commercially, both of which we wished to avoid. Hence swabbing was chosen, but care taken to swab a relatively large area, and to use very firm pressure on the wet and dry swabs.

The saving in materials effected by the loop-tile method is impressive. If both methods were used on 720 samples, each diluted to 10^{-5} and plating the initial swab sample and five decimal dilutions in duplicate the reference method as applied (strictly the full reference method requires two separate dilution series to be made on each sample whereas only one was made here) would use 8640 agar plates, 3600 bottles of diluent (each 9 ml) 3600 1 ml pipettes and 720 pipettes or dropping needles to deliver known volumes of each dilution to the surface of the agar. The loop-tile method required 18 bottles of diluent (each 250 ml) and 2160 agar plates.

There is additionally a considerable saving of time e.g. in labelling plates,

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preparing and dispensing diluent, transfer of diluent to plates and in washing-up dilution bottles (cf. Kramer & Gilbert, 1978).

The same methods have been applied by others, after a short initial period of training, to survey the bacterial numbers on beef and pork carcasses at nine abattoirs in Norway. In that survey beef carcasses were consistently cleaner than pork; the interior of carcasses was cleaner than the exterior; abattoirs where pork carcasses were singed as a separate step were cleaner than those where singeing was combined with dehairing; there was no difference in bacterial numbers on beef carcasses dressed vertically or on cradles; and an abattoir with consistently poorer performance was identified (Johanson *et al.* 1983).

Both methods of making bacterial counts were remarkably consistent in that very similar results were obtained on six separate occasions. The loop-tile method produced almost identical results to the reference method and could reasonably replace it under the circumstances tested. Considering additionally that only four sites on each of 10 carcasses need to be sampled at each visit suggests a cheap, but relatively efficient, scheme for monitoring hygiene in commercial abattoirs.

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