J. Hyg., Camb. (1978), **81**, 151
Printed in Great Britain

Enumeration of micro-organisms in food: a comparative study of five methods

By J. M. KRAMER and R. J. GILBERT
Food Hygiene Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, England

(*Received* 23 *December* 1977)

SUMMARY

Five methods for the enumeration of micro-organisms in food (pour plate, surface spread plate, surface drop, agar droplet, and microdilution) were used in parallel to examine 100 samples selected from a wide range of food products.

Statistical analyses of the results showed that the regression and correlation coefficients between the methods were highly significant; the lowest correlation coefficient derived for any pair of methods was 0·979. A variation in count between the methods of less than 0·5 $\log_{10}$ cycles was given by 98 % of the samples.

Analysis of the operation times and material requirements of each method showed that substantial savings in cost, time, space and support labour were achieved with the microdilution and agar droplet techniques as compared with the conventional pour plate and spread plate methods.

INTRODUCTION

Increasing attention is being paid to microbiological quality control in food processing establishments throughout the world and a regular appraisal of the techniques used is necessary. The International Commission on Microbiological Specifications for Foods (ICMSF) was formed in 1962 in response to the need for internationally acceptable and authoritative decisions on microbiological limits for foods commensurate with public health safety, and particularly for foods in international commerce.

This Commission reviewed current methods for the microbiological examination of foods and published interim recommendations for their use (Thatcher & Clark, 1968). For the enumeration of mesophilic aerobic micro-organisms in food the ICMSF favoured the pour plate or standard plate count method. This procedure was used extensively and its merits and limitations were well documented (American Public Health Association, 1967), in contrast to the relative absence of such information on alternative methods.

Since the pour plate method is both time-consuming and costly, laboratories committed to the examination of large numbers of samples have sought more rapid and economical procedures for estimating the numbers of bacteria in food. Of these the surface spread plate method is now widely used in Europe and is

recommended by the International Standards Organization (1976) and the British Standards Institution (1976). The surface drop method (Miles & Misra, 1938) is also popular in many laboratories. More recent procedures include the plate loop (Thompson, Donnelly & Black, 1960), microdilution techniques (Fung & Kraft, 1968; Kramer, 1977), the agar droplet (Sharpe & Kilsby, 1971; Sharpe *et al.* 1972) and the loop-tile method (Ingram & Roberts, 1974).

The purpose of the present study was to compare the pour plate, surface spread plate, surface drop, agar droplet and microdilution methods for the enumeration of bacteria in food.

MATERIALS AND METHODS

Equipment

The Colworth 'Droplette' machine was obtained from A. J. Seward, Bury St Edmunds, Suffolk, 'Microtiter-V' plates from Dynatech Laboratories, Billinghurst, Sussex, and the variable-stroke micropipettor (Gilson 'Pipetman' P.200) from Anachem Ltd, Luton, Beds. Standard 9 cm diameter Petri dishes (Sterilin Ltd, Teddington, Middlesex) were used throughout the work.

Medium

Plate count agar (PCA; Oxoid) from a single production batch was used in all the methods tested. For the pour plate and agar droplet (Colworth 'Droplette') techniques, the agar was melted and cooled to 46 °C in a water bath before use. Pre-poured plates were required for the other methods and these were dried thoroughly at 36 °C.

Preparation of samples

Ten gram samples of the food were blended with 90 ml volumes of quarter-strength Ringer solution for 30 s in a Colworth 'Stomacher 400' (A. J. Seward, Bury St Edmunds, Suffolk). The 1/10 homogenized suspension of each sample was common to all five methods. Individual serial decimal dilutions were prepared from this for each method up to a $1/10^6$ dilution of the original food sample. Quarter-strength Ringer solution was used as the diluent in four of the methods and molten PCA in the agar droplet procedure. All dilutions were plated. After inoculation, plates were incubated at 30 °C for 24 h (agar droplet), 48 h (surface spread plate, surface drop and microdilution methods) or 72 h (pour plate).

Experimental design

One operator examined ten samples of each of ten types of food using the five plating methods. The foods used were: sausage meat, minced beef, cooked meat and poultry, salami, frozen cooked prawns, dried shrimp, dried egg, cheese, cream cakes and boiled rice. Samples were either those submitted to the laboratory for routine investigation, or were purchased from local shops.

The plating methods used were:

(1) Pour plate (Thatcher & Clark, 1968).
(2) Surface spread plate (Thatcher & Clark, 1968), modified by applying 0·1 ml volumes of each dilution to half plates.

Table 1. *Comparison of colony counts on ten food products determined by five methods*

Food product	No. of samples examined	Mean (and range) $\log_{10}$ colony count/g determined by Pour plate	Spread plate	Surface drop	Agar droplet	Micro-dilution
Sausage meat	10	7·52 (6·58–8·16)	7.56 (6·60–8·17)	7·60 (6·63–8·21)	7·49 (6·46–8·10)	7·55 (6·54–8·07)
Minced beef	10	7·32 (6·07–8·40)	7·50 (6·81–8·33)	7·49 (6·80–8·26)	7·40 (6·62–8·23)	7·47 (6·78–8·24)
Cooked meat and poultry	10	6·09 (3·78–7·79)	6·13 (3·81–7·76)	6·23 (3·88–7·80)	6·06 (3·66–7·75)	6·18 (3·38–7·84)
Salami	10	7·61 (7·01–8·31)	7·46 (6·87–8·10)	7·54 (6·95–8·19)	7·41 (6·63–8·13)	7·50 (6·96–8·22)
Frozen cooked prawns	10	6·22 (4·90–8·12)	6·16 (4·94–8·08)	6·30 (4·97–8·11)	6·10 (4·72–7·83)	6·21 (4·88–8·02)
Dried shrimp	10	4·98 (3·85–6·16)	4·88 (3·78–6·10)	5·10 (3·81–6·33)	4·88 (3·69–5·92)	4·96 (3·74–5·99)
Dried egg	10	4·08 (3·28–4·72)	4·06 (3·04–4·85)	4·17 (3·18–4·93)	4·03 (3·26–4·81)	4·12 (3·30–4·90)
Cheese	10	6·62 (5·10–7·94)	6.49 (4·81–7·80)	6·58 (5·00–7·90)	6·54 (4·86–7·83)	6·55 (4·94–7·88)
Cream cakes	10	5·67 (4·14–6·91)	5·57 (4·06–6·77)	5·63 (4·09–6·94)	5·38 (3·99–6·86)	5·66 (4·04–6·84)
Boiled rice	10	4·91 (3·37–6·78)	4·95 (3·50–6·80)	5·02 (3·57–6·98)	4·93 (3·46–6·93)	5·05 (3·61–6·85)

(3) Surface drop (Miles & Misra, 1938), modified as described by Thatcher & Clark (1968).
(4) Agar droplet (Sharpe & Kilsby, 1971).
(5) Microdilution (Kramer, 1977).

The methods were used in parallel to examine individual samples and their order of use was varied between examinations. Colony counts were transformed into $\log_{10}$ values. Corresponding results obtained by the various methods were paired and analysed by computer for regression and correlation.

RESULTS

Comparison of colony counts

Table 1 shows the range and mean colony counts obtained when ten samples of each of ten food products were examined by five methods. A variation in count between the methods of less than 0·5 $\log_{10}$ cycles was given by 98 % of the samples.

The results of each method were analysed by regression against the results obtained with the other four methods. The regression and correlation coefficients between all methods together with the standard error of the regression coefficients are presented in Table 2. Correlation between any two methods was good (r = 0·979–0·994), and there was a high degree of significance in the association of the paired results ($P < 0·001$).

https://doi.org/10.1017/S0022172400053857 Published online by Cambridge University Press

Table 2. *Regression and correlation coefficients for each pair of methods**

Method 1	Method 2	Regression coefficient (β)	Intercept	Standard error (S.E.$_\beta$)	Multiple correlation coefficient
Pour plate	Spread plate	0·988	0·062	0·018	0·992
Pour plate	Surface drop	0·991	0·076	0·011	0·990
Pour plate	Agar droplet	1·001	−0·103	0·015	0·982
Pour plate	Microdilution	0·985	0·112	0·019	0·987
Spread plate	Surface drop	0·994	0·004	0·016	0·993
Spread plate	Agar droplet	1·012	−0·150	0·014	0·985
Spread plate	Microdilution	0·998	0·040	0·017	0·992
Surface drop	Agar droplet	1·007	−0·159	0·013	0·986
Surface drop	Microdilution	0·994	0·028	0·010	0·994
Agar droplet	Microdilution	1·002	−0·134	0·016	0·979

* Results from Table 1; $n = 100$.

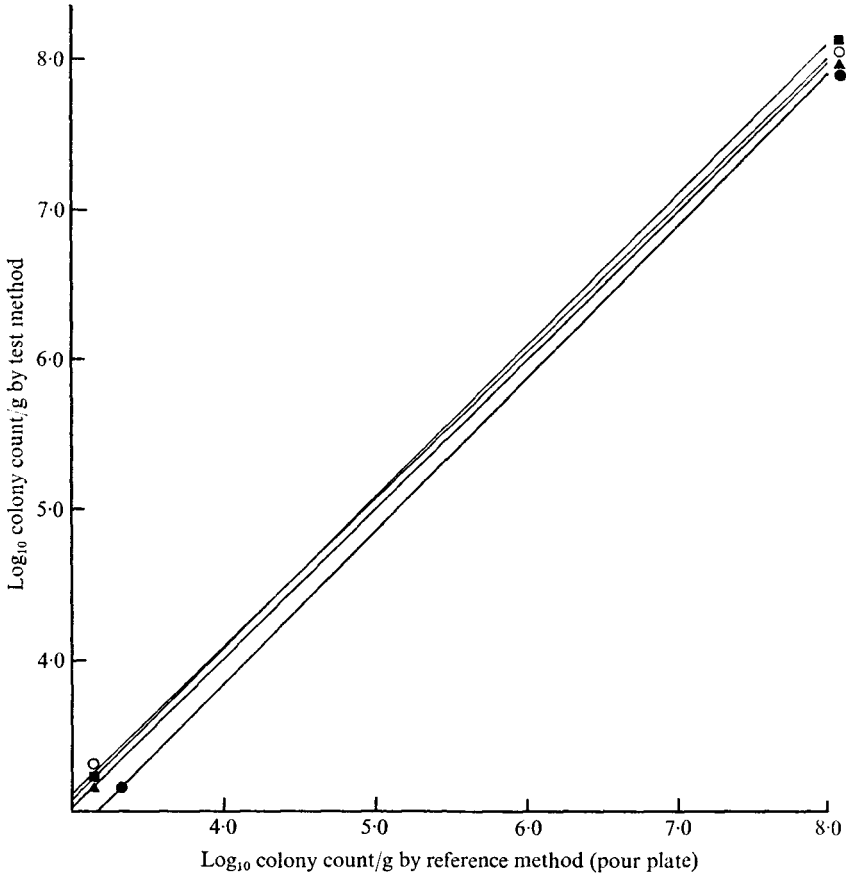

Fig. 1. Lines of regression for bacterial enumeration methods. Regression of colony count data obtained by the pour plate method (X) on corresponding results by the: (▲), surface spread plate [$Y = 0{\cdot}988X + 0{\cdot}062$]; (■), surface drop [$Y = 0{\cdot}991X + 0{\cdot}076$]; (●), agar droplet [$Y = 1{\cdot}001X - 0{\cdot}103$] and (○), microdilution [$Y = 0{\cdot}985X + 0{\cdot}112$] methods.

https://doi.org/10.1017/S0022172400053857 Published online by Cambridge University Press

Table 3. *Media and equipment requirements for the examination of* 100 *samples of food by five methods*

	Method				
Media/equipment	Pour plate	Spread plate	Surface drop	Agar droplet	Microdilution
Plate count agar (l)	24·0	12·0	4·0	4·5	4·0
Quarter-strength Ringer soln. (l)	4·5	4·5	4·5	Nil	0·09
Plastic Petri dishes	1200	600	200	100	200
Dilution vessels (d)	Nil	Nil	Nil	Nil	6 (a)
Dilution vessels (n/d)	500	500	500	500	Nil
Pipettes (d)	Nil	Nil	Nil	600 (b)	600 (c)
Pipettes/droppers (n/d)	600	600	600	Nil	Nil
Additional items	Nil	Glass spreaders	Nil	Viewer-dispenser machine	Variable-stroke micro-pipettor

d, disposable; n/d, non-disposable. (a), As micro-agglutination trays. (b), As plastic straws. (c), As plastic micropipettor tips.

For all methods tested, the lines of regression fit almost ideally to the line of equality (Fig. 1). The graph indicates that slightly lower counts would be expected from the agar droplet method than from the pour plate method. However, this discrepancy is small, and well within normally accepted tolerances for colony enumeration methods.

In the present study the initial sample preparation was common to all methods. Individual sample preparation could be expected to increase the variations observed.

Comparison of material requirements

The media and equipment requirements for the examination of 100 samples of food by each of the five methods is given in Table 3. The figures shown are based on dilutions taken up to $1/10^6$ of the original food sample, but do not include materials such as the Stomacher machine, and the 90 ml volumes of diluent used for the preparation of the 1/10 homogenate. The pour plate and spread plate methods both required large numbers of Petri dishes, pipettes and dilution vessels, and large volumes of agar and diluent. Substantial savings of these materials were achieved when the agar droplet and microdilution procedures, and to a lesser extent the surface drop method, were used for the enumeration of bacteria in food.

In addition, the materials required for the pour plate method were found to occupy 8–12 times the operating and storage space needed for the surface drop, agar droplet or microdilution methods.

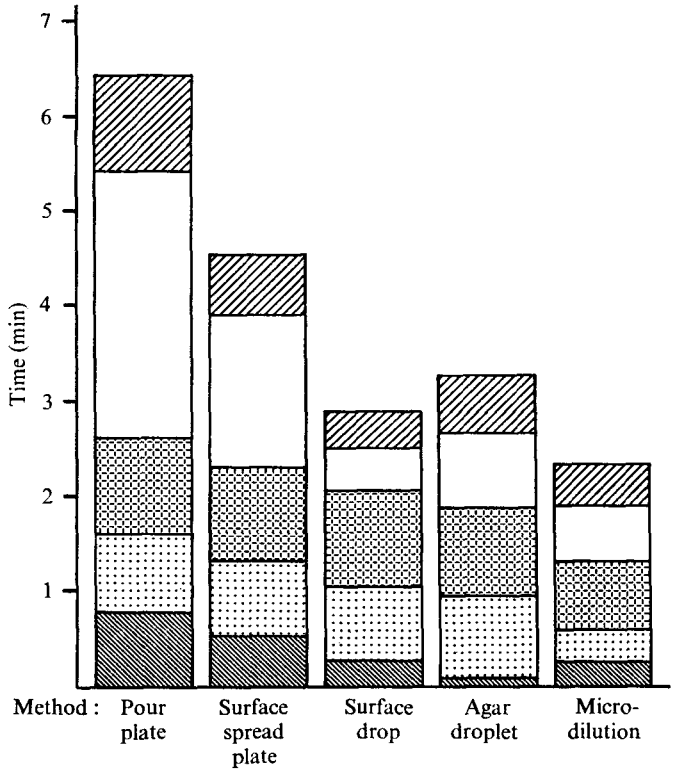

Fig. 2. Comparison of the bench labour requirements of the five methods studied. The height of each column represents the mean total operation time per sample for the method indicated. Subdivisions show the breakdown of the total operation time into five components: ▧, labelling of plates; ⊡, dispensing of diluent; ▩, serial dilution of sample; □, transfer of dilutions to plates (with mixing or spreading, where appropriate) and ▨, the colony count.

Comparison of labour requirements

Figure 2 compares the bench labour requirements per sample of food for each of the five methods. The times shown represent mean values derived from the examination of ten samples.

In the time taken to test one sample by the pour plate method, two samples could be processed by the surface drop and agar droplet methods, and three by the microdilution technique. Nearly half of the bench labour with the pour plate method was concerned with the transfer of dilutions to Petri dishes and the subsequent thorough mixing with molten agar. The large numbers of plates used in the procedure, together with the manual counting of up to 300 colonies per plate, contributed further to a lengthy procedure. In contrast, the surface drop, agar droplet and microdilution methods offered significant savings in both time and effort required to carry out the colony plate count.

All the methods examined relied on support labour, both for the preparation of culture media, diluent and sterile equipment, and for the disposal of used materials. However, the amount of support labour required by each method varied from

considerable (pour plate method) to minimal (microdilution technique). The surface spread plate, surface drop and agar droplet methods ranked mid-way. The magnitude of the variations between the methods can be seen from the information presented in Table 3.

DISCUSSION

To estimate the numbers of viable bacteria in large numbers of food samples an efficient, simple, rapid and relatively inexpensive method is desirable. The present work compared three conventional methods (pour plate, surface spread plate and surface drop) and two (agar droplet and microdilution) recently developed procedures. There was no significant difference between the colony counts obtained by these methods. Other factors such as labour and material requirements should therefore be considered before the decision is made to adopt a particular method for routine use. In this respect, the agar droplet and microdilution procedures had the obvious advantages of lower costs for materials and labour when compared to the pour plate and surface spread plate methods. For laboratories examining large numbers of samples of food such savings would be significant.

Furthermore, the flexibility of the surface drop and microdilution methods enables the simultaneous differential count of, for example, presumptive *Staphylococcus aureus* to be carried out with ease. The master dilutions used to inoculate the plate count medium are also applied to an appropriate range of selective media; the increase in work-load is relatively small. The surface spread method is also suitable for this purpose, although following inoculation of the plates, each dilution must then be spread. In contrast, the pour plate and agar droplet methods lack such flexibility. For examinations in which both total and differential count results are required these methods in general are unsuitable for use with selective media, and must be supplemented with an appropriate surface plate technique.

The advantages and limitations of four of the methods used (pour plate, surface spread plate, surface drop and agar droplet) are well documented by, for example, Hartman & Huntsberger (1961), Barraud *et al.* (1967), Clark (1967), Thatcher & Clark (1968), Sharpe *et al.* (1972) and Koller & Jelinek (1976). The microdilution technique (Kramer, 1977) was developed by this laboratory in response to the requirement for a simple, rapid and economical routine bacterial counting procedure. The method should be of interest to those who undertake large numbers of colony plate counts, but it is not suited for the occasional examination of one or two samples. The comments by Thatcher & Clark (1968) on the surface drop method also apply to the microdilution technique.

Less conventional techniques for the rapid estimation of microbial populations, although not designed specifically for use in food microbiology, have been adapted for this purpose. These include bioluminescence (Sharpe, Woodrow & Jackson, 1970), microcalorimetry (Sacks & Menefee, 1972; Rowley *et al.* 1974), radiometry (Previte, 1972; Rowley *et al.* 1974) and impedance monitoring (Ur & Brown, 1975; Hardy *et al.* 1977). All of these techniques may eventually serve as rapid screening tests for microbial counts in food, but at the present time their application is limited with respect to sensitivity, and most require expensive equipment and

skilled operators. However, a most useful development in recent years has been the Spiral Plate Maker (Gilchrist *et al.* 1973) and the spiral plate count method is now accepted in the U.S.A. by the Association of Official Analytical Chemists (1977) as an official procedure for the examination of foods and cosmetics. Reports on the spiral plate method, and comparisons with conventional methods have shown that the procedure has many advantages which recommend its use in a routine laboratory (Donnelly *et al.* 1976; Gilchrist *et al.* 1976; Peeler *et al.* 1977; Jarvis, Lach & Wood, 1977). Results from this laboratory (J. M. Kramer & M. Kendall, unpublished data) endorse these findings.

To the food microbiology laboratory committed to the routine monitoring of high numbers of samples, the economics of the procedures used are a major consideration. In this respect, our findings indicate that conventional pour and surface spread plate methods for determining bacterial numbers in food should not be universally accepted for the purpose of international standards and specifications without reappraisal.

We are grateful to Mrs Margaret Hurley of the Epidemiological Research Laboratory for help with the statistics.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION (1967). *Standard Methods for the Examination of Dairy Products*, 12th edition. Washington, D.C.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1977). Changes in methods: Method no. 46.C10-46.C16. *Journal of the Association of Official Analytical Chemists* **60**, 493.

BARRAUD, C., KITCHELL, A. G., LABOTS, H., REUTER, G. & SIMONSEN, B. (1967). Standardisierung der aeroben Gesamtkeimzahlbestimmung in Fleisch und Fleischerzeugnissen. *Die Fleischwirtschaft* **47**, 1313.

BRITISH STANDARDS INSTITUTION (1976). Methods for microbiological examination of meat and meat products. Part 1. Enumeration of micro-organisms: colony count at 30 °C. *British Standard BS* 5393: *Part* 1. London.

CLARK, D. S. (1967). Comparison of pour and surface plate methods for determination of bacterial counts. *Canadian Journal of Microbiology* **13**, 1409.

DONNELLY, C. B., GILCHRIST, J. E., PEELER, J. T. & CAMPBELL, J. E. (1976). Spiral plate count method for examination of raw and pasteurised milk. *Applied and Environmental Microbiology* **32**, 21.

FUNG, D. Y. C. & KRAFT, A. A. (1968). Microtiter method for the evaluation of viable cells in bacterial cultures. *Applied Microbiology* **16**, 1036.

GILCHRIST, J. E., CAMPBELL, J. E., DONNELLY, C. B., PEELER, J. T. & DELANEY, J. M. (1973). Spiral plate method for bacterial determination. *Applied Microbiology* **25**, 244.

GILCHRIST, J. E., DONNELLY, C. B., CAMPBELL, J. E. & PEELER, J. T. (1976). A collaborative study of the spiral plate method on two food and one cosmetic sample. *Abstracts 90th Annual Meeting of A.O.A.C.*, no. 163a.

HARDY, D., KRAEGER, S. J., DUFOUR, S. W. & CADY, P. (1977). Rapid detection of microbial contamination in frozen vegetables by automated impedance measurements. *Applied and Environmental Microbiology* **34**, 14.

HARTMAN, P. A. & HUNTSBERGER, D. V. (1961). Influence of subtle differences in plating procedure on bacterial counts of prepared frozen foods. *Applied Microbiology* **9**, 32.

INGRAM, G. C. & ROBERTS, T. A. (1974). The loop-tile method of bacterial counting: a minimal apparatus method suitable for field work. *Meat Research Institute Memorandum*, no. 25.

INTERNATIONAL STANDARDS ORGANIZATION (1976). *Draft International Standard ISO/DIS* 4833. Geneva.

JARVIS, B., LACH, V. H. & WOOD, J. M. (1977). Evaluation of the spiral plate maker for the enumeration of micro-organisms in foods. *Journal of Applied Bacteriology* **43**, 149.

KOLLER, W. & JELINEK, J. A. (1976). Die kulturelle Keimzahlbestimmung im Agartropfen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene.* 1. Abt. Orig. A **235**, 527.

KRAMER, J. M. (1977). A rapid microdilution technique for counting viable bacteria in food. *Laboratory Practice* **26**, 675.

MILES, A. A. & MISRA, S. S. (1938). The estimation of the bactericidal power of the blood. *Journal of Hygiene* **38**, 732.

PEELER, J. T., GILCHRIST, J. E., DONNELLY, C. B. & CAMPBELL, J. E. (1977). A collaborative study of the spiral plate method for examining milk samples. *Journal of Food Protection* **40**, 462.

PREVITE, J. J. (1972). Radiometric detection of some food-borne bacteria. *Applied Microbiology* **24**, 535.

ROWLEY, D. B., PREVITE, J. J., WELLS, R. E., LAMPI, R. R. & MIKELSON, D. A. (1974). Radiometry and microcalorimetry – techniques for the rapid detection of foodborne microorganisms. *Food Technology* **28**, 52.

SACHS, L. E. & MENEFEE, E. (1972). Thermal detection of spoilage in canned foods. *Journal of Food Science* **37**, 928.

SHARPE, A. N., WOODROW, M. N. & JACKSON, A. K. (1970). Adenosinetriphosphate (ATP) levels in foods contaminated by bacteria. *Journal of Applied Bacteriology* **33**, 758.

SHARPE, A. N. & KILSBY, D. C. (1971). A rapid, inexpensive bacterial count technique using agar droplets. *Journal of Applied Bacteriology* **34**, 435.

SHARPE, A. N., DYETT, E. J., JACKSON, A. K. & KILSBY, D. C. (1972). Technique and apparatus for rapid and inexpensive enumeration of bacteria. *Applied Microbiology* **24**, 4.

THATCHER, F. S. & CLARK, D. S. (1968). *Microorganisms in Foods: Their Significance and Methods of Enumeration.* Canada: University of Toronto Press.

THOMPSON, D. I., DONNELLY, C. B. & BLACK, L. A. (1960). A plate loop method for determining viable counts of raw milk. *Journal of Milk and Food Technology* **23**, 167.

UR, A. & BROWN, D. F. J. (1975). Impedance monitoring of bacterial activity. *Journal of Medical Microbiology* **8**, 19.