

Comparison of Two Bacteriological Swab Techniques

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INTRODUCTION

At the 22nd European Meeting of Meat Research Workers in 1976 (1) a new swab technique was presented (Swab/Agar Plate method = SAPM). This technique by which the bacteria are transferred directly to a pre-poured agar surface, has later been further described and discussed (2), and experience has shown it to be very useful in investigations at the Danish Meat Research Institute (DMRI) as well as in routine quality control.

Now a comparison has been made between this technique and another more traditional swab technique (3). In that method the sampling area is swabbed first with a moistened and then with a dry cotton wool ball. The samples are then mixed, diluted and plated (Swab/Dilution Technique = SDT).

PROCEDURE

Samples were taken on beef carcasses at four of DMRI's standard sampling sites: Site No 3 - neck, site No 4 - forerib, site No 5 - brisket and site No 9 - medial face of round. For each site ten samples were taken on different carcasses (five on left and five on right side), every time beginning with the SAPM (1 cm²) within the same area where the samples for the SDT (50 cm²) were taken immediately after, starting with wiping off the SAPM-template with the moist cotton wool ball.

The investigation was carried out at three visits in each of two different abattoirs (a total of 4 x 3 x 2 = 24 series of 10 samples for each technique).

RESULTS

The results for the SAPM are given in point values, which are converted into bact./sample (1 point = 1/3 log₁₀ unit). Table 1 shows the results for each series of 10 samples obtained by both techniques, i.e. means and standard deviations. The means for the SAPM are given in both point values and in log bacteria per sample (points/3).

Table 1: Bacterial counts (in points/sample and log bact./cm²) and Standard deviations (in log units) - Surface samples from beef carcasses.

Site	Visit	Quick method (SAPM)			Traditional method (SDT)	
		points	points/3	stand.dev.	log.bact.	stand.dev.
3	1	7.80	2.60	0.86	3.25	0.62
	2	7.00	2.33	0.47	3.20	0.32
	3	7.40	2.47	0.71	3.11	0.65
		7.40	2.47	0.70	3.19	0.55
4	1	8.10	2.70	0.96	3.38	0.75
	2	6.30	2.10	0.47	2.93	0.55
	3	7.20	2.40	0.52	3.07	0.63
		7.20	2.40	0.69	3.13	0.65
5	1	9.10	3.03	0.79	3.58	0.48
	2	10.3	3.43	0.69	3.94	0.41
	3	9.60	3.20	0.71	3.64	0.60
		9.67	3.22	0.73	3.72	0.51
9	1	8.50	2.83	0.45	3.69	0.81
	2	9.60	3.20	0.71	3.61	0.51
	3	9.30	3.10	0.72	3.59	0.51
		9.13	3.04	0.64	3.63	0.62
3	1	8.00	2.67	1.17	3.54	1.41
	2	5.60	1.87	0.48	2.40	0.51
	3	6.11	2.04	0.54	2.65	0.69
		6.57	2.19	0.79	2.87	0.96
4	1	8.00	2.67	0.63	2.85	0.38
	2	6.40	2.13	0.63	2.72	0.71
	3	6.70	2.23	0.86	2.76	0.78
		7.03	2.34	0.72	2.77	0.65
5	1	6.70	2.23	0.52	2.88	0.29
	2	6.33	2.11	0.58	2.62	0.40
	3	7.50	2.50	0.57	2.82	0.39
		6.84	2.28	0.56	2.78	0.36
9	1	6.10	2.03	0.64	2.59	0.44
	2	7.33	2.44	0.41	2.92	0.52
	3	6.80	2.27	0.54	3.03	0.49
		6.74	2.25	0.54	2.85	0.49

Average counts and standard deviations are calculated for each site/abattoir. These counts have been converted to bacteria per sample and are listed in table 2, where the sites are ranked according to magnitude. The statistical correlation is dealt with later under DISCUSSION. The mean counts obtained by the SDT are higher than those by the SAPM, on average six times higher. For abattoir A, sites No 9 and 5 have higher counts than sites No 4 and 3; for abattoir B the counts are of the same level for all four sites.

Table 2: Average counts, converted from table 1

Site	Method: Quick (SAPM)		Traditional (SDT)	
	Unit: bact./sample		bact./cm ²	
Abatt.:	A	B	A	B
No. 4	170	150	1.300	590
No. 3	200	110	1.500	740
No. 9	750	120	4.300	710
No. 5	1.200	130	5.200	600

Table 3: Distribution of standard deviations

Method: Unit:	Quick (SAPM) log ₁₀	Traditional (SDT) log ₁₀
Range of standard deviation		
0.26-0.50	5 (21%)	9 (38%)
0.51-0.75	14 (58%)	13 (54%)
0.76-1.00	4 (17%)	1 (4%)
over 1.00	1 (4%)	1 (4%)
average s.d.	0.68	0.62

It has been debated, whether the sampling area should be large (50-100 cm²) or small (1-10 cm²), the point being that if a small area is used, the risk of "missing" a spot with a very high count is too high. If this was an important factor, it should show in this material. In this case the standard deviation for the SDT (50 cm²) would be higher than for the SAPM (1 cm²). Table 3 shows the distribution and the average of the standard deviations from table 1. The level of standard deviation is very much the same for the two methods, if any difference it is slightly lower for the SDT. This indicates that the size of the sampling area is not of significant importance.

DISCUSSION

Owing to the sample-to-sample variation within sites and visits, the standard deviation of the material as a whole was considerably higher when based on single values instead of means of the ten replicates per site and visit. Thus, in the former case, - according to table 3 - the standard deviation was 0.68 and 0.62 for the two techniques while for the latter it was 0.43 and 0.42, respectively. In accordance with this, the coefficient of correlation between the two methods was 0.67 when calculated from single samples, and 0.92 when calculated from the average of ten samples per site per visit.

Figure 1 shows the relationship between the two sets of results. The regression curve is determined according to (4), taking into consideration that both variables are subjected to error, not just the one of them. The "line of best fit" is of the linear equation $y = 1.01x - 0.62$.

Besides that, another 0.17 log unit has to be added because there is a deviation from zero of $+\frac{1}{2}$ point = 1/6 log unit when results are converted from point values to bact./sample. This is due to the fact that the table after which the numbers of colonies are determined into groups (v points) is based on the end values of the group, while the table for converting point values to bact./sample is based on the middle value of the group. So, the total difference between log bact./cm² (SDT) and log bact./sample (SAPM) on average is 0.79 log units and the corresponding factor is 6.1.

A slope of 1.01 shows that - within the examined range - this factor is not dependent on the count level.

In the description of the SAPM (1., 2.) it is stated that results under 3.5 and over 10.9 points should be given as "lower than 10" and "higher than 3.000", respectively, without giving a specific number. These limits can now be converted to bact./cm² (SDT), namely 60 and 18.000, respectively. Results outside these limits will presumably fall outside the regular limits of the SAPM.

This does, however, not mean that results outside these limits should not be recorded. It is, of course, of interest to know whether a result is low or high or even "very high". Such a result should, however, not be stated with a figure.

SAMPLING TIME

In connection with this investigation it was registered how much time was consumed for each of the two methods.

The sampling was performed by two persons who are highly experienced in this job, hence doing it at a quite high rate. The time required for taking the actual samples by the SDT was approx. three times that by the SAPM (30 to 40 samples versus 90 to 120 samples per hour, respectively, or $1\frac{1}{2}$ to 2 minutes versus $\frac{1}{2}$ to $\frac{2}{3}$ of a minute per sample). In addition the samples had to be processed further in the laboratory when using the SDT (mixed, diluted and plated). This was estimated to take ten minutes per sample. This limits the number of samples which can be taken per day considerably. Especially if there is no laboratory at the abattoir or if a mobile laboratory is not available.

Counting/estimating the number of colonies took on average approximately $1\frac{1}{2}$ to 2 minutes and $\frac{1}{2}$ to $\frac{2}{3}$ of a minute per sample, respectively. This gives the following calculation per sample:

Method:	Quick (SAPM)	Traditional (SDT)
Sampling (2 persons): $2 \times (\frac{1}{2} \text{ to } \frac{2}{3}) =$	1 to 4/3 min.	$2 \times (1\frac{1}{2} \text{ to } 2) =$ 3 to 4 min.
dilution etc:		about 10 min.
reading results:	$\frac{1}{2} \text{ to } \frac{2}{3} \text{ min}$	$\frac{1\frac{1}{2} \text{ to } 2 \text{ min.}$
total:	$1\frac{1}{2} \text{ to } 2 \text{ min.}$	14-16 min.

CONCLUSIONS

There is a very good correlation between the results obtained by the two techniques. If based on the average per site per visit (series of ten samples) the correlation coefficient was found to be 0.92, if based on single observations it was 0.67.

On average, the counts per cm^2 obtained by the traditional technique are six times higher than the counts per sample obtained by the quick method. Within the examined range this factor is not dependent on the count level.

Samples for which the counts per cm^2 obtained by the traditional technique are higher than 18.000 or lower than 30 will presumably fall outside the regular limits of the quick method.

There is no indication that the use of a 50 cm^2 sampling template rather than a 1 cm^2 template is advantageous.

The total time required for taking and preparing the samples and reading the results was found to be 14 to 16 minutes per sample for the traditional technique, compared to $1\frac{1}{2}$ to 2 minutes per sample for the quick method.

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REFERENCES

1. Ølgaard, K. (1976): Determination of relative bacterial levels on carcasses and meats - a new quick method, 22nd European Meeting of Meat Research Workers, L2, Malmö, Sweden.
2. Ølgaard, K. (1977): Determination of relative bacterial levels on carcasses and meats - a new quick method. *Journal of Applied Bacteriology*, **42**, 321-329.
3. Kitchell, A.G., Ingram, G.C. & Hudson, W.R. (1973): Microbiological sampling in abattoirs. In: *Sampling - Microbiological Monitoring of Environments* (ed. R.G. Board and D.W. Lovelock), p. 48. Society for Applied Bacteriology Technical Series No. 7, London: Academic Press.
4. Sprent, P. (1969): *Models in Regression and Related Topics*, pp. 29-45, Methuen & Co., London.

Figure 1
 Traditional method (log bact./cm²) versus Quick method (points/sample)

