# A comparison of selected public health criteria in milk from milk-shops and from a national distributor

M More O'Ferrall-Berndt<sup>a</sup>

## **ABSTRACT**

Selected public health criteria of pasteurised milk available to the consumer from milk-shops in a pre-defined area of Pretoria compared with a national distributor's milk was evaluated. Of the 135 milk samples purchased from milk-shops, 87 % were not fit for human consumption on the basis of the minimum standards prescribed in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972). The national distributor's milk (n =79) did not contain any pathogens, toxins nor inhibitory substances and passed all the criteria laid down in the Act. Even though milk-shop milk was sold as having been pasteurised, 38.5 % of samples were alkaline phosphatase positive, indicating probable inadequate pasteurisation. Milk-shop milk quality varied between milk-shops and between sampling days and differed significantly (P < 0.05) from the national distributor's milk. Total aerobic plate and coliform counts were generally high for all milk-shop milk samples. Somatic cell counts of milk-shop milk differed significantly (P < 0.05) from the national distributor's milk. Escherichia coli was detected in 1 ml of 17 % of milk-shop milk, 95 % of which originated from milk which was alkaline phosphatase positive. Salmonella spp. could not be detected in  $1\,\mathrm{m}\ell$  in any of the E. coli-positive milk tested. Staphylococcus aureus was isolated from  $40\,\%$  of milk-shop milk samples, and S. aureus enterotoxins from 7.8 % of 51 cultures. Inhibitory substances were detected in  $54.1\,\%$  of milk-shop milk. The presence of inhibitory substances and the isolation of E. coli and S. aureus (some of which were able to produce enterotoxins) indicated potentially unsafe milk and poses a serious public health risk to

**Key words**: milk hygiene, milk-shops, national distributor, pathogens, Pretoria, toxins, veterinary public health.

More O'Ferrall-Berndt M A comparison of selected public health criteria in milk from milk-shops and from a national distributor. *Journal of the South African Veterinary Association* (2003) 74(2): 35–40 (En.). Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

## INTRODUCTION

Cow milk is a highly nutritious and valuable human food, but its nutrient composition also makes it an ideal medium for bacterial growth<sup>8,9,19</sup>. Although many contaminating organisms only spoil the product, thereby reducing its shelf-life, other bacteria are pathogenic to man and can transmit disease if the milk is left untreated<sup>19,33</sup>. Unlike meat and meat products, milk is less likely to be subjected to any subsequent heating by the consumer before consumption, and therefore contaminated milk is potentially more dangerous<sup>34</sup>.

There have been numerous outbreaks of milk-borne disease in humans with pathogens such as *Staphylococcus aureus, Escherichia coli, Campylobacter* spp. and *Salmonella* spp., especially since mass production came into effect <sup>9,36</sup>. Most of these

<sup>a</sup>Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: romamof@icon.co.za

Received: December 2002. Accepted: March 2003.

outbreaks have occurred in raw milk, but there have also been outbreaks of disease after consuming pasteurised milk due to a failure in the pasteurisation process or post-pasteurisation contamination<sup>13,30,31</sup>.

Appropriate epidemiological statistics on milk-borne diseases in South Africa are not readily available. Unless data were to become available to prove to the contrary, it seems realistic to assume that milkborne diseases are probably at least as prevalent in South Africa as in other countries under conditions of industrialised mass production and distribution of raw and pasteurised dairy products. Surveys conducted on raw milk samples in other developing countries showed that on the whole the quality was bad 1-4,26,28,29. As a result of the deregulation of the South African dairy industry in the early 1990's, 'milk-shops' have become a common retail outlet for milk which would not qualify for sale to large national distributors, especially in the lower socio-economic areas. Milk from mainly smaller farms is

sold directly to the public who collect the milk from these outlets in mainly their own containers, usually at a lower price<sup>25</sup>. In Pretoria, milk-shops have developed rapidly from none in January 1996 to over 55 in January 2000. Sampling of milk-shop milk by environmental health officers in this city was reduced from 3 times a week in 1997 to once a week in 2000, due to budgetary constraints.

The aim of this study was to evaluate the safety and potential shelf-life of pasteurised milk available to the consumer in a predetermined area of Pretoria, comparing 2 different marketing systems. Firstly, milk from a large national distributor, who buys quality milk at a premium from farmers, was evaluated. Processing and packaging took place at a plant under strict hygienic conditions before distribution. Secondly, milk purchased from 'milk-shop' distributors who buy milk from farmers on volume alone, with no incentives paid for quality, was also evaluated. Milk-shop milk is purportedly pasteurised in the shop before sale to the public, but not necessarily packaged. All milk was evaluated to determine whether it fell within the parameters laid down by law according to the Foodstuffs, Cosmetics and Disinfectants Act, No. 54 of 1972: Regulations relating to milk and dairy products, No. R.1555; Government Gazette No. 18439, 21 November 1997 hereafter referred to as 'the Act' 17.

## **MATERIALS AND METHODS**

Study design

One hundred and thirty-five milk samples were obtained over a 6-week period from June to August 1998 from 4 randomly chosen milk-shops (Milk-shops 1, 2, 4 and 5) and from 1 selected milk-shop (Milk-shop 3). Seventy-nine samples of milk, originating from a well-known national distributor's commercial brand of milk were purchased from 3 supermarkets (Supermarkets 1, 2 and 3), and were used as the reference control milk. Milk-shop 3 and Supermarket 3 were situated on the same premises, selling both milk originating from a bulk tank as well as milk from the national distributor. This outlet was

Table 1: Temperature of the milk (°C) at the time of purchase and potential hazards present in the milk.

Origin	No. of samples tested	Range (°C)	Number ≤ 5 °C (%)	ALP <sup>b</sup> positive	<i>E. coli</i> positive in 1 mℓ	S. aureus positive in 1 mℓ	Positive for inhibitory substances
Milk-shop 1	27	3.5–10.5	13 (48.1)	27	21	26	15
Milk-shop 2	27	4.0-7.5	9 (33.3)	0	1	4	25
Milk-shop 3	27	5.0-10.0	1 (3.7)	0	0	5	10
Milk-shop 4	27	6.5-11.0	0 (0)	25	2 (11)°	8	14
Milk-shop 5	27	4.5-9.0	3 (11.1)	0	0 ` ´	11	9
Supermarket 1 <sup>a</sup>	25	1.5-7.0	20 (80.0)	0	0	0	0
Supermarket 2 <sup>a</sup>	27	2.5-8.5	9 (33.3)	0	0	0	0
Supermarket 3ª	27	4.5-8.0	2 (74.4)	0	0	0	0

<sup>&</sup>lt;sup>a</sup>Milk from the same national distributor, purchased at 3 different outlets.

chosen because the management of the milk with respect to the cold chain should have been the same as both types of milk were kept in the same display cabinet. All shops were situated in the northwestern parts of Pretoria.

## Laboratory procedures

The temperature of the milk was taken within 5 minutes of purchase by decanting approximately 100~mt of milk into a separate plastic container and measuring the temperature using a calibrated electronic thermometer. The decanted milk was then discarded. The balance of the milk was kept on ice in a cool box until it was analysed in the laboratory. All microbiological analyses were carried out within 4 hours of the milk being purchased. Milk samples were kept in a household refrigerator until they were processed.

Standard procedures for the use of 3M Petrifilm aerobic count plates were used for aerobic colony count, and plates were incubated at 32 °C for 48 hours. Standard procedures for the use of the 3M Petrifilm *E. coli*/coliform count plates were used for the *E. coli* counts. An incubation temperature of 32 °C, and not 35 °C as prescribed by the Petrifilm manufacturers, was used as this was according to the method described in the Act<sup>17</sup>. Coliform counts were evaluated using 3M Petrifilm rapid coliform count plates. Single-use disposable pipettes were used for each of the serial dilutions.

The Aschaffenburg and Mullen alkaline phosphatase test was performed, using standard methods as described in the Act<sup>17</sup>. The somatic cell count was determined using the Fossomatic apparatus, using standard operating procedures. Antibiotics and other antimicrobial residues were tested for using the Brilliant Black Reduction Test (Laboratorium Enterotox, Germany) following standard procedures<sup>6</sup>. The Brucella milk ring test was used to identify *B. abortus* antibodies

in milk using the standards compiled by the South African Institute of Medical Research.

Staphylococcus aureus isolation was done on Baird Parker Agar Base. A positive colony was confirmed as being S. aureus by means of the Staphylase test (Oxoid Limited, Basingstoke, Hampshire, England). Colony-forming units were not enumerated. Discrete S. aureus colonies were subcultured onto Tryptone Soya Broth and incubated overnight at 37 °C, and subsequently tested for the presence of Staphylococcal enterotoxins A, B, C and D by means of reversed passive latex agglutination, using the SET-RPLA Staphylococcal enterotoxin test kit (Oxoid). The Staphylococcal enterotoxin test was done on all positive S. aureus cultures. Fifteen milk samples from the national distributor were also tested, 1 from each day of sampling. These samples were centrifuged for 15 minutes at 3400 rpm and the sediment was discarded. Enterotoxin detection was carried out on the supernatant.

## Data analysis

Data were analysed using the statistical computer package SAS (SAS Institute Inc., NC). Sigma Plot (Jandel Scientific) was used to generate the graphs. Data on bacterial enumerations were converted to  $\log_{10}$  values because of their non-normal distribution. Significance was accepted at P < 0.05.

## **RESULTS AND DISCUSSION**

The temperature of milk was below  $5 \,^{\circ}$ C in only 26.6 % of samples purchased (Table 1). Maintenance of the cold chain is an important factor influencing the safety and keeping quality of milk, especially in a country with a warm climate like South Africa. To delay the growth of microorganisms, it is recommended to hold the milk at  $\leq 5 \,^{\circ}$ C<sup>23</sup>. Lück *et al.*<sup>24</sup> reported that when the storage temperature is increased to  $7 \,^{\circ}$ C the standard plate count of

a milk sample after 7 days may be as much as 1000 times higher than on a comparable sample stored at 4-5 °C. Gruetzmacher and Bradley<sup>18</sup> cited several authors who found that a 3 °C rise in temperature decreases the shelf-life of milk by half. The normal cold chain can, however, only contribute to a limited improvement of the shelf-life of pasteurised milk when the products contain large numbers of postprocessing contaminants which grow at cold chain temperatures<sup>23</sup>. At elevated temperatures the growth of pathogenic organisms such as *S. aureus, Bacillus* spp. and enterotoxin-producing E. coli is increased and can therefore cause health hazards<sup>23</sup>.

Fifty-two of the 135 milk-shop milk samples tested (38.5 %), were alkaline phosphatase positive indicating inadequate pasteurisation (Table 1)<sup>27</sup>. One of the 2 milk-shops with alkaline phosphatase positive samples, had no negative alkaline phosphatase results over the entire 6-week period. Significantly, all the milk-shops in the study had a High-Temperature-Short-Time (HTST) pasteuriser present and displayed 'Pasteurised milk' signs. The national distributor's milk was always alkaline phosphatase negative. Milk-shops 1 and 4 either did not pasteurise at all or the pasteuriser did not work efficiently. The fault in pasteurisation was an ongoing problem over a 6-week period. The Act states that if pasteurisation is carried out according to the high-temperature short-time method, thermographic recordings of pasteurisation temperatures must be made and kept for at least 4 weeks, and the apparatus used must be calibrated monthly<sup>17</sup>. A positive alkaline phosphatase result may also indicate the possible addition of raw milk to pasteurised milk or reactivation of the phosphatase enzyme by high bacterial numbers in the milk.

Standard plate counts or total aerobic colony counts are used to estimate viable bacterial populations in the pasteurised

<sup>&</sup>lt;sup>b</sup>ALP = alkaline phosphatase.

cE. coli suspect.

milk and reflect the hygienic practices used in the production, processing and handling of the milk<sup>22</sup>. They give a crude indication of the milk's shelf-life. Figure 1 shows that the standard aerobic plate count for milk-shop milk (n = 129) varied greatly over the 6-week sampling period. Counts ranged from  $1.0 \times 10^2$  to  $2.66 \times$  $10^7$  cfu/m $\ell$ , with a median value of 41 000 cfu/m $\ell$  (legal limit < 50 000 cfu/m $\ell^{17}$ ). Individual samples, however, showed that 74 % of samples had counts lower than 50 000 cfu/ml and were therefore within the legal limits. Standard aerobic plate counts for the national distributor's milk (n = 79) varied from 700 to 8700 cfu/mℓ with a median count of 2200 (Fig. 1). The standard plate counts of milk from Milk-shop 3 and from Supermarket 3 differed significantly from each other, indicating that the origin and treatment of the milk is important in determining its quality.

Coliform counts in milk-shop milk (n =129) varied greatly between milk-shops over the 6-week period, ranging from 0 to  $3.4 \times 10^5$  coliforms per m $\ell$  (Fig. 2), with 88 (68 %) samples having counts lower than 20 coliforms per ml, which is the maximum number allowed when the Petrifilm method of counting is used. The median value for milk-shop milk was 30 coliforms per ml. However, if one excludes the 2 milk-shops which probably sold raw milk, the median coliform count in the remaining milk-shops was below the 20 coliforms per m $\ell$  limit allowed for in the Act<sup>17</sup>. Nevertheless, milk-shop owners need to be made more aware of basic hygiene measures when handling the milk, as coliforms are destroyed by pasteurisation, and therefore their presence after correct pasteurisation is indicative of bacterial contamination post-pasteurisation  $^{10,38}$ . In Milk-shops 1 and 4, the coliform counts ranged from 51 to 9000/ml and from 0 to 34 000/ml, respectively. The other milk-shops which pasteurised correctly had variations between 0 and 1100 coliforms per m $\ell$ . The results showed that there was a significant difference in the coliform count between those shops that pasteurised and those that did not. Coliform counts for the national distributor's milk were always zero (Fig. 2).

Escherichia coli is a faecal indicator organism, whose recovery from milk suggests that other organisms of faecal origin, including pathogens such as Salmonella and Campylobacter, may also be present<sup>10</sup>. It may also be isolated from the milk of mastitic animals. Out of 135 milk-shop milk samples tested for E. coli, 24 (17.7 %) were positive in 1 m $\ell$ , and a further 11 (8.1 %) were suspect for the organism (Table 1). Over 95 % of isolates

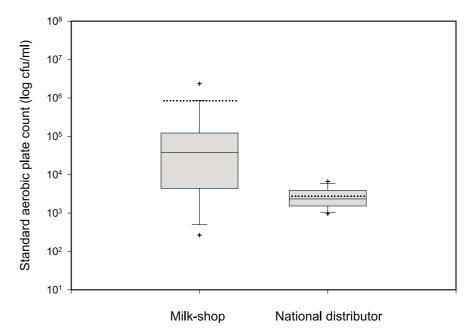


Fig. 1: Standard aerobic colony counts of milk-shop milk and that of a national distributor.

originated from milk which was alkaline phosphatase positive. Of the 27 samples of milk purchased from Milk-shop 1, 21 (77.8 %) were E. coli positive. Unfortunately, on many of the plates containing 1 ml of undiluted milk from Milk-shop 4, it was impossible to accurately determine whether or not *E. coli* was present. These plates contained so many coliforms that all that could be observed were very large gas bubbles under the film. These were considered suspect samples. This is a drawback of the dry rehydrated film method for coliform and E. coli counts, since high coliform numbers obliterate E. coli organisms. Other methods such as the Modified Eijkman Test for E. coli, although more laborious and time-consuming to perform, might be more useful

in such cases. Milk-shop 4 sold 14 (51.9 %) samples which were *E. coli* negative. The remaining thirteen (48.1 %) samples were either positive or suspected to be positive for E. coli. The high prevalence of E. coli in Milk-shops 1 and 4 is possible since the milk from these 2 milk-shops was not pasteurised correctly<sup>21,32</sup>. Milk-shop 2 sold 1 sample that was positive for E. coli (Table 1), possibly indicating human contamination after pasteurisation by handlers who practice poor personal hygiene or by contact with water containing sewage. The national distributor's milk was always negative for E. coli in 1 ml (Table 1).

Fifty-four (40 %) of all milk-shop milk samples purchased contained the organism *S. aureus* in 1 mt (Table 1). One third

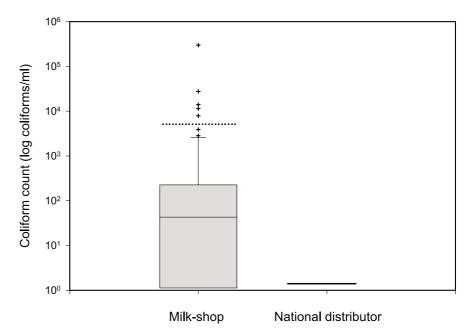


Fig. 2: Coliform counts of milk-shop milk and that of a national distributor.

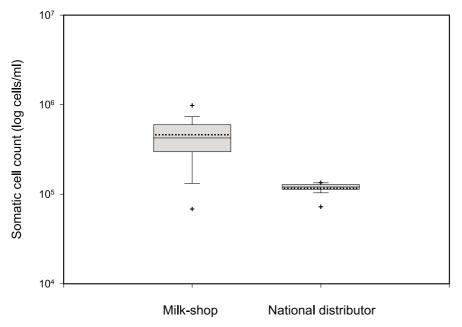


Fig. 3: Somatic cell counts of milk-shop milk and that of a national distributor.

of these organisms was found in correctly pasteurised milk and the other two-thirds in milk which was not correctly pasteurised. S. aureus in the latter group may have originated from animals with subclinical mastitis, as S. aureus is the dominant mastitis organism in South Africa, being prevalent in at least 75 % of South African herds<sup>16,35</sup>. S. aureus in raw milk may also have originated from human carriers. Where the organism was isolated from milk which had been correctly pasteurised, it must have originated from the people who handle the milk, since this organism is destroyed by pasteurisation<sup>5</sup>. Surveys have shown that up to 60 % of humans are nasal carriers of this organism, and that between 5 % and 20 % of people carry the organism as part of their normal skin flora<sup>5</sup>. The national distributor's milk did not contain any S. aureus (Table 1).

If milk is not refrigerated, several strains of S. aureus can produce heat-stable enterotoxins that survive the pasteurisation process and cause food poisoning in man<sup>14</sup>. Of the 51 S. aureus-positive cultures which were tested for the production of enterotoxins, 4 (7.83 %) produced heat-stable staphylococcal enterotoxins A (SEA), B (SEB), D (SED) or a combination of these. All the toxin producing strains isolated originated from Milkshop 1. SEA/SEB was produced by 2 S. aureus strains and SEA/SEB/SED by the other 2 strains. Bolstridge and Roth<sup>7</sup> reported that 18.9 % of S. aureus isolates from both raw and processed dairy products purchased in South Africa were found to be enterotoxigenic, with most producing enterotoxins A or C or a combination of A and C. Most food poisoning outbreaks involve enterotoxins A and D

as they are produced under a much wider range of environmental conditions than B and  $C^5$ . No *S. aureus* enterotoxin could be detected in 15 national-distributor milk samples tested. The production of enterotoxin by staphylococci can be completely managed by temperature control as multiplication of the bacteria and toxin formation are almost completely inhibited below 7 °C $^5$ .

Of public health importance was the fact that 73 of 135 (54 %) milk-shop milk samples purchased contained some type of inhibitory substance (Table 1). Residues are illegal in terms of the Act<sup>17</sup>. Since the milk was not analysed further to determine which substances were present they could consist of antibiotics or other antimicrobials such as formalin or hydrogen peroxide which may have been (illegally) added to the milk to increase the shelf-life. The results showed that the national distributor's milk never contained any inhibitory substances (Table 1). The prevalence of inhibitory substances in milk-shop milk was high, ranging from 33.3 % in Milk-shop 5 to 92.6 % in Milk-

The Act<sup>17</sup> states that milk should not contain any inflammatory product which may render the milk unfit for human consumption. Cows in very early or very late lactation, or cows with a low-grade or latent udder infection, are likely to produce milk containing an excessive number of somatic cells, consisting mainly of leucocytes and some epithelial cells<sup>20</sup>. Milk-shop milk somatic cell counts varied between  $1.2 \times 10^4$  and  $1.6 \times 10^6$  cells per m $\ell$ , with a median count of  $4.2 \times 10^5$  cells (Fig. 3). Only 18.7 % (25 of 135 samples) of somatic cell counts were above the legal limit of 500 000 cells/m $\ell$ . The

national distributor's milk always had somatic cell counts of less than 150 000 cells per m $\ell$  (Fig. 3) and differed significantly from all the milk-shops except for Milk-shop 4. Somatic-cell counts are decreased in the clarifying process which is done at larger dairies and processing plants, and this may be the reason why the somatic cell count of the national distributor were so constant and so low over the 6-week period.

All milk samples tested by means of the brucella milk ring test (BMRT) were negative for antibodies to *Brucella abortus* which is a zoonosis and has not yet been eradicated from cattle in South Africa. Commercial pasteurisation effectively kills *B. abortus* <sup>15,32</sup>. As all milk samples were tested at least 2–3 times per week it is unlikely that there could have been false negatives.

Seventeen *E. coli*-positive samples were further tested for the presence of *Salmonella* spp. in 1 m*l*, but these samples were all negative for the organism.

## **CONCLUSIONS**

In conclusion, of the 135 pasteurised milk samples purchased from milk-shops, 117 (87 %) were not fit for human consumption on the basis of all the criteria laid down in the Foodstuffs, Cosmetics and Disinfectants Act<sup>17</sup>. Milk-shop 1 never sold milk that was fit for human consumption, whereas the remaining 4 milk-shops, only complied with the Act between 4 % and 33 % of the time. All of the 79 samples purchased from a large national distributor passed all the criteria laid down in the Act.

The results showed that milk-shop milk differed significantly from the milk that originated from the national distributor and varied greatly between milk-shops and between sampling days over the 6-week period. Consumers are therefore unwittingly exposed to unnecessary health risks by drinking unsafe milk. These findings are similar to those found after a survey in South Africa in 1995 by the Department of Health<sup>11</sup> which concluded that 73 % of pasteurised milk samples did not comply with all the regulations. Their results included the milk of national distributors. In this study it was found that all the samples purchased from the national distributor consistently passed all the criteria laid down in the Act, and therefore samples that were obtained from national distributors in the national study may have improved the results to some extent.

The fact that nearly 40 % of milk samples were most probably incorrectly pasteurised, and the high prevalence of *E. coli* and *S. aureus* in these raw milk samples proves

the greater risk of raw milk. Susceptibility to food-borne pathogens varies greatly from person to person. High risk people who may be particularly susceptible to infection include immunocompromised people whose immune systems are deficient either because of an immunodeficiency disorder or because of treatment with immunosuppressive drugs<sup>37</sup>. These would include pregnant women, transplant recipients, AIDS and cancer patients, very young infants, steroid users, and patients with chronic renal disease<sup>12</sup>. South Africa has a high prevalence of HIV-positive people and milk-shop milk could be a real hazard to their health. Not only can unsafe milk affect the health of the consumer, but it may also have economic implications such as medical and hospitalisation costs, mortality costs, productivity losses, and the long-term reduction in quality of life. This could place a burden on primary health care services, the employers and employees due to absenteeism.

To produce safe, sound and wholesome milk for the consumer entails good production practices throughout the chain from the cow to the consumer. This includes the milking of healthy animals, the use of clean and hygienic equipment on the farm and during processing, maintenance of the cold chain throughout the production process, effective pasteurisation and prevention of post-pasteurisation contamination. People handling milk should be educated in safe food-handling techniques and proper personal hygiene practices including hand washing after using the lavatory. Training programmes for staff working in milk-shops is essential as these people work with food and are often ignorant of basic hygiene principles. Milk-shop owners (and dairy farmers) should institute hygiene programmes on the farm and in the shop that should consist of good manufacturing processes, quality control, hazard analysis and critical control point (HACCP) principles. There is also a need for more stringent control over milk-shops by the relevant authorities. Questions must be asked as to whether or not the local authority ever analysed the milk and if so, why they did not do anything about the results. A suggestion might be that people who work with perishable foods such as milk or meat that could affect the health of the consumer, would need to undergo some type of compulsory training before being able to work in a specific field, and that this training would include a component on the regulations concerning that industry as well as some knowledge of the processes involved. Public health aspects should also be part of the training. However, public education is also needed as legislation alone is insufficient.

## **ACKNOWLEDGEMENTS**

I thank Prof. C M Veary for his helpful comments on the manuscript, Prof. P J Jooste for technical advice on the project, Prof. GJJ van Zyl and Mrs Rina Owen for statistical analysis and Mr J J T K van der Toorn for advice and assistance in the preparation and carrying out of the research work. My sincere thanks to the University of Pretoria and the National Research Foundation for financial assistance, to Ms Karin Betz of 3M for the donation of Petrifilms, to Oxoid Products for the donation of the Staphylococcal enterotoxin test kits and to Rhine Ruhr for the donation of the Brilliant Black Reduction tests.

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