Rapid Estimation of Microbial Numbers on Meat and Poultry by the Direct Epifluorescent Filter Technique

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ABSTRACT

The direct epifluorescent filter technique (DEFT) for rapid estimation of microbial numbers was evaluated by comparison with the plate count on a variety of uncooked red meat and poultry samples. Good agreement [correlation coefficient \( r = 0.95-0.96 \)] was obtained from samples with plate counts of \( 5 \times 10^3 \text{ g}^-1 \) and above from red meat carcasses (surface swabbed), aerobic or vacuum packed chill-stored joints (surface sampled - stomachered) and frozen beef (thawed stomachered). For stored and unstored raw poultry sampled by skin scraping or stomaching of muscle and skin good overall correlation \( r = 0.88-0.89 \) was obtained between the DEFT count and the plate count in the ranges \( 1.1 \times 10^3 \text{ to } 1.3 \times 10^7 \text{ cm}^-2 \) (skin scraping) and \( 1 \times 10^4 \text{ to } 9.5 \times 10^6 \text{ g}^-1 \) (muscle and skin) even though the DEFT always overestimated counts on samples on which no growth had occurred (plate count \(<7 \times 10^4 \text{ cm}^-2 \) or \(<1 \times 10^5 \text{ g}^-1 \)). However, good linearity between DEFT and plate counts allowed use of the regression equation to obtain a good estimate of the plate count on these samples. The DEFT was unsuitable for application to poultry neck skin sampled by shaking because particulate material interfered with counting. This was also a problem with Mechanically Recovered Meat although the DEFT gave a fair estimate \( r = 0.72 \) of the plate count on certain types (beef and veal) of this product. The DEFT was capable of providing counts within 35 to 45 min and its applicability to the rapid estimation of bacterial numbers in meat and poultry is discussed.

Conventional culture methods for determining total viable counts on meat and meat products take at least 1 d to produce a result. In meat factories this time lag precludes an immediate release scheme for raw materials which have to be stored until microbiological tests are completed. It also delays distribution of products to retail outlets when it is necessary first to ensure the required shelf life by determining bacterial levels. In addition, online microbiological testing cannot be used to permit quick corrective action when a trend of increasing contamination is occurring. Finally, it is impossible to make a quick assessment of the microbiological condition of meats following transportation, as in international trade.

Similar constraints throughout the food industry have led to development of several techniques for rapid estimation of bacterial counts in foods. Most of these are indirect, measuring either a component of bacteria as in the ATP technique \( (14) \) or detecting changes produced by bacteria in media following inoculation with a portion of the food sample as in impedance or conductance \( (4,12) \), microcalorimetry \( (7) \), and radiometry \( (7) \) methods. Direct microscopic enumeration is possible using the direct epifluorescent filter technique (DEFT) originally developed for rapid enumeration of bacteria in milk \( (10) \) and later modified for application to other foods including meats \( (9) \). The DEFT appears particularly advantageous for use in the meat industry as it combines a short result time (approx. 30 min) with the ability to give a good estimate of microbial numbers at levels down to \( 10^4 \text{ g}^-1 \).

Evaluation of the DEFT for estimation of bacterial numbers in meats has been limited to a small number of fresh and frozen meat samples \( (9) \), ground meat \( (3,11) \), and heat-treated sausages \( (11) \), and has only included stomached samples. To establish its broader applicability we have evaluated the DEFT on a wider variety of sample types including poultry and Mechanically Recovered Meat (MRM), employing all the sampling techniques (swabbing, scraping, shaking, stomaching) in current use.

METHODS

A total of 300 samples categorized into seven groups (Table 1) according to meat type and sampling technique were analyzed. Large blocks (27 kg) of uncomminuted manufacturing meat provided the samples of frozen beef, half of which were thawed, kept at 0°C for 7 d (to obtain high counts) and stored at -10°C for 2 months before examination. The chicken neck skin samples were taken at a commercial processing plant from air-chilled carcasses 2.5 h after slaughter. All meat was of commercial origin except for some of the sampled beef and pig carcasses which had been slaughtered at the Institute of Food Research - Bristol Laboratory abattoir. Stored samples were...
TABLE 1. Categories of sample and their preparation for DEFT counting.

<table>
<thead>
<tr>
<th>Type of meat</th>
<th>No. of samples</th>
<th>Sampling method</th>
<th>Diluent volume (ml)</th>
<th>Dispersal method</th>
<th>Dilution and volume (ml) used for DEFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen beef</td>
<td>60</td>
<td>100-200 g thawed in microwave oven</td>
<td>Equal to weight of sample&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stomaching for 2 min</td>
<td>None (3 or 6) or 10&lt;sup&gt;-1&lt;/sup&gt; (3) or 10&lt;sup&gt;-3&lt;/sup&gt; (3) or 10&lt;sup&gt;-4&lt;/sup&gt; (3)</td>
</tr>
<tr>
<td>Vacuum packed or aerobically stored beef joints</td>
<td>70</td>
<td>3 × 7 cm² areas cut from surface</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stomaching for 1 min</td>
<td>None (6) or 10&lt;sup&gt;-1&lt;/sup&gt; (3) or 10&lt;sup&gt;-2&lt;/sup&gt; (3) or 10&lt;sup&gt;-3&lt;/sup&gt; (3)</td>
</tr>
<tr>
<td>Beef, pork and lamb carcasses and aerobically stored beef joints</td>
<td>50</td>
<td>50 or 100 cm² area swabbed</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Swabs compressed with pipette</td>
<td>None (6) or 10&lt;sup&gt;-1&lt;/sup&gt; (3)</td>
</tr>
<tr>
<td>Chicken carcasses after chilling</td>
<td>29</td>
<td>10 g neck skin removed</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Hand-shaken for 3 min</td>
<td>10&lt;sup&gt;-4&lt;/sup&gt; (3)</td>
</tr>
<tr>
<td>Thawed chicken thighs (some stored aerobically)</td>
<td>49</td>
<td>25 g skin and muscle excised</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stomaching for 1 min</td>
<td>10&lt;sup&gt;-1&lt;/sup&gt; (3)</td>
</tr>
<tr>
<td>Thawed chicken thighs (some stored aerobically)</td>
<td>41</td>
<td>7.55 cm² area scraped</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Surface scraped in presence of diluent</td>
<td>None (3) or 10&lt;sup&gt;-1&lt;/sup&gt; (3)</td>
</tr>
<tr>
<td>Mechanically recovered meat</td>
<td>50</td>
<td>25 g sample examined</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sample placed in fine-meshed nylon-lint bag and stomached for 1 min</td>
<td>10&lt;sup&gt;-1&lt;/sup&gt; (3) or 10&lt;sup&gt;-2&lt;/sup&gt; (3)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Diluent: 1% peptone, pH 7.0
<sup>b</sup>Diluent: 0.1% peptone, 0.85% NaCl, pH 7.0

Figure 1. Relationship between DEFT count and plate count for frozen meat. Line represents fitted regression line (y = 1.85 + 0.74x; r = 0.95).

DEFT count and plate count were always made on the same sample suspension.

Every sample suspension (10 ml) was prefiltered as described by Pettipher and Rodrigues (9) except that 25-mm diameter 5-μm polypropylene filter discs (Foss Electric (UK) Ltd. York, England) were used in place of sheet 5-μm nylon mesh. When a count of <10<sup>6</sup>/ml of suspension was expected, the sample was filtered undiluted. Undiluted suspensions from MRM and chicken muscle and skin blocked the prefilters and those from chicken neck skin samples blocked the filters at the later staining stage. These were therefore decimally diluted before prefiltration, as were suspensions with an expected count >10<sup>6</sup>/ml to obtain distinct clumps in the DEFT preparation. Dilutions used for each category of sample are shown in Table 1.

Three- or six-milliliter portions of prefiltered suspensions were treated with trypsin and Triton X-100, filtered, and stained for DEFT counting as described by Pettipher et al. (10), except where indicated. The volume of acridine orange stain was reduced to 1 ml and staining time to 30 s. Two milliliters of citrate-NaOH buffer (0.1 M; pH 3) were used for rinsing and isopropanol (1 ml instead of 2.5 ml used previously) was employed for the final rinse. These modifications improved the quality of the DEFT preparations (9,13). For all preparations clumps of orange-red-fluorescing bacteria were counted in 20 microscope fields of view taken at random. The apparatus used was the bio-Foss Automated Microbiology System (Foss Electric (UK) Ltd, Bishopthorpe, York) which includes purpose-built reagent and filtration equipment and an epifluorescence microscope fitted with a specially modified television scanner linked to a microprocessor-controlled image analyser for automatic counting.

Plate count

Sample suspensions were diluted (diluent: 0.1% peptone, 0.85% NaCl; pH 7.0) and plated on plate count agar (PCA, Oxoid) + 1% NaCl using the 'loop-tile' technique as described by Hudson et al. (5). This technique gives counts comparable with those obtained using the ISO reference method (5). Colonies were counted after 5 d of incubation at 20°C.
RESULTS AND DISCUSSION

Frozen beef

The sampling technique used for frozen beef in which a relatively large portion (100-200 g) was stomached intact after microwave thawing was designed for rapidity. With this technique it was also possible to use a high meat to diluent ratio (1:1) for stomaching to increase the precision of the DEFT on samples with low counts. No problems were encountered in filtering up to 6 ml of the undiluted suspensions where necessary and good DEFT preparations were obtained with few visible meat particles. Half the samples had been stored before freezing to obtain a wide range of counts and there was good agreement between the DEFT counts and plate counts over the range $6.3 \times 10^3$ to $2.5 \times 10^{10}$/g detected in the 60 samples, with a correlation coefficient ($r$) of 0.95 (Fig. 1). Pettipher and Rodrigues (9) also found that the DEFT count agreed well with the plate count for frozen meat and it is clear that the DEFT is suitable for the rapid estimation of microbial numbers in this type of sample.

Chill-stored beef joints

Microbial contamination and growth on intact forms of meat such as carcasses or joints occurs almost entirely on the surface which is commonly sampled for microbiological analysis by removing a known area using a cork borer (6). At the dilution rate used for homogenization in this study (21 cm$^2$ area stomached in 100 ml of diluent) good DEFT preparations were obtained using 6 ml of the suspensions. There was good agreement between the DEFT count and the plate count over the range $1.6 \times 10^4$ to $2.3 \times 10^8$/cm$^2$, with a correlation coefficient ($r$) of 0.96 (Fig. 2). Three samples with plate counts of $10^5$/cm$^2$ or less all had DEFT counts $>0.5 \log_{10}$ unit higher, indicating a loss of sensitivity by the DEFT. However, in routine quality control loss of precision at these low contamination levels would be of little practical importance. Alternatively, use of a larger sample volume should enhance precision at these lower levels.

Swab samples from carcasses and beef joints

Swab sampling for microbiological analysis is often used when disfigurement of the meat is to be avoided, as for carcasses. The dispersal of the contents of swabs from 50- or 100-cm$^2$ areas in 20 ml of diluent as used in this study is typical for the technique. Such swab suspensions (6 ml) from lean surfaces of joints gave good DEFT preparations, but non-microbial particulate material, some of which stained orange-red, was evident in some of the preparations from carcass swab suspensions. This, together with loss of sensitivity, may explain why the DEFT counts from carcasses appreciably exceeded ($>0.5 \log_{10}$ unit higher) plate counts on most samples with a plate count $<5 \times 10^5$/cm$^2$ (Fig. 3). This would be of little practical importance in quality control and there was good agreement between the DEFT counts and plate counts over the range $5 \times 10^3$ to $9.1 \times 10^6$/cm$^2$, with a correlation coefficient ($r$) for all swab samples of 0.95. Again, use of a larger sample size could well be beneficial at lower counts.
Neck skin samples from chicken carcasses

The neck flap can be removed from poultry carcasses without causing damage and provides a suitable sample for determination of the level of contamination after processing. The shake system of dispersal employed can be used to obtain an estimate of the number of bacteria on the sample, and was chosen in place of homogenization to reduce the level of fine particulate matter and fat which is released from poultry skin and which would interfere with the DEFT. However, even this technique produced suspensions which were still very cloudy after prefiltration and treatment with surfactant and trypsin, and blockage of the bacterial filter occurred when they were used without dilution. Suspensions were filterable when used as a 10⁻¹ dilution, but the quality of the DEFT preparations was poor with a large amount of debris (some stained orange-red) from which it was very difficult to distinguish bacterial cells. This almost certainly accounts for the poor agreement between DEFT and plate counts (Fig. 4). It is also possible that the DEFT count included a few non-viable or damaged cells as a result of the 50°C hot water ‘scalding’ treatment given to the carcasses, as Pettipher and Rodrigues (8) have shown that some types of bacteria killed by heat treatment remain acridine orange-stainable. A further possibility was that some cells included in the DEFT were anaerobic organisms not recovered by aerobic plate counting. However, anaerobic incubation of some suspensions on a reduced medium [VL1f agar (1) containing 1 μg of haemin/ml] did not increase the plate count which discounts this as a major factor. Before the DEFT can be assessed further for application to this type of sample it will be necessary to develop an alternative pre-treatment system to remove the debris interfering with counting.

Mixed skin and muscle samples from chicken

Bacterial contamination levels on poultry joints are commonly determined using a weighed mixed muscle and skin sample taken from the surface. At the dilution rate used in homogenization (25 g stomached in 100 ml of diluent) suspensions of these samples could not easily be pre-filtered and 10⁻¹ dilutions were therefore used for the DEFT, giving preparations of good quality. Plate counts on the samples were between 1 × 10^3 and 9.5 × 10^6/g and there was good overall agreement (r = 0.89) between DEFT and plate count (Fig. 5). It was, however, evident that the DEFT count appreciably exceeded (>0.5 log units higher) the plate count on all samples with a plate count <1 × 10⁵/g. This cannot be explained on the basis of poor sensitivity because DEFT counts on the suspensions from these samples were between 6.8 × 10^3 and 2.8 × 10^4/ml in which range sensitivity is acceptable. All these samples were either unstored or had been stored for such a short period (4 d at 0°C) that little or no growth had occurred and the count must have been largely composed of the initial contaminating flora. It seems possible that the DEFT overcounted on these samples because it included non-viable cells from that flora killed either by hot water ‘scalding’ treatment or during subsequent frozen storage before sampling.

The direct use of the DEFT count would, therefore, give a good estimation of the plate count on skin and muscle samples from poultry on which appreciable growth had occurred. However, overestimation of the plate count may occur with unstored samples or samples stored for a short period. Nevertheless, reasonably good linearity was obtained between DEFT and plate counts.
Scrape samples from chicken skin
Scraping skin in the presence of diluent enclosed in a cylinder is a suitable method for the non-destructive sampling of poultry carcasses or joints (2). Suspensions produced by this technique could be filtered undiluted as 3-ml samples and gave good DEFT preparations with little debris. Plate counts on the sampled skin areas were between $1.1 \times 10^3$ and $1.3 \times 10^4$/cm² and there was good overall agreement ($r = 0.88$) between DEFT and plate count (Fig. 6). However, the DEFT count exceeded the plate count on all samples with a plate count < $7 \times 10^4$/cm², overcounting by 10- to 100-fold on samples with plate counts between $10^3$ and $10^4$/cm². This cannot be explained on the basis of poor sensitivity because DEFT counts on the suspensions from these samples were between $1.2 \times 10^3$ and $1.5 \times 10^5$/ml in which range sensitivity is good. Nearly all the samples on which overcounting occurred were unstored and it again seems probable that the DEFT count on these samples included non-viable cells. Nevertheless, reasonably good linearity was obtained between DEFT and plate counts (Fig. 6) and the regression equation can once more be justifiably used to produce a more accurate estimate of the plate count if this is required.

Mechanically Recovered Meat
Suspensions from Mechanically Recovered Meat (MRM) were very cloudy despite the containment of most MRM particles in the inner nylon-lint bag during stomachering, and blockage of the prefilter occurred when they were used for the DEFT without dilution. A $10^1$ dilution of suspensions could be filtered but many of the preparations (particularly from chicken, lamb and pork MRM) were of poor quality with a large amount of debris, some of which was stained orange-red. In some preparations particles of MRM had completely blocked many of the pores in the polycarbonate membrane. It was therefore not surprising that there was poor overall correlation ($r = 0.486$) between the DEFT and plate counts (Fig. 7). There was particularly poor agreement for samples of chicken, lamb and pork MRM; the DEFT overcounting by 5- to 230-fold for chicken, 20- to 300-fold for lamb, and 20- to 1,200-fold for pork. However, the DEFT gave a fair estimate of the plate count for beef and veal samples, the correlation coefficient for these samples alone being 0.72, with the DEFT only overcounting by a maximum of 7- and 14-fold for veal and beef MRM, respectively.

These observations indicate that the DEFT as performed in this study may have potential for estimation of plate counts in beef and veal MRM, although evaluation on a larger number of samples with a wider range of plate counts is required. Before the DEFT can be evaluated further for application to other types of MRM it will be necessary to develop an improved pretreatment system to remove or break down MRM particles before filtration for staining.
Applicability of the DEFT to meat and poultry

This study has confirmed the suitability of the DEFT for rapid estimation of microbial numbers in frozen or chill-stored lean meat, and has also shown that it is applicable to microbiological examination of red meat carcasses sampled by swabbing. In each instance automatic counting can be used and the DEFT count gives good agreement with the plate count over a range adequate for routine quality evaluation (5 × 10^5/g or cm^2 and above).

The DEFT has not previously been evaluated for application to poultry but it is evident from our study that this is not as straightforward as for red meats. Carcass neck skin samples contain much particulate material which is not removed by the standard DEFT pretreatment system, modification of which will be necessary before the DEFT can be applied to this type of sample in the rapid estimation of bacterial numbers on carcasses after processing. There is also evidence that the DEFT count includes non-viable cells when poultry on which little or no growth has occurred is examined. Nevertheless, we have shown that it is possible to use the DEFT to obtain a good estimate of bacterial numbers on both stored and unstored poultry. This can be achieved by employing exterior skin scraping or stomaching of muscle and skin as the sampling technique and by using a pre-determined regression equation to estimate the viable count from the DEFT count.

Comminuted meats are the most unsuitable types of sample for examination by the DEFT because of their high content of particulate material. To minimize this problem Boisen (3) used a nylon-lint bag to retain most particles during stomaching and then briefly centrifuged the sample suspension before the standard pre-treatment filtration stage. Good DEFT preparations were obtained and both manual and automatic DEFT counts agreed well with the plate count over the range 5 × 10^5 to 2.6 × 10^6/g in a variety of raw minced meat samples. Qvist and Jakobsen (11) replaced centrifugation with a 30-min settlement stage and found good agreement between the DEFT count and the plate count for raw ground beef with plate counts of ca. 10^6/g, but were unable to use automatic counting of the DEFT preparations, presumably because of the fluorescent background material. Because manual counting is not a practical option for routine commercial use, only automatic counting was employed in the present study in which MRM was examined as an example of a comminuted meat. Using the nylon-lint bag during stomaching, but with no centrifugation or settlement stage, DEFT preparations from 3 of the 5 types of MRM examined were unsuitable for accurate enumeration. Taken together, these findings show that extra or alternative pretreatment stages, possibly including centrifugation, are necessary when applying the DEFT to comminuted meats, particularly when using automatic counting. Before employing these routinely it must, however, be demonstrated that viable bacteria are not removed from the sample suspension.

Cooked meats were not included in this study but Qvist and Jakobsen (11) found a very poor relationship between the DEFT and plate counts for heat treated sausages examined soon after production. They attributed this to the inclusion in the DEFT count of cells killed during heat treatment, but commented that this ‘total’ count is informative as an indicator of the microbiological status of the product before heat processing. However, good agreement between the DEFT count and the plate count was obtained for stored samples at the end of their shelf-life when growth had occurred to level of 10^7 to 10^9/g. Pettipher and Rodrigues (9) presented results for two samples of cooked meats for which good agreement between the DEFT count and the plate count were obtained. The DEFT, therefore, could be used for rapid microbiological examination of heat treated meat products, but it appears necessary to establish the significance of the DEFT count for each product type before using the technique routinely.

Although modifications to the pretreatment procedure will be necessary in some instances, it is clear that the DEFT is suitable for rapid estimation of bacterial numbers in many types of meat and poultry samples. The result time is short - in the present study counts were obtained on batches of five samples with 35-45 min - a period allowing very rapid assessment of ingredient and product microbiological quality. The technique is demanding on a technician’s time and does not permit testing of large numbers of samples per day, but these disadvantages could be lessened by automating the pretreatment and staining stages. Further enhancements to the technique, in particular the ability to selectively count specific pathogens and spoilage organisms, would make the DEFT even more valuable in both the quality control and research laboratory.

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REFERENCES


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