A COMPARISON OF SELECTED PUBLIC HEALTH CRITERIA IN MILK FROM MILK-SHOPS AND FROM A NATIONAL DISTRIBUTOR

by

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Submitted in partial fulfilment of

the requirements for the degree of

M.Med.Vet. (Hyg.)

in the

Faculty of Veterinary Science,

University of Pretoria

PRETORIA

MARCH 2000

"Milk is nearly a perfect food" - Hippocrates

Dedicated to:

my husband, RORY and our son, SEBASTIAN

ACKNOWLEDGEMENTS

The help, support and co-operation of a number of people have made this work possible. I express my sincere thanks, appreciation and gratitude to the following people and organisations:

My husband, Rory, for the many hours of help in the laboratory, as well as for his infinite patience, support, encouragement and assistance during the my post-graduate studies and at all other times;

Professor C M Veary, my promoter, for his valuable advice, guidance and encouragement during this project and throughout my post-graduate studies;

Mr JJTK van der Toorn for his invaluable advice and assistance in the preparation and carrying out of the research work, as well as for the data input and help with analysing the data. Special thanks too for his efforts in obtaining donations from the various companies;

Professor P J Jooste, my co-promoter, for his invaluable advice and input in the writing of the protocol and dissertation;

Professor A Guthrie for his assistance in the design of the study;

Ms Jane Nurton for her encouragement and support in acquiring the skills to use a new computer programme;

Mrs Antoinette Lourens for her help in obtaining numerous journal articles from other libraries both nationally and internationally;

Professor G J J van Zyl and Mrs Rina Owen for their assistance with the statistics and statistical analyses;

My sincere thanks to the University of Pretoria, Faculty of Veterinary Science and the National Research Foundation (previously the Foundation for Research and Development (FRD)) for the financial assistance which was received for this research project;

My sincere thanks to Ms Karin Betz of 3M for the donation of Petrifilms, to Oxoid products for the donation of the Staphylococcal and *Escherichia coli* enterotoxin test kits and to Rhine Ruhr for the donation of the Brilliant Black Reduction tests. Their donations of laboratory material was very much appreciated; and

Finally, I thank my parents, Günther and Christina, who provided me with a sound educational foundation and supported me throughout my studies.

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SUMMARY

A COMPARISON OF SELECTED PUBLIC HEALTH CRITERIA IN MILK FROM MILK-SHOPS AND FROM A NATIONAL DISTRIBUTOR

by

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Selected public health criteria of pasteurised milk available to the consumer from milk-shops in a selected area of Pretoria compared with a national distributor's milk were evaluated. Pasteurised milk samples were obtained from five randomly selected milk-shops in the north-western part of Pretoria over a six-week period from June to August 1998. Milk from a well-known national distributor was also obtained from three supermarkets in the same area during the same time period to act as the control milk.

Mean total aerobic bacterial counts, coliform counts and psychrotrophic bacterial counts were determined. The presence of *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Salmonella* species, staphylococcal enterotoxins, inhibitory substances, alkaline phosphatase and somatic cells in milk were also determined. 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 (Act 54 of 1972). In contrast, 100% of the 79 control milk samples passed all the safety criteria laid down in the Act.

Milk-shop milk quality varied between milk-shops and between sampling days. All milk-shop milk was sold as having been pasteurised, yet 38.5% of milk samples purchased failed the alkaline phosphatase test, indicating that they had not been pasteurised correctly or were contaminated with raw milk. The total aerobic plate counts were generally high for all milk-shop milk samples ranging from 1.0×10^2 to 2.7×10^7 CFU/mℓ with a median value of 41 000 CFU/mℓ, whereas for the control milk it ranged from 7.0×10^2 to 8.7×10^3 CFU/mℓ, with a median value of 2 200 CFU/mℓ. Coliform counts varied from 0 to 3.4×10^4 per mℓ in milk-shop milk, with 68% of samples having counts lower than 20 coliforms/mℓ, which is the maximum number allowed when the Petrifilm method of counting is used. Coliforms could not be detected in 1 mℓ of control milk samples. *E. 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 mℓ in any of the *E. coli*-positive milk tested.

Psychrotrophic bacterial counts done after pre-incubation of milk-shop milk were extremely high, and ranged from 3×10^5 to 2.2×10^8 CFU/mℓ, with a median value of 2.4×10^7 CFU/mℓ. In Europe the psychrotrophic count may not be greater than 100 000 CFU/mℓ. None of the milk-shop milk passed this European standard, whereas 98.7% of the milk obtained from the national distributor fell within the prescribed parameters.

S. aureus was isolated from 54 (40%) milk-shop milk samples, and four (7.8%) of 51 isolates tested produced staphylococcal enterotoxins A (SEA), B (SEB), D (SED) or a combination. Control milk did not contain any *S. aureus* and 15 milk samples tested for the enterotoxin gave a negative result.

All control milk was negative for inhibitory substances, but these were detected in 54.1% of milk-shop milk. Somatic cell counts varied between 1.2×10^4 and 1.6×10^6 cells/mℓ in the milk-shop milk, with a median count of 4.2×10^5 cells/mℓ. Only 18.7% of samples had counts above the legal limit of 500 000 cells/mℓ. The national distributor's milk always had counts less than 150 000 cells/mℓ.

The results showed that milk-shop milk differed significantly ($p < 0.05$) from the national distributor's milk, and that the quality of milk purchased from milk-shop outlets was generally of a poor bacteriological quality. 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 posed a serious public health risk to consumers.

SAMEVATTING

'n VERGELYKING VAN GESELEKTEERDE VOLKSGESONDHEIDSMAATSTAWWE VAN MELK VANAF MELKWINKELS EN VANAF 'n NASIONALE VERSPREIDER

deur

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Geselekteerde volksgesondheidsmaatstawwe vir gepasteuriseerde melk beskikbaar aan verbruikers vanaf melkwinkels in 'n uitgesoekte gebied van Pretoria, en vergelyk met die melk van 'n nasionale melkverspreider, is geëvalueer. Gepasteuriseerde melkmonsters vanaf vyf ewekansig geselekteerde melkwinkels in die noord-westelike deel van Pretoria, is oor 'n ses-weke periode vanaf Junie tot Augustus 1998 verkry. Melk, vanaf 'n bekende nasionale melkverspreider, is ook vanaf drie selfbedieningswinkels in dieselfde gebied en oor dieselfde tydperk verkry om as kontrolemelk te dien.

Die gemiddelde totale aërobiese bakteriese telling, kolivormtellings en psigrotrofiese bakteriese tellings is bepaal. Die teenwoordigheid van *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spesies, stafilokokkusenterotoksiene, inhiberende middels, alkaliese fosfatase en somatiese selle in melk is ook bepaal. Uit 135 melkmonsters wat vanaf melkwinkels aangekoop is, was 87% op grond van die minimum standaarde voorgeskryf in die Wet op Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels, (Wet 54 van

1972), nie geskik vir menslike verbruik nie. In vergelyking het 100% van die 79 kontrole melkmonsters in al die veiligheidsbepalings neergelê in die Wet geslaag.

Die kwaliteit van melk verskil tussen melkwinkels en ook tussen bemonsteringsdae. Alhoewel al die melkwinkels melk as gepasteuriseer verkoop het, het 38.5% van die aangekoopte melkmonsters nie die alkaliese-fosfatase toets geslaag nie, wat aandui dat dit nie doeltreffend gepasteuriseer is nie, of dit was besoedel deur ongepasteuriseerde melk. Die totale aërobiese plaattellings was oor die algemeen hoog vir al die melkwinkel melkmonsters, en het gevarieer vanaf 1.0 x 10^2 tot 2.7 x 10^7 KVE/mℓ met 'n mediaan waarde van 41 000 KVE/mℓ, terwyl dit vir die kontrolemelk tussen 7.0 x 10^2 tot 8.7 x 10^3 KVE/mℓ met 'n mediaan waarde van 2 200 KVE/mℓ was. Kolivormtellings het gewissel vanaf 0 tot 3.4 x 10^4 per mℓ in die melkwinkel melk, en 68% van die monsters het tellings laer as 20 kolivorme per mℓ getoon, wat die hoogste aantal toelaatbaar is wanneer die Petrifilm metode van telling gebruik word. Kolivorme kon nie in 1 mℓ van die kontrole melkmonsters gewaar word nie. *E. coli* is gewaar in 1 mℓ in 17% van melkwinkel melk, waarvan 95% hul oorsprong gehad het in melk wat positief vir alkaliese fosfatase was. Geen *Salmonella* spp. kon in die positiewe *E. coli* melk wat getoets is gewaar word nie.

Die psigrotrofiese bakteriese tellings na voor-inkubasie in melkwinkel melk was uiters hoog, en het gevarieer vanaf 3 x 10^5 tot 2.2 x 10^8 KVE/mℓ, met 'n mediaan waarde van 2.4 x 10^7 KVE/mℓ. In Europa mag die psigrotrofiese telling nie hoër as 100 000 KVE/mℓ wees nie. Terwyl geen melk van die melkwinkels voldoen het aan hierdie Europese standaard nie, het 98.7% van die melk aangekoop vanaf die nasionale melkverspreider binne die voorgeskrewe parameters geval.

S. aureus is in 54 (40%) van die melkwinkel melkmonsters geïsoleer, en vier (7.8%) van die 51 stamme getoets, het stafilokokkusenterotoksiene A (SEA), B (SEB), D (SED) of 'n kombinasie geproduseer. Kontrolemelk het geen *S. aureus* bevat nie, en 15 melkmonsters het negatief getoets vir die enterotoksien.

Alle kontrolemelk was negatief vir inhiberende middels, maar is in 54.1% van die melkwinkel melk gevind. Somatiese seltellings het gewissel tussen 1.2 x 10^4 en 1.6 x 10^6 selle/mℓ in die melkwinkel melk, met 'n mediaantelling van 4.2 x 10^5 selle/mℓ. Slegs 18.7% van monsters het seltellings hoër as die wettige limiet van 500 000 selle/mℓ gehad. Die melk van die nasionale verspreider het altyd tellings laer as 150 000 selle/mℓ getoon.

Die resultate toon dat die melkwinkel melk aansienlik ($p < 0.05$) verskil het van die nasionale verspreider se melk, en dat die kwaliteit van die melk aangekoop van melkwinkels in die reël van 'n swak bakteriologiese kwaliteit was. Die teenwoordigheid van die inhiberende middels en die isolasie van *E. coli* en *S. aureus* (waarvan sommiges enterotoksiene geproduseer het) is aanduidend van moontlike, ongesonde melk, en hou die gevaar in van 'n ernstige volksgesondheidsrisiko vir die verbruiker.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

BACKGROUND

Cow's milk has long been considered a highly nutritious and valuable human food, and is consumed by millions daily in a variety of different products. Its nutrient composition makes it an ideal medium for bacterial growth, and therefore it can be considered one of the most perishable agricultural products because it can so very easily be contaminated (Bryan 1983, Bramley & McKinnon 1990, Heeschen 1994). Many contaminating organisms only spoil the product, thereby reducing its shelf-life. Some, such as lactic acid bacteria, are useful in milk processing, causing milk to sour naturally. Other bacteria, such as those listed in Table 1, are pathogenic to man and can transmit disease if the milk is consumed untreated (Sharp *et al.* 1985, Heeschen 1994). Unlike meat and meat products, milk is less likely to be subjected to any subsequent heating by the consumer before consumption and contaminated milk is therefore potentially more dangerous (Steele *et al.* 1997). The high fat content of milk protects pathogens against gastric acid, while its fluid nature ensures a fairly short retention time in the stomach (Potter *et al.* 1984, Sharp *et al.* 1985).

Raw milk of good hygienic quality is necessary to produce milk products of good quality and adequate shelf-life and to provide a safe, sound and wholesome food for the consumer. Since milk is a liquid, it is in contact with some type of equipment or surface from the time it is removed from the cow until it is consumed. Milk freshly drawn from a disease-free udder contains small numbers of bacteria (500 to 1 000 bacteria per mℓ) which derive from organisms colonizing the teat canal (Bramley & McKinnon 1990). Milk quality starts to deteriorate immediately after milking due to bacteria entering the milk from a wide variety of sources. These bacteria may originate from soil, water and faeces that collect on the skin of the cow and unavoidably end up in the milk. Once micro-organisms get into the milk they multiply rapidly. The speed at which milk quality declines depends on the hygiene of the

milker, milking equipment and bulk tank, as well as the temperature and length of time that milk is stored before sale to the consumer or treatment at a factory (Lück 1986). Microbial growth can be controlled by cooling the milk, as most micro-organisms reproduce more slowly in colder environments.

Pathogenic bacteria may also be present in raw milk as a direct consequence of clinical or subclinical mastitis (Giesecke *et al.* 1994). In 1989 Giesecke *et al.* reported that subclinical mastitis was prevalent in at least 75.5% of South African dairy herds which were affected at levels ranging from moderate to very serious. Mastitis affects a variety of compositional parameters of milk which in turn may affect the dairy technological usefulness, the nutritional and hygienic characteristics of milk (Giesecke *et al.* 1994). Among the organisms commonly producing mastitis, *Streptococcus agalactiae*, *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* are pathogenic for man (Bramley & McKinnon 1990).

PATHOGENS FOUND IN MILK

There have been numerous outbreaks of milk-borne disease in humans with pathogens such as *S. aureus, E. coli, Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. being incriminated during the past century, especially since mass production came into effect (Bryan 1983, Vasavada 1988). Most of these 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 system or post-pasteurisation contamination (Porter & Reid 1980, Fahey *et al.* 1995). Raw milk may contain micro-organisms pathogenic to man which originated either from within or outside the udder (see Table 1).

Human carriers may also be the source of infection in milk-borne outbreaks, as reported for *Salmonella* infections, and for cases of scarlet fever or septic sore throat due to *Streptococcus pyogenes* (Bryan 1983, Bramley & McKinnon 1990). Fortunately, all these pathogens can be destroyed by pasteurisation, but problems arise if the milk is contaminated after the heat process (Bramley & McKinnon 1990, D'Aoust *et al.* 1988).

Table 1: Diseases transmissible to man through milk *(Source: Heeschen, 1994)*

Disease	Principal Sources of Infection		
	Man	Milk Animal	Environment
BACTERIAL			
Anthrax*		●	●
Botulism (toxin)			●
Brucellosis		●	
Cholera	●		
Coli infections (pathogenic strains of *E. coli*)	●	●	
Clostridium perfringens (welchii) infection			●
Diphtheria	●		
Enteritis* (non-specific, from large numbers of killed or living coli, proteus, pseudomonas, etc.)			●
Leptospirosis*		●	
Listeriosis*		●	
Paratyphoid fever	●	●	
Rat-bite fever	●	●	
Salmonellosis (other than typhoid and paratyphoid fevers)	●		
Shigellosis	●	●	
Staphylococcal enterotoxic gastroenteritis	●	●	
Streptococcal infections	●	●	
Tuberculosis	●	●	
Typhoid fever	●		
VIRAL			
Infections with adenoviruses*	●		
Infections with enteroviruses (including polioviruses and the Coxsackie groups)	●		
Foot-and-mouth disease		●	
Infectious hepatitis*	●		
Tick-borne encephalitis		●	
RICKETTSIAL			
Q fever		●	
PROTOZOAL			
Amoebiasis*	●		
Balantidiasis*	●		●
Giardiasis*	●		
Toxoplasmosis*		●	

* *Not conclusively incriminated as milk-borne, but epidemiologically probable or suspect.*

The most important and serious human diseases resulting from the consumption of contaminated raw milk are tuberculosis and brucellosis (Bramley & McKinnon 1990). In both diseases the causative organism, *Mycobacterium bovis* and *Brucella abortus* respectively, may be excreted in the milk from infected animals. Often with *Brucella* infections, there is little change in the composition of the milk or udder tissue, i.e. mastitis is not present. Under normal circumstances, pasteurisation destroys both *Mycobacterium bovis* and *Brucella abortus*, so rendering the milk safe for consumption.

In South Africa, *Staphylococcus aureus* has been found to be the dominant mastitis-associated organism (Swartz *et al.* 1984, I M Petzer, Faculty of Veterinary Science, Onderstepoort, pers. comm. 1998, L Fourie, Ermelo Provincial Veterinary Laboratory, pers. comm. 1998). Staphylococcal mastitis of the cow poses a direct threat to public health, because a proportion of bovine strains produce enterotoxins (Asperger 1994). Consumption of food containing *S. aureus* enterotoxin leads to food poisoning (Bryan 1983). As the enterotoxin is heat stable, subsequent pasteurisation of the toxin contaminated milk or any heat treatment attempted by the consumer will not make it safe for consumption. Staphylococcal enterotoxin formation can be prevented by cooling the raw milk timeously, maintaining the cold chain and then effectively pasteurising the product (Asperger 1994).

S. aureus may also be present in the milk due to post-pasteurisation contamination. This can occur if people involved with the processing of the milk have colds, skin infections, diarrhoea or stomach disorders (Bryan 1983). Different surveys have shown that between 4% and 60% of humans are nasal carriers of *S. aureus*, and that between 5% to 20% of people carry the organism as part of the normal skin flora (Asperger 1994). A study done in South Africa in 1985 found that 18.9% of all *S. aureus* isolates from milk were toxigenic (Bolstridge & Roth 1985).

Numerous outbreaks of enteritis caused by *Campylobacter jejuni* (*C. jejuni*) have been associated with the consumption of unpasteurised cow's milk (Porter & Reid 1980, Jones *et al.* 1981, Taylor *et al.* 1982, Finch & Blake 1985, Hutchinson *et al.* 1985, Kornblatt *et al.* 1985, Klein *et al.* 1986). This organism can be isolated from the faeces of cattle infected or

colonized with the bacteria (Svedhem & Kaijser 1981, Oosterom *et al.* 1982, Potter *et al.* 1983, Humphrey & Beckett 1987), and has been shown to cause asymptomatic bovine mastitis in which the organism is excreted directly through the milk of an infected cow (Hudson *et al.* 1984, Hutchinson *et al.* 1985, Morgan *et al.* 1985, Orr *et al.* 1995). In most outbreaks however, *Campylobacter* could not be isolated from the milk after an outbreak (Porter & Reid 1980, Jones *et al.* 1981, Taylor *et al.* 1982, Potter *et al.* 1983, Finch & Blake 1985, Kornblatt *et al.* 1985, Fahey *et al.* 1995). Nevertheless, a survey done in England in 1988 showed that 5.9% of raw milk samples were positive for *C. jejuni* (Humphrey & Hart 1988). It was also found that there was a significant association between the presence of *E. coli* in the milk and that of *C. jejuni* (Humphrey & Hart 1988).

Campylobacters can produce symptomless and persistent infection or colonisation in milking herds without any detectable contamination of the milk (Robinson & Jones 1981), and the excretion of *C. jejuni* can be intermittent (Humphrey & Beckett 1987). *C. jejuni* does not multiply in the milk, but can survive for at least 24 hours at room temperature, and for up to three weeks at 4 °C (Doyle & Roman 1982, de Boer *et al.* 1984). *C. jejuni* is killed by proper pasteurisation (Gill *et al.* 1981, D'Aoust *et al.* 1988).

Outbreaks involving inadequately pasteurised milk have been described in England (Porter & Reid 1980, Fahey *et al.* 1995). Failure in the public electricity supply and a faulty pasteuriser were identified as the causes of the problem. In the case of the faulty pasteuriser, the indicator lights showed that the pasteurisation process was under way, even though the milk was not being heated to a high enough temperature to ensure complete pasteurisation (Fahey *et al.* 1995).

In developing countries, including South Africa, *C. jejuni* infection has been shown to be hyperendemic, with an age-related decrease in incidence of infection (de Mol & Bosmans 1978, Bokkenheuser *et al.* 1979, Glass *et al.* 1983). Acquired immunity could be important in preventing infection or preventing illness after infection (Blaser *et al.* 1987). Nevertheless, immuno-compromised people are at risk of contracting the infection (Johnson *et al.* 1984).

Faecal contamination of unpasteurised milk, a failure in the pasteurisation of milk and post-pasteurisation contamination of milk have all been associated with *E. coli* 0157:H7 outbreaks (Chapman *et al.* 1993, Upton & Coia 1994, Tast *et al.* 1997). *E. coli* 0157:H7 has been associated with haemorrhagic colitis and haemolytic uraemic syndrome (HUS) (Rea & Fleming 1994, Tast *et al.* 1997). *E. coli* 0157:H7 will not survive high-temperature short-time pasteurisation, but if inadequate pasteurisation or post-pasteurisation contamination does take place, *E. coli* 0157:H7 can grow in milk at 8 °C, which is not an uncommon temperature for the holding of refrigerated milk in consumer's homes (Wang *et al.* 1997).

Unpasteurised milk has also been implicated in outbreaks of human salmonellosis (Marth 1969, Bryan 1983, Barrett 1986, Ryan *et al.* 1987, El-Gazzar & Marth 1992). Salmonellas usually contaminate milk as a result of faecal contamination. The largest outbreak of salmonellosis ever identified in the United States involved between 168 791 and 197 581 people, mostly children, and was traced back to pasteurised milk (Ryan *et al.* 1987). A strain had persisted in the pasteurising plant and had repeatedly contaminated milk after pasteurisation.

Susceptibility of food-borne pathogens varies greatly from person to person. Milk often is an important component of the diets of the young and the elderly and, unfortunately, young children, the elderly, pregnant women and the immuno-compromised are most at risk from food-borne pathogens (Wang *et al.* 1997). The immune systems of these groups of individuals are often not sufficiently responsive to prevent infection by pathogenic bacteria (Johnson *et al.* 1984, Wang *et al.* 1997). For these reasons greater emphasis should be placed on the safety of milk. However, food-borne pathogens can also cause disease in all other segments of the population, as they do not only lead to the classic, acute syndromes, but may often result in serious chronic sequelae such as cholecystitis, colitis, endocarditis, meningitis, myocarditis, septicaemia, haemolytic-uraemic syndrome and pancreatitis (Mossel 1987).

EPIDEMIOLOGY OF MILK-BORNE DISEASES

No appropriate epidemiological statistics on milk-borne diseases in South Africa are readily available. Unless data were to become available to prove to the contrary, it seems realistic to assume that milk-borne diseases are probably at least as prevalent in South Africa as in other countries where there is 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. Even if this milk is later pasteurised, the process will not guarantee a perfectly safe, sound and wholesome product as the bacteriological quality of the raw product has an influence on the shelf-life of the finished product (Lück *et al.* 1977, Antila 1982). Similar results to those of the other developing countries may be expected on some South African dairy farms, especially the smaller ones, who sell their milk to the milk-shops as their milk does not qualify for sale to the large distributors.

Surveys of raw milk samples in Trinidad showed that they were of a poor bacteriological quality. Between 20% and 75% were positive for *E. coli* and between 94% and 100% contained *S. aureus* of which 8% to 40% produced enterotoxins (Adesiyun 1994, Adesiyun *et al.* 1995).

In Kenya, *S. aureus* strains were isolated from 183 out of 300 raw milk samples collected. Ninety-seven of these 183 strains were assayed for the production of enterotoxins, and 74.2% of them were found to be enterotoxigenic (Ombui *et al.* 1992).

Surveys of raw milk purchased from street vendors and dairy shops in Egypt all showed high total colony and coliform counts, indicating contamination in the various stages of production and handling (Aboul-Kheir *et al.* 1986, Morgan *et al.* 1989, Abd El-Ghani 1993). These authors showed that between 27% and 61% of samples were contaminated with *E. coli* and 65% of samples contained staphylococci.

In Thailand, raw milk obtained from farms and collection centres, also showed poor bacteriological results with respect to total bacterial counts and coliform counts (Saitanu *et al.* 1996).

A study done on the milk from smallholder farmers in Zimbabwe showed satisfactory results on the standard plate count, with seven out of ten samples having counts of less than 100 000 colony forming units (CFU) per mℓ (Mutukumira *et al.* 1996). Coliforms were however, present in large numbers, and as *E. coli* was not specifically looked for, these coliforms may also have been faecal in origin.

Pasteurisation is the most common process used to destroy bacteria in milk. In pasteurisation, the milk is heated to a temperature sufficient to kill all pathogenic bacteria and most spoilage organisms. Correct pasteurisation reduces the prevalence of diseases generally associated with raw milk, especially raw milk produced and handled under unhygienic conditions (Holsinger *et al.* 1997). Thermally treated milk has, however, also been implicated as a source of human illness where inadequate pasteurisation or post-pasteurisation contamination has taken place (Porter & Reid 1980, Upton & Coia 1984, Fahey *et al.* 1995). Therefore the most important control measures to ensure milk safety are proper pasteurisation and avoiding post-pasteurisation contamination.

MARKETING OF MILK IN SOUTH AFRICA

Statistics on milk sales show that 60% of all dairy products are sold through hypermarkets, supermarkets and chain stores. Smaller grocery stores and cafes distribute some 35% of dairy products whilst the remaining 5% are direct milk sales from "milk-shops" (Theron 1997). Large national distributors such as Clover, Dairybelle and Parmalat base their payments to the farmer on milk received not only on the volume produced, but also on bacterial and somatic cell counts, as well as compositional quality standards such as butterfat and protein levels. Table 2 shows the criteria set by one of the distributors for the payment of milk (Jooste 1996).

Table 2: Criteria for the payment of milk set by one of the national distributors

Standard Plate Count (aerobic)	
<20 000 CFU/ mℓ	a premium is paid
20 000 to 50 000 CFU/ mℓ	no premium, no penalty
50 000 to 200 000 CFU/mℓ	3c per ℓ penalty
>200 000 CFU/mℓ	6c per ℓ penalty
Somatic Cell Count	
< 200 000 cells/mℓ	premium of 2c per ℓ
250 000 to 1 million cells/mℓ	no premium, no penalty
> 1 million cells/mℓ	penalty of 2c per ℓ

All milk is tested as it arrives at the distributor by means of the so-called "platform tests". On arrival, the temperature of the milk in the tanker is taken, which must be below 7°C. The tanker milk also undergoes a test to measure the freezing point of the milk to determine whether or not water was added to the milk. The bacterial quality is measured by means of the standard plate count and the milk is tested for the presence of inhibitory substances. Every individual supplier's milk also undergoes tests to determine the total bacterial count, the somatic cell count, butterfat, protein and lactose levels, as well as the freezing point of the milk. Large distributors usually have their own laboratories to do the quality control tests on the milk.

MILK-SHOPS IN SOUTH AFRICA

Since the first free elections in South Africa in 1994, the South African economy has undergone a fundamental restructuring. This was encouraged by a number of factors, the most important of which were:

1. the opening up of South African business which led to stiff competition on the world markets. This forced companies to "right size" so as to compete internationally and to become "world class". This was seen in Pretoria where ISCOR (Iron and Steel

Corporation) shut down its loss making operations, which led to a large number of retrenchments.

2. the army which the new Government inherited was enormous and sapping vital cash reserves, which it (the Government) felt could be better utilized in the area of health and education. A concerted effort was made to reduce the size of the military and one way was to offer retrenchments packages. As Pretoria had a number of military units in quite close proximity, there were a number of military personnel who accepted these packages on offer.

3. one of the key economic polices of the Government was to reduce the inflation rate in South Africa so as to bring it closer to that of South Africa's trading partners, and thus to relieve the pressure on South Africa's currency, the Rand. One way to achieve this was to be fairly aggressive in curtailing the supply of money available to the public by increasing the interest rates. This resulted in the economic growth rate slowing down to almost 0% over the last years of the 1990's. This slow down in the economic growth rate exacerbated the unemployment problem in Pretoria, as well as in the rest of the country.

Some of those people who were retrenched used that money to purchase small farms or smallholdings, and started to farm with amongst other things, dairy cattle, which can be considered a cash crop.

In the early 1990's the dairy industry was deregulated, which brought about a new, and rapidly developing practice in urban areas, especially in the lower socio-economic areas, of direct bulk milk sales to the public. These points of sale have been defined as "milk-shops" in this study. Milk-shops serve as an outlet for the relatively small amount of milk produced by the smaller farmers, and are run by farmers or businessmen who sell milk directly to the public. Consumers collect the milk in their own containers.

The premises where these milk-shops are located often range from small depots in shopping centres to fruit and vegetable shops, supermarkets and general dealers. Milk-shops have even been found in garage driveways (Jooste 1996). Many milk-shop owners do not have sufficient technical and scientific knowledge, both in dairy science and dairy microbiology, for large-scale collection and distribution of saleable milk, and often run these depots without any knowledge of milk hygiene or dairy technology.

As a result of the economic depression in South Africa, many people started looking for cheaper sources of staple products. The high prices of commercially pasteurised milk forced many consumers to buy milk from milk-shops. Virtually every household consumes milk on a daily basis as an important basic foodstuff. Few families, especially in the poorer socio-economic areas, can however afford to spend about R3.00 a day on one litre of pasteurised milk. Milk-shops became a cheap alternative to the high milk prices elsewhere. Milk-shops usually also market their products as being "fresh milk" or "fresh farm milk", giving the impression that it is full of goodness and safe!

Farmers also benefit from selling to milk-shops. At the time of this research project (June 1998), farmers received approximately R1.30 per litre if they sold their milk to one of the larger national distributors, whereas if they sold directly to a milk-shop they received approximately R1.45 per litre. Presently (January 2000), farmers receive only about R1.20 per litre from the national distributors. Another advantage to farmers of selling their milk to milk-shops, is that their milk is not analysed by the milk-shop owner, as it would have been if it had been sold to the national distributor, as milk-shops pay on volume alone and not on quality.

In Pretoria, milk-shops have, in a short period, developed rapidly. Table 3 shows the increase in the number of milk-shops situated in Pretoria from January 1996 to date.

In 1997, milk-shops in Pretoria were sampled three times per week by environmental health officers from the Pretoria Municipality. Due to severe budget restraints, milk-shops are currently only tested once a week.

A survey done by Jooste on the milk quality in South Africa in 1994 (Jooste 1996), found that pasteurised milk samples from larger distributors had significantly lower mean total bacterial counts, as well as coliform counts when compared to milk which was sold to the public from milk-shops.

Table 3: The number of milk-shops in Pretoria 1996 - 2000

Date	Number of milk-shops
January 1996	None
November 1996	14
February 1997	20
October 1997	37
March 1998	42
January 2000	Approximately 55

LEGISLATION REGARDING MILK

Milk is a product widely consumed by the public, especially by infants and children. As it has the potential to contain pathogens which can affect the health of those who consume it, standards have been established and promulgated into an Act to protect the public.

The only Act in South Africa which governs the safety of milk *per se*, and which sets the standards to which milk and dairy products must conform to, is the Foodstuffs, Cosmetics and Disinfectants Act, No. 54 of 1972: Regulations relating to milk and dairy products. This Act is administered by the Directorate of Food Control of the Department of Health.

According to this Act, pasteurised milk may not be sold if it contains any antibiotics or antimicrobials in amounts that exceed the maximum residue levels stipulated in the Maximum Limits for Veterinary Medicine and Stock Remedy Residues Regulations. It may also not contain pathogenic organisms, extraneous matter or any inflammatory product or other substance which may render the milk unfit for human consumption.

Pasteurised milk must pass the Aschaffenburg and Mullen phosphatase test. Bacteriologically it may not contain more than 20 coliforms (using the dry rehydrated film method also known as the Petrifilm plate for coliforms), or any *E. coli* per mℓ. On subjection to the standard plate count it may not contain more than 50 000 bacterial CFU per mℓ.

The Regulations further stipulate that all pasteurised milk shall, immediately after pasteurisation, be cooled and maintained at a temperature not exceeding 5°C.

Compositional standards of milk are controlled by the Agricultural Product Standards Act, No.119 of 1990: Regulations relating to dairy products and imitation dairy products, as amended. They were not dealt with in this study as these components had no direct effect on the safety of milk, or the health of the consumer.

JUSTIFICATION

The aim of this research project was to evaluate the quality of milk available to the consumer, comparing two different marketing systems. The sampled milk was evaluated to determine the bacteriological quality, as well as to look at the prevalence of selected pathogens and toxins and for the presence of inhibitory substances. Current South African regulations (The Foodstuffs, Cosmetics and Disinfectants Act, No. 54 of 1972: Regulations relating to milk and dairy products, 21 November 1997) were used as reference to the standards which had to be adhered to. The hypothesis was that:

H_O There is no difference in quality at point of sale between milk sold from "milk-shops" and milk which originates from a commercial national distributor

H_A There is a statistically significant difference in quality at point of sale between milk sold from "milk-shops" and milk which originates from a commercial national distributor

In South Africa, there was no data published in the last ten years on the bacteriological quality of milk to which the consumer was exposed. There was also no published data on the prevalence of inhibitory substances in milk at point of sale. The milk was therefore sampled so as to evaluate:

1. **The safety of the milk for human consumption**:

Milk shops are generally situated in the poorer socio-economic areas, where consumers buy the milk because it is cheaper. If consumers are unwittingly exposed to unnecessary health risks and become ill as a result of drinking unsafe milk, it will not only affect their health, but also their productivity. This could put burdens on primary health care services and on employers.

2. **The potential shelf-life of the milk:**

The shorter the shelf-life of the milk, the quicker it will deteriorate, especially in the absence of adequate refrigeration facilities which some consumers do not have in their homes. However, milk with a short shelf-life will also deteriorate in a refrigerator. Many consumers end up throwing milk away which has become sour or has an off-flavour, in effect increasing the price they pay for a litre of milk.

The objectives of the research project were to:

1. Compare aspects of safety and the potential shelf-life of the milk available to the consumer in a selected area of Pretoria between two different distribution chains who either do or do not pay the farmer for the quality of milk produced namely:
   * a commercial national distributor who buys milk from farmers on which a premium is paid for quality. Processing and packaging takes place at the plant under strict hygienic conditions before distribution.
   * "milk-shop" distributors who buy milk from farmers on volume alone with no incentives paid for quality. The milk is processed in the shop before sale to the

public, but not necessarily packaged. Hygienic conditions may vary depending on the level of training which the staff who work with the milk, have received. It is sold to the consumer in the consumer's own container or it may be bottled in the shop.

2. Determine whether the milk fell within the parameters laid down by law according to the Foodstuffs, Cosmetics and Disinfectants Act, 1972: Regulations relating to milk and dairy products of 21 November 1997.

3. Determine whether the milk was safe for human consumption.

4. Determine whether the potential shelf-life of the milk was adequate.

5. Determine whether there was a difference in milk quality on different days of the week.

CHAPTER 2

MATERIALS AND METHODS

One hundred and thirty-five milk samples were obtained over a six-week period from June to August 1998 from five milk-shops in the north-western part of Pretoria. Seventy-nine samples of milk were also purchased from three supermarkets which sold a well-known national distributor's commercial brand of milk, and this milk was used as a reference control milk. The milk-shop milk was either purchased prepackaged or bottled at point of sale by the milk-shop. The national distributor's milk was packaged in sachets.

STUDY DESIGN

Milk samples were purchased from four randomly selected retailers (out of eight available within the specified area) who distributed their milk directly to the consumer from a bulk tank (known as "Milk-Shops"). These shops were all located within a radius of 20 km of Onderstepoort in the north-western part of Pretoria. Two shops were randomly chosen in a poor socio-economic area and two shops in a more middle class area. This was determined by looking at the price of houses in the two areas. These areas were selected because of the good demographic cross-section of the population they represented. Each of the milk-shops looked clean and tidy and did not give an impression of being unhygienic. The owners were all very friendly, giving the shop a personal touch and making the consumer feel happy to return.

Milk samples were also purchased from a selected retailer (a supermarket) who sold both milk from a bulk tank, as well as milk from a commercial national distributor. The milk from the commercial national distributor acted as the control milk. This supermarket was chosen because it sold both bulk tank milk and control milk, and therefore the management of the

shop with respect to the cold chain, should have been the same as both types of milk were kept in the same display cabinet.

A further two samples of "control milk", also from the same commercial national distributor described above, were purchased from two other large supermarkets in the area. An attempt was made to ensure that all the national distributor's milk samples had the same expiry date and were therefore processed on the same day. This was not always possible as sometimes a shop had not bought fresh milk and was still selling milk with an older expiry date. The three supermarkets did however, always sell milk within the "sell-by date".

The farms of origin of the milk were unknown to the researcher as the study attempted to look at the quality of milk available to the consumer, without creating any suspicion on the part of the milk-shop owners. Therefore no direct questions could be asked as to where the milk came from or how many farmers supplied milk to the shop.

Milk was purchased over a six-week period, on alternate days, so that in one week purchases were made on a Tuesday and Thursday, and the following week on a Monday, Wednesday and Friday. From the second week onwards, two milk samples were purchased at each point of sale so as to decrease the variance in the data. There were therefore 8 shops x 2 samples (= 16 samples) purchased for each sampling day over the remaining five-week sampling period. On one of the sampling days, one of the supermarkets selling the national distributor's milk (Supermarket 1), had a power failure and therefore the electricity supply was off and the shop did not open its doors to consumers. Only 25 samples were thus purchased from this particular shop over the six-week sampling period.

All milk samples on sale to the public were kept in their original packaging until they were analysed. The national distributor's milk was packaged in 1 ℓ plastic sachets. Milk-shops usually sell their milk to clients who bring their own containers, thereby keeping down the cost of packaging. All the milk-shops also bottled milk in plastic 500 mℓ or 1 ℓ containers for members of the public who had not brought their own containers. They either pre-packaged them in advance, storing the milk in a refrigerator, or bottled them on demand from the bulk

tank as requested. These pre-packaged milk bottles were purchased at milk-shops so as not to create suspicion by bringing sterile glass bottles to the shop. The temperature of the milk was taken within five minutes of purchase, by decanting approximately 100 mℓ of milk into a separate plastic container and measuring the temperature using an electronic thermometer calibrated to the nearest 0.5 °C. The decanted milk was then discarded. Care was taken not to contaminate the balance of the milk after decanting, and this milk was kept on ice in a cooler box until it was analysed in the laboratory.

The time of purchase was taken as zero hour, and all microbiological analyses were carried out within four hours of the zero hour. The time of sampling and the time of processing in the laboratory were recorded.

At the time of purchase, all the milk samples were labelled and the following data was entered on a separate sheet (see Addendum X):

* sample number
* date and time of purchase
* place of purchase
* purchase price
* temperature of the milk at purchase
* whether the milk was sold as having been pasteurised or not
* whether the milk had been labelled or not
* expiry date.

LABORATORY WORK

Milk samples were kept in a household refrigerator until they were processed for bacterial counts. All milk samples were mixed by inverting them gently in their original containers a minimum of ten times before processing in the laboratory.

Petrifilm plates (dry rehydrateable films)

Petrifilm (3M) plates were used for aerobic, psychrotrophic, coliform and *E. coli* counts. Petrifilm plates are a commercially available, time and labour-saving method for enumerating bacteria by eliminating the need to prepare media. This sample-ready system consists of nutrients and/or selective and differential agents coated onto two dry rehydratable films along with a cold-soluble gelling agent and tetrazolium indicator dye which facilitates colony enumeration (Bishop & Juan 1988, Blackburn *et al.* 1996). Colonies growing on Petrifilm appear pink owing to the reduction of the dye. Coliform colonies, including *E. coli*, which grow on the Petrifilm produce gas by the fermentation of lactose. Petrifilm plates are ready to receive 1 mℓ of milk with no sterilization or plate pouring required. There is therefore no between-lot medium variation and less incubator space is needed. They are particularly useful in quality assurance laboratories which have limited facilities.

The Petrifilm aerobic count and the Petrifilm *E. coli* and coliform counts were found to be suitable and practical alternatives to standard methods used in enumerating bacteria in raw milk (Ginn *et al.* 1984, Betts *et al.* 1994, Blackburn *et al.* 1996). The different authors found that there was a good correlation between the Petrifilm aerobic count and the standard aerobic colony count method; the Petrifilm coliform colony count and the Standard Violet Red Bile Agar plating method, and the Petrifilm *E. coli* count and modified Eijkman test. Blackburn *et al.* (1996) found that the aerobic count Petrifilm and the coliform count Petrifilm both showed better repeatability than the standard methods. In South Africa an investigation into using the Petrifilm as an alternative for the evaluation of aerobic micro-organisms, coliforms and *E. coli* in fresh and pasteurised milk was conducted by Vermaak and Petzer (1996), who also concluded that the Petrifilm technique compared well with, and was even more sensitive than present conventional counting methods.

All milk samples were subjected to the following tests:

pH of the milk

The pH of the milk on the day of purchase and after incubating for 18 hours at 21 °C was determined by means of the Orion SA 250 pH meter autocalibrated with buffers at 7.00, 4.01 and 10.01 in accordance with the manufacturer's specifications.

Standard aerobic plate count / Total aerobic count (Petrifilm 3M)

The standard plate count is used to estimate viable bacterial populations in the milk and reflects the hygienic practices used in the production and handling of the milk (Houghtby *et al.* 1994).

Standard procedures for the use of the dry rehydrated film method for standard colony count were used (see Addendum I). The method used was according to the Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972: Regulations relating to milk and milk products, No R.1555. *Government Gazette* No 18439, 21 November 1997, 4-29.

Serial milk dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made for the standard plate count. Plates were incubated at 32 °C for 48 hours. A few standard plate count values are missing for some of the milk-shops during the first few days as insufficient dilutions were made and the bacterial numbers present were too numerous to count. Thereafter dilutions as described above, even though far above what is acceptable, were made so that exact counts could be made. Single-use disposable pipettes were used for each dilution.

Modified psychrotrophic bacterial count (Petrifilm 3M)

Milk samples were plated onto an aerobic Petrifilm plate as described in Addendum I, except that approximately 80 mℓ of milk was first pre-incubated at 21 °C for 18 hours, after which 1 mℓ of the serial dilutions were placed onto Petrifilms and incubated at 21 °C for 48 hours (Bishop & Juan 1988, Byrne *et al.* 1989, Phillips & Griffiths 1990). This method has shown good correlations with the psychrotrophic bacterial count done at 7 °C for 10 days.

Phillips & Griffiths (1990) described a similar method whereby milk was pre-incubated at 15 °C for 25 hours and then plated onto Petrifilms for 48 hours at 21 °C. This method showed a high correlation with the European Economic Community (EEC), now the European Union (EU), directive which requires incubation at 6 °C for 5 days, and specifies that the bacterial count of pasteurised milk shall not be greater than 100 000 CFU per mℓ after incubation (Council Directive of 5 August 1985 on health and animal health problems affecting intra-community trade in heat-treated milk (85/397/EEC). *Journal of the European Communities* No. L226, 13, cited by Phillips & Griffiths 1990 and Suhren & Heeschen 1990).

A pre-incubation temperature of 21 °C was chosen for this study as one incubator could then be used for both the pre-incubation of the milk and the incubation of the Petrifilm plates. A water bath was not used.

Serial milk dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were used for the modified psychrotrophic bacterial count. A new single-use disposable pipette was used for each dilution so that there would be no carry-over of milk from one dilution to another. Psychrotrophic values are missing for some of the milk-shops during the first few days as insufficient dilutions were made and the bacterial numbers present were too numerous to count. Thereafter dilutions as described above, even though far above what is acceptable, were made so that exact counts could be made.

E. coli and coliform count (Petrifilm 3M)

Coliforms in milk are suggestive of unsanitary conditions or practices during production, processing or storage. Coliforms are destroyed by pasteurisation, and therefore their presence after correct pasteurisation are indicative of bacterial recontamination post-pasteurisation (Christen *et al.* 1992).

Escherichia coli is a faecal indicator organism, whose recovery from milk suggests that other organisms of faecal origin, including pathogens, may also be present (Christen *et al.* 1992).

Standard procedures for the use of the dry rehydrated film method for coliform and *E. coli* count were used (see Addendum II). The method used was according to the Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972: Regulations relating to milk and milk products, No R.1555. *Government Gazette* No 18439, 21 November 1997, 4-29. This Act prescribes an incubation temperature of 32°C and not 35°C as prescribed by the Petrifilm manufacturers. Coliform and *E. coli* counts were tested in 1 mℓ of milk using the combined coliform and *E. coli* petrifilm plates.

Serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} were also made and the milk was plated onto petrifilm rapid coliform count plates. These plates only give a coliform count. Serial dilutions were made because some of the milk-shops had counts that were too numerous to count in 1 mℓ of milk. The petrifilm rapid coliform count plates, and not the combined coliform and *E. coli* petrifilm plates, were used for the serial dilutions as they were donated by the manufacturer 3M.

Aschaffenburg and Mullen alkaline phosphatase test

Pasteurisation is universally used to kill pathogens, and the alkaline phosphatase test is used to determine the effectiveness of pasteurisation (Murthy *et al.* 1992). The presence of alkaline phosphatase may also indicate the possible addition of raw milk to pasteurised milk. The

thermal resistance of alkaline phosphatase is greater than that of non-spore-forming pathogenic micro-organisms, and therefore milk heated to 62.8°C for 30 minutes or to 71.7°C for 15 seconds, will inactivate alkaline phosphatase, and will also kill all non-spore-forming pathogenic micro-organisms (Murthy *et al.* 1992).

The Aschaffenburg and Mullen alkaline phosphatase test was performed, using the method specified in the Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972: Regulations relating to milk and milk products, No R.1555. *Government Gazette* No 18439, 21 November 1997, 4-29 (see Addendum III).

Somatic cell count

The Regulations (Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972) state 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 (Hinz *et al.* 1992).

The somatic cell count was determined using the Fossomatic apparatus. Standard operating procedures for counting somatic cells in milk by means of the Fossomatic were used (see Addendum IV).

Brilliant Black Reduction Test for the detection of antibiotic and other antimicrobial residues

The Regulations (Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972) do not specify any specific tests to determine whether inhibitory substances are present in the milk. The Brilliant Black Reduction Test was chosen as it is highly sensitive to a number of antibiotics (see Addendum V). It is also the test of choice used by some of the European Union countries

(Suhren & Heeschen 1996). This test is easy to use, cheap and detects a broad spectrum of antibiotics for screening purposes. The test principle is based on the detection of growth inhibition, noticed visually by interpreting the colour of a pH-indicator in the test medium. *Bacillus stearothermophilus*, a thermophilic organism which is very sensitive to penicillin, is used as the test organism.

To avoid the influence of the micro flora of the sample on the test micro-organisms, a high incubation temperature of 64°C is used, because only minor parts of the milk flora can multiply and metabolize at this temperature (Suhren 1995, Suhren & Heeschen 1996).

Brucella milk ring test

Brucella abortus is a zoonosis which causes undulant fever in man. Brucellosis in animals has not yet been eradicated in South Africa. Commercial pasteurisation effectively kills *B. abortus* with a large margin of safety (Flowers *et al.* 1992).

Standard procedures for the brucella milk ring test were used to identify *B. abortus* antibodies in the milk (see Addendum VI).

Isolation of *Staphylococcus aureus* on Baird-Parker Agar Base and confirmation by means of the Staphylase test

Staphylococcus aureus isolation was done on Baird Parker Agar Base containing Potassium Tellurite solution and Egg Yolk Emulsion (see Addendum VII). One mℓ of milk was used, and a positive colony was confirmed as being *S. aureus* by means of the Staphylase test (see Addendum VIII). No counts were performed.

Detection of Staphylococcal enterotoxins

Several strains of *S. aureus* can produce heat stable enterotoxins which survive the pasteurisation process and cause food poisoning in man (Flowers *et al.* 1992). Individual *S. aureus* cultures were inoculated in 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) (see Addendum IX). This kit was used as the sensitivity of the assay was found to be 0.25 ng/mℓ in pasteurised milk, which was more sensitive than that claimed by the manufacturer (2 ng/mℓ) (Rose *et al.* 1989, Park & Szabo 1986).

The Staphylococcal enterotoxin test was done on all positive *S. aureus* cultures. Fifteen milk samples from the national distributor were also tested, one from each day of sampling. These samples were centrifuged for 15 minutes at 3 400 rpm and the sediment was discarded. Enterotoxin detection was carried out on the supernatant.

Detection of *Salmonella* spp.

One milk sample from every pair of milk samples purchased which was positive for *E. coli* was cultured for *Salmonella* spp. Samples were inoculated for pre-enrichment onto a non-selective medium and incubated at 37°C for 24 hours. They were then inoculated onto Rappaport Vassiliadis Soya Broth and incubated at 42°C for 24 hours, after which they were inoculated into a selective solid agar medium (Brilliant green, Bismuth sulphite and XLD) at 37°C for 24 hours for presumptive identification of *Salmonella* spp.

DATA ANALYSIS

A data sheet was completed for each day of sampling (see Addendum X), and all the raw data, including all the results of the different dilutions was completed on this sheet.

The Department of Information Technology and the Department of Statistics of the University of Pretoria analysed the data using the statistical computer package SAS (SAS Institute Inc., CarY, NC).

Comparisons were made between milk bought from "Milk-shops" and milk bought from the commercial national distributor. All count data were transformed to $\log_{10}$ values because of their non-normal distribution. Frequency distributions were made and correlation analyses were performed to provide association between different variables.

Analysis of variance (ANOVA) was done to determine whether the week number, day of the week, place of purchase, temperature at purchase, presence or absence of alkaline phosphatase, presence or absence of inhibitory substances, and the pH of the milk (independent variables) from the five milk-shops and the milk from the commercial national distributor differed with regard to aerobic standards plate count, psychrotrophic bacterial count, coliform count and somatic cell count (dependent variables). Probability (p) values were reported, and any value less than or equal to ($\leq$) 0.05 was reported as significant.

The computer spreadsheet packages of Sigma Plot (Jandel Scientific, San Rafael, CA) and Microsoft Excel 97 (1985-1997 Microsoft Corporation) were used to make the graphs.

CHAPTER 3

RESULTS

During a six-week period of investigation from June to August 1998, a total of 214 milk samples were purchased and analysed in the Department of Animal and Community Health's food laboratory. One hundred and thirty-five of these samples originated from five "milk-shops". The remaining 79 samples were purchased at three different supermarkets who all sold the same brand of commercially available milk from a large national distributor. Milk-shop 3 and Supermarket 3 are the same shop which sold both milk from a bulk tank and milk from a large commercial national distributor.

The results obtained from the following tests are described:

1. Temperature of the milk at purchase
2. Aschaffenburg and Mullen phosphatase test
3. Standard aerobic plate count
4. Psychrotrophic bacterial count
5. Coliform count
6. Presence of *Escherichia coli* in 1 mℓ
7. Presence of *Staphylococcus aureus* in 1 mℓ and production of *S. aureus* enterotoxins
8. Presence of inhibitory substances
9. Somatic cell count
10. Presence of *Brucella abortus* antibodies
11. Isolation of *Salmonella* spp. from 1 mℓ *E. coli* positive milk
12. pH of the milk

Temperature of the Milk at Purchase

Table 4: Temperature of the milk (°C) at the time of purchase

Origin	Number of samples tested	Mean temp (°C)	Standard deviation	Range (°C)	Number ≤ 5°C	Percent ≤ 5°C
Milk-shop 1	27	6.3	1.9	3.5 - 10.5	13	48.1
Milk-shop 2	27	5.9	1.1	4.0 - 7.5	9	33.3
Milk-shop 3	27	7.0	1.3	5.0 - 10.0	1	3.7
Milk-shop 4	27	8.6	1.2	6.5 - 11.0	0	0[b]
Milk-shop 5	27	6.8	1.3	4.5 - 9.0	3	11.1
Supermarket 1 * **National distributor X**	25	4.0	1.3	1.5 - 7.0	20	80.0[a]
Supermarket 2 * **National distributor X**	27	5.7	1.5	2.5 - 8.5	9	33.3
Supermarket 3 * **National distributor X**	27	6.4	1.0	4.5 - 8.0	2	7.4
Totals	**214**				**57**	**26.6**

* milk from the same national distributor, purchased at three different outlets
[a] differed significantly from all other outlets
[b] differed significantly from all other outlets

The Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972) stipulates that the temperature of milk should immediately be brought down to 5°C or less after pasteurisation (processing), and maintained at this temperature to retard bacterial growth. Retailers are obliged to keep milk at a temperature of less than 5°C. Table 4 and Figure 1 show that this target was only met in 26.6% of samples purchased. Only one of the larger retailers (Supermarket 1) consistently kept the temperature of their milk under 5°C, averaging 4°C during the six-week sampling period. The temperature of the milk from this supermarket differed significantly ($p < 0.05$) from all the other outlets. All the other shops had averages greater than 5°C, with one milk-shop (Milk-shop 4) never attaining a temperature below 5°C. This shop also differed significantly ($p < 0.05$) from all the other shops selling milk.

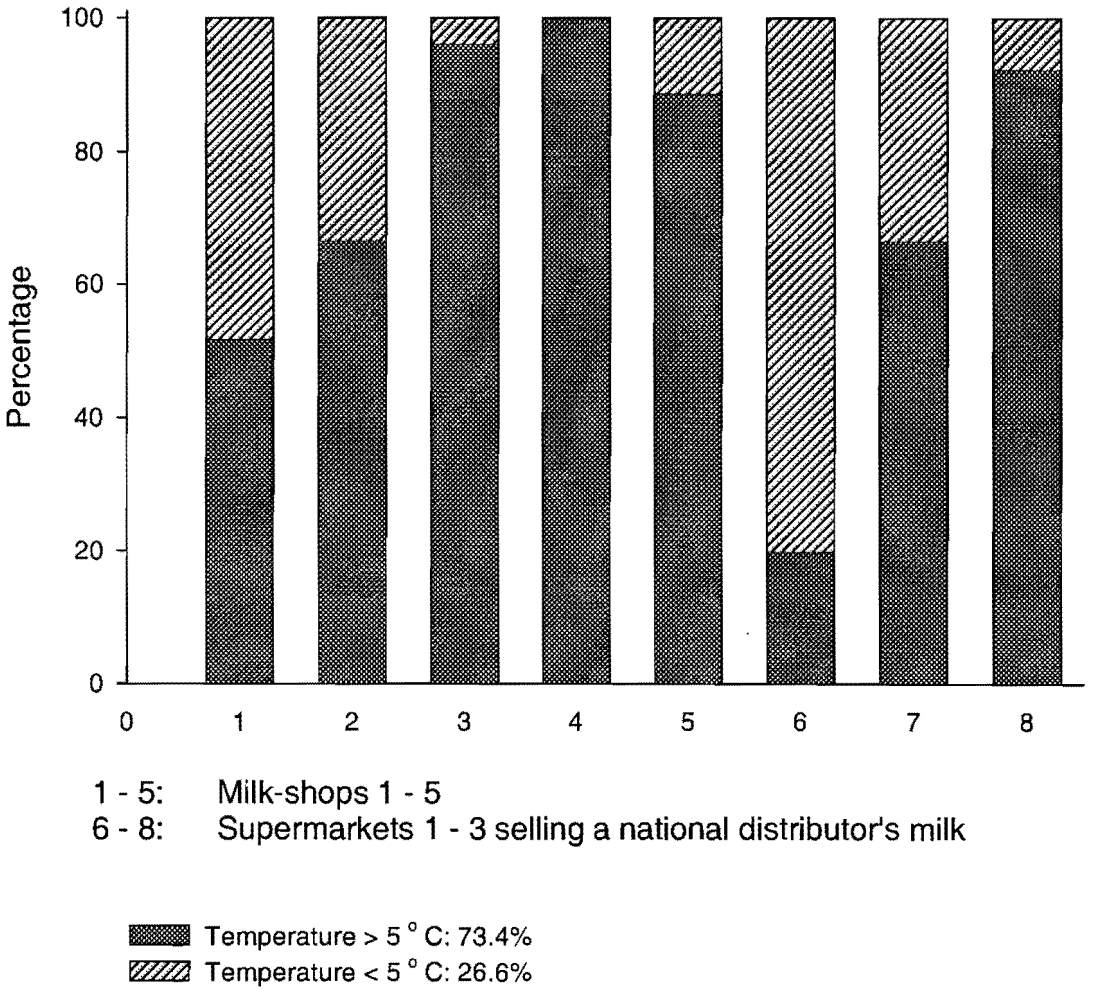


Figure 1: Percentage of milk samples with a temperature greater than or less than 5°C at each of the different outlets

Aschaffenburg and Mullen Phosphatase Test

Table 5: Indication of the alkaline phosphatase status of the milk, and whether it was labelled as pasteurised

Origin	Number of samples tested	Labelled as pasteurised	Alkaline phosphatase positive
Milk-shop 1	27	19 (70.4)[a]	27 (100)[a]
Milk-shop 2	27	20 (74.1)	0 (0)
Milk-shop 3	27	27 (100)	0 (0)
Milk-shop 4	27	27 (100)	25 (92.6)
Milk-shop 5	27	0 (0)	0 (0)
Supermarket 1 * **National distributor X**	25	25 (100)	0 (0)
Supermarket 2 * **National distributor X**	27	27 (100)	0 (0)
Supermarket 3 * **National distributor X**	27	27 (100)	0 (0)

* milk from the same national distributor, purchased at three different outlets
[a] indicates percentage of total

Of the 135 milk-shop milk samples tested, 52 (38.5%) were alkaline phosphatase positive (Table 16). These samples all originated from two of the five milk-shops, one of which had no negative alkaline phosphatase results in the samples taken over the six-week period (Table 5). The national distributor's milk was always alkaline phosphatase negative (Tables 5 and 16).

Milk-shops did not always indicate whether the milk was pasteurised or not. Milk-shop 5's milk was never labelled at all, and Milk-shops 1 and 2 only labelled their milk 70.4% and 74.1% of the time respectively over the six-week period (Table 5).

BACTERIAL COUNTS

Standard Aerobic Plate Count

Table 6: Standard aerobic plate counts (CFU/mℓ) over the six-week sampling period

Origin	Number tested	Median count (CFU/mℓ)	Geometric mean (CFU/mℓ)	Range (CFU/mℓ)
Milk-shop 1	25	15 600	22 929	2 200 - 990 000
Milk-shop 2	27	900	1 524	100 - 43 000
Milk-shop 3	27	52 000	36 434	1 200 - 131 000
Milk-shop 4	23	303 500	101 109	100 - 26 600 000
Milk-shop 5	27	103 000	117 809	7 900 - 1 140 000
Supermarket 1 * **National distributor X**	25	1 900	2 357	900 - 6 500
Supermarket 2 * **National distributor X**	27	2 200	2 219	700 - 5 600
Supermarket 3 * **National distributor X**	27	2 300	2 707	800 - 8 700

* milk from the same national distributor, purchased at three different outlets

Pasteurised milk may not contain more than 50 000 CFU/mℓ of milk as measured by the standard aerobic plate count (Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972). Tables 6 and 14, and Figures 2, 3 and 4 show that the standard aerobic plate count for milk-shop milk (n = 129) varied tremendously over the six-week sampling period. Counts ranged from 1.0×10^2 to 2.66×10^7 CFU/mℓ, with a median value of 41 000 CFU/mℓ (the median is the middle value when a sample of observations are arranged in order of magnitude, and is insensitive to extreme values). Standard aerobic plate counts for the national distributor's milk always ranged below 9 000 CFU/mℓ (Tables 6 and 15).

Standard aerobic plate counts showed no significant ($p > 0.05$) difference between each week over the trial period of six weeks. There was however, a significant ($p < 0.05$) difference between milk purchased on Mondays compared with milk purchased on Fridays, with milk purchased on Fridays having a lower standard aerobic plate count than milk purchased on Mondays. No significant ($p > 0.05$) differences were found between the other days of the week.

There was no significant ($p > 0.05$) difference in standard aerobic plate count between the national distributor's milk purchased at the three different supermarkets, and also no significant ($p > 0.05$) difference between the national distributor's milk and the milk purchased from Milk-shop 2. There was however a significant ($p < 0.05$) difference between the national distributor's milk, and milk purchased from the remaining four milk-shops. After excluding the national distributor's milk samples, there was also a significant ($p < 0.05$) difference between Milk-shop 2 and all the other milk-shops. Milk-shops 1, 3, 4 and 5 did not differ significantly ($p > 0.05$) from each other.

There was also no significant ($p > 0.05$) difference in the standard aerobic plate count between those milk-shops which pasteurised correctly and those that did not.

There was no significant ($p > 0.05$) difference in the standard aerobic plate count between milk which contained inhibitory substances and milk which did not contain any.

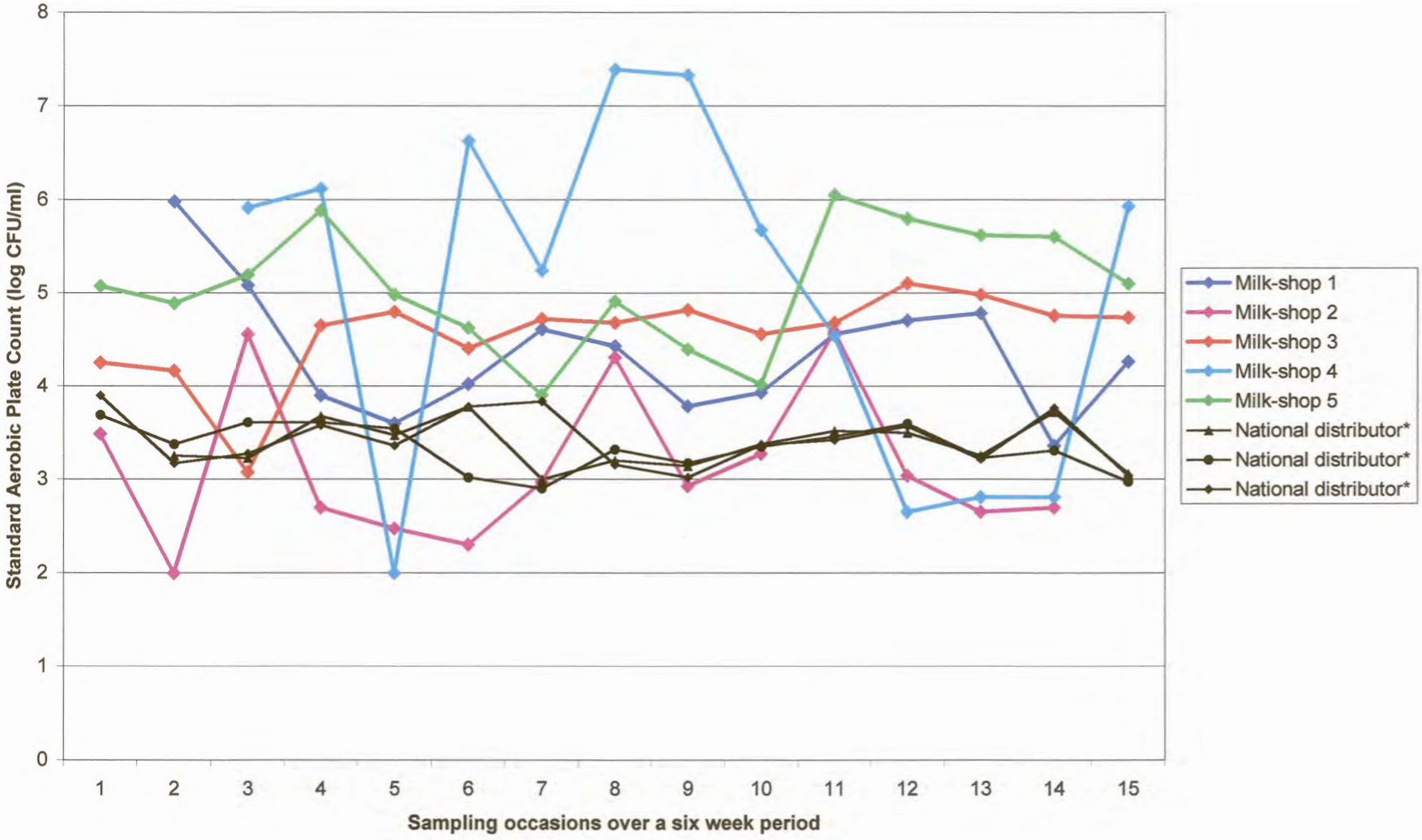


Figure 2: Standard aerobic plate counts over the sampling period

* the same brand of a national distributor's milk was purchased at three different supermarkets

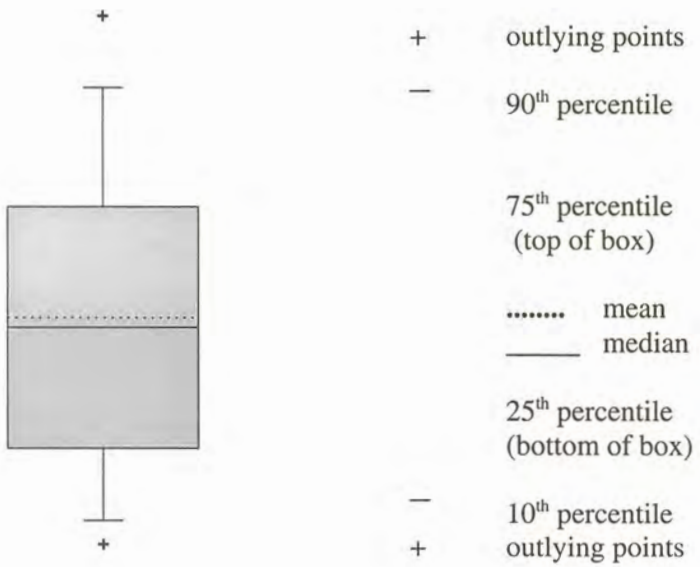


Figure 3: Explanation of a box plot representation of colony counts

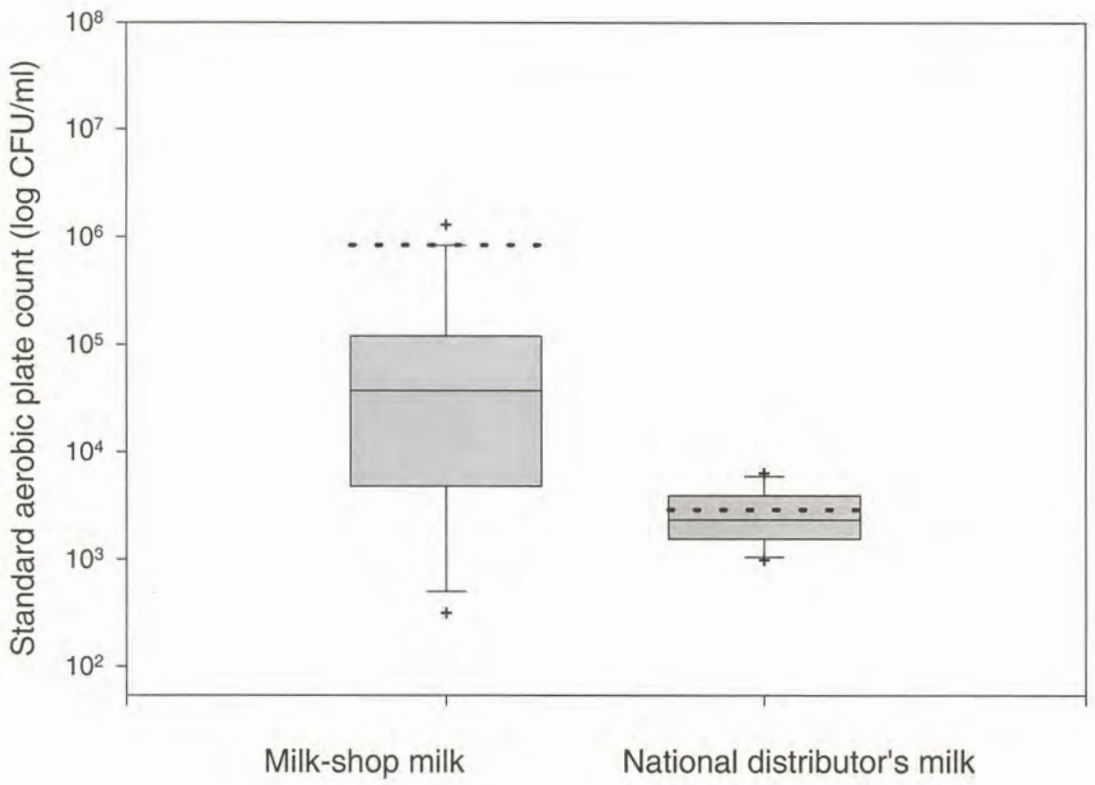


Figure 4: Total aerobic colony counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor

Psychrotrophic Bacterial Count

Table 7: The psychrotrophic bacterial count (CFU/mℓ) over the six-week sampling period

Origin	Number tested	Median count (CFU/mℓ)	Geometric mean (CFU/mℓ)	Range (CFU/mℓ)
Milk-shop 1	22	65 500 000	68 731 417	15 500 000 - 221 000 000
Milk-shop 2	22	19 700 000	14 055 862	500 000 - 101 000 000
Milk-shop 3	22	5 400 000	5 198 318	300 000 - 183 000 000
Milk-shop 4	22	20 750 000	17 257 115	960 000 - 150 000 000
Milk-shop 5	22	25 350 000	31 028 908	3 200 000 - 212 000 000
Supermarket 1 * **National distributor X**	25	8 400	8 606	1 600 - 43 000
Supermarket 2 * **National distributor X**	27	13 600	16 731	3 300 - 87 000
Supermarket 3 * **National distributor X**	27	8 000	13 817	1 900 - 480 000

* milk from the same national distributor, purchased at three different outlets

Psychrotrophic bacterial counts in milk-shop milk (n = 110) differed significantly ($p < 0.05$) from those of the national distributor, and were extremely high, ranging from 3×10^5 CFU/mℓ to 2.2×10^8 CFU/mℓ, with a median count of 2.4×10^7 CFU/mℓ (Tables 7 and 14, Figures 5 and 6). The national distributor's milk had a median psychrotrophic bacterial count of 9 200 CFU/mℓ, ranging from 1 600 CFU to 480 000 CFU/mℓ (Tables 7 and 15, Figure 5). The milk-shops differed significantly ($p < 0.05$) from each other with respect to the psychrotrophic bacterial count, but the national distributor's milk did not differ significantly ($p > 0.05$) between outlets.

There was no significant ($p > 0.05$) difference in the psychrotrophic bacterial count between the different days of the week or between each week over the trial period of six weeks.

There was a significant ($p < 0.05$) difference in the psychrotrophic bacterial counts between milk containing inhibitory substances and milk not containing any, as well as between milk which was pasteurised correctly (alkaline phosphatase negative) and milk which was not pasteurised correctly (alkaline phosphatase positive).

After excluding the national distributor's milk, there was a significant ($p < 0.05$) difference in the psychrotrophic bacterial count between Milk-shop 3 and all the other milk-shops. Milk-shop 1 also differed significantly ($p < 0.05$) from Milk-shops 2 and 3.

There were correlations between the aerobic standard plate count and the psychrotrophic bacterial count in Milk-shops 2 and 4, as well as in the national distributor's milk purchased from Supermarket 1 (Table 8).

Table 8: Correlation between the Aerobic Standard Plate Count ($\log_{10}$CFU/mℓ) and the Psychrotrophic Bacterial Count ($\log_{10}$CFU/mℓ) over the six-week sampling period

Origin	**Pearson correlation coefficient (r^2)**
Milk-shop 1	0.1
Milk-shop 2	0.54
Milk-shop 3	0.001
Milk-shop 4	0.95
Milk-shop 5	0.37
Supermarket 1* (National distributor X)	0.61
Supermarket 2* (National distributor X)	0.27
Supermarket 3* (National distributor X)	0.12

* milk from the same national distributor, purchased at 3 different outlets

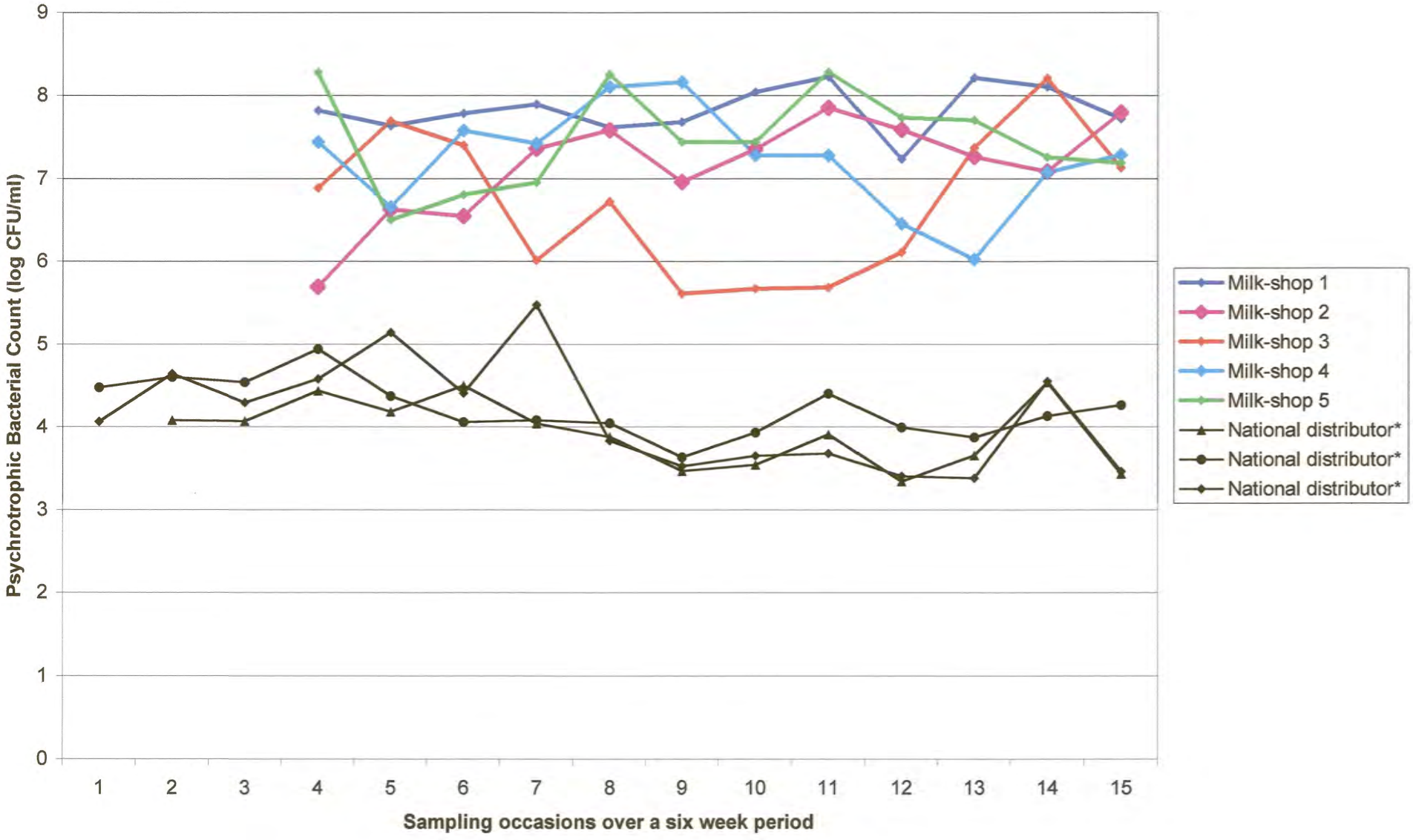


Figure 5: Psychrotrophic Bacterial Counts over the sampling period

* the same brand of a national distributor's milk was purchased at three different supermarkets

Figure 6: Psychrotrophic bacterial counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor

Coliform Count

Table 9: Coliform counts over the six-week sampling period

Origin	Number tested	Median count (coliforms/mℓ)	Geometric mean (coliforms/mℓ)	Range (coliforms/mℓ)
Milk-shop 1	26	180	233	51 - 9 000
Milk-shop 2	26	16	20	0 - 1 110
Milk-shop 3	27	0	2	0 - 50
Milk-shop 4	23	130	184	0 - 34 000
Milk-shop 5	27	20	25	0 - 1 090
Supermarket 1* **National distributor X**	25	0	-	0
Supermarket 2* **National distributor X**	27	0	-	0
Supermarket 3* **National distributor X**	27	0	-	0

* milk from the same national distributor, purchased at three different outlets

Coliform counts in milk-shop milk (n = 129) ranged from 0 to 3.4 x 10^5/mℓ (Table 9 and Figure 7), 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 (Table 14). Coliform counts for the national distributor's milk were always zero (Tables 9 and 15).

Coliform counts in Milk-shop 1 ranged from 51 to 9 000/mℓ and in Milk-shop 4 they ranged from 0 to 34 000/mℓ (Table 9). The other milk-shops which pasteurised correctly had variations between 0 and 1 100 coliforms per mℓ.

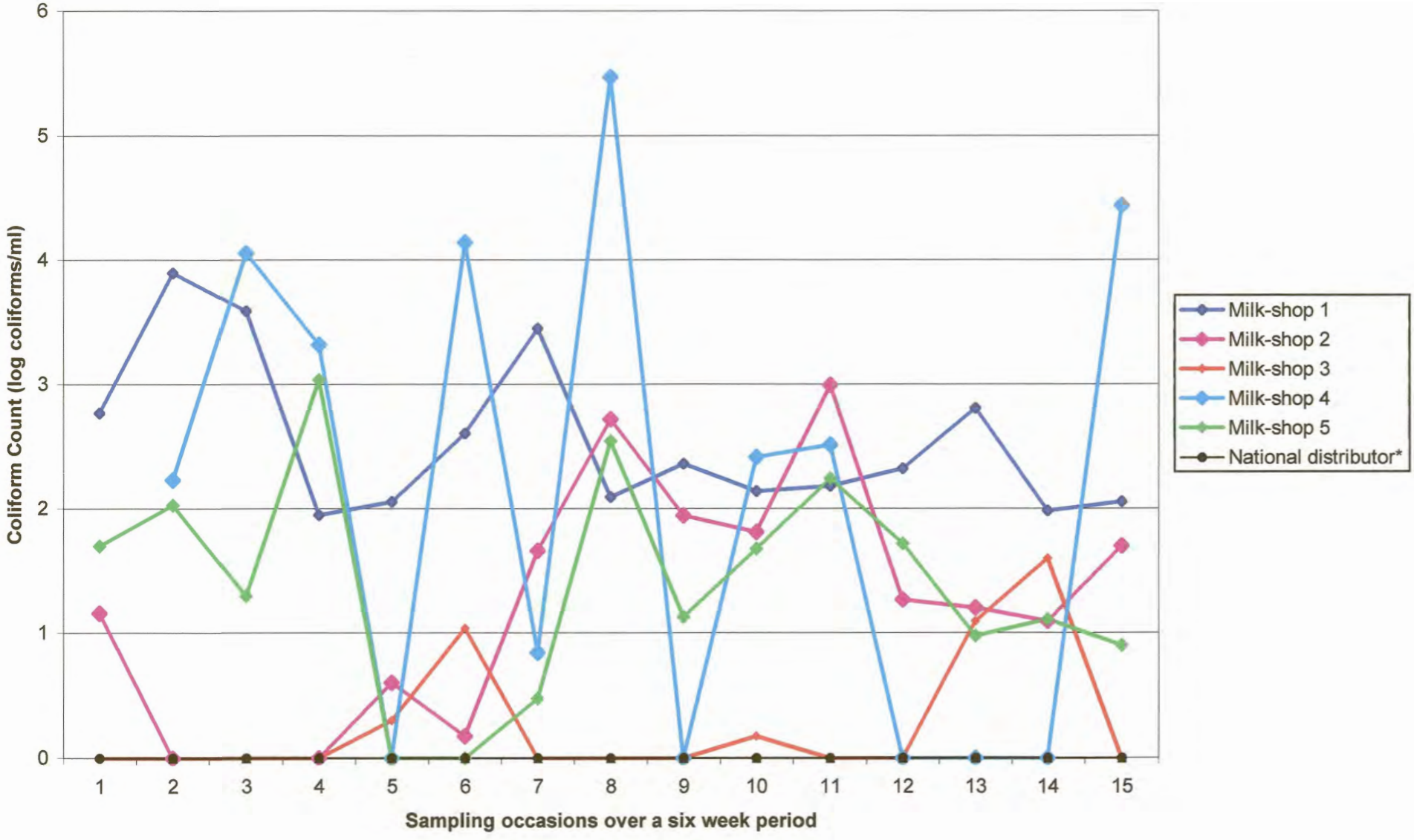


Figure 7: Coliform counts over the sampling period

* the same brand of a national distributor's milk was purchased at three different supermarkets

There was a significant ($p < 0.05$) difference in the coliform count between the different days of the week with Mondays differing from Wednesdays and Fridays.

The national distributor's milk never contained any coliform bacteria, and differed significantly ($p < 0.05$) from all the milk-shops except Milk-shop 3 (Figure 8). After excluding the national distributor's milk, Milk-shop 3 also differed significantly ($p < 0.05$) from all the other milk-shops. Milk-shop 1 and Milk-shop 4 did not differ significantly ($p > 0.05$) from each other with respect to the coliform count, but they differed significantly ($p < 0.05$) from all the other milk-shops.

There was a significant ($p < 0.05$) difference in coliform counts between milk containing inhibitory substances and milk not containing any, as well as between milk which was pasteurised correctly (alkaline phosphatase negative) and milk which was not pasteurised correctly (alkaline phosphatase positive).

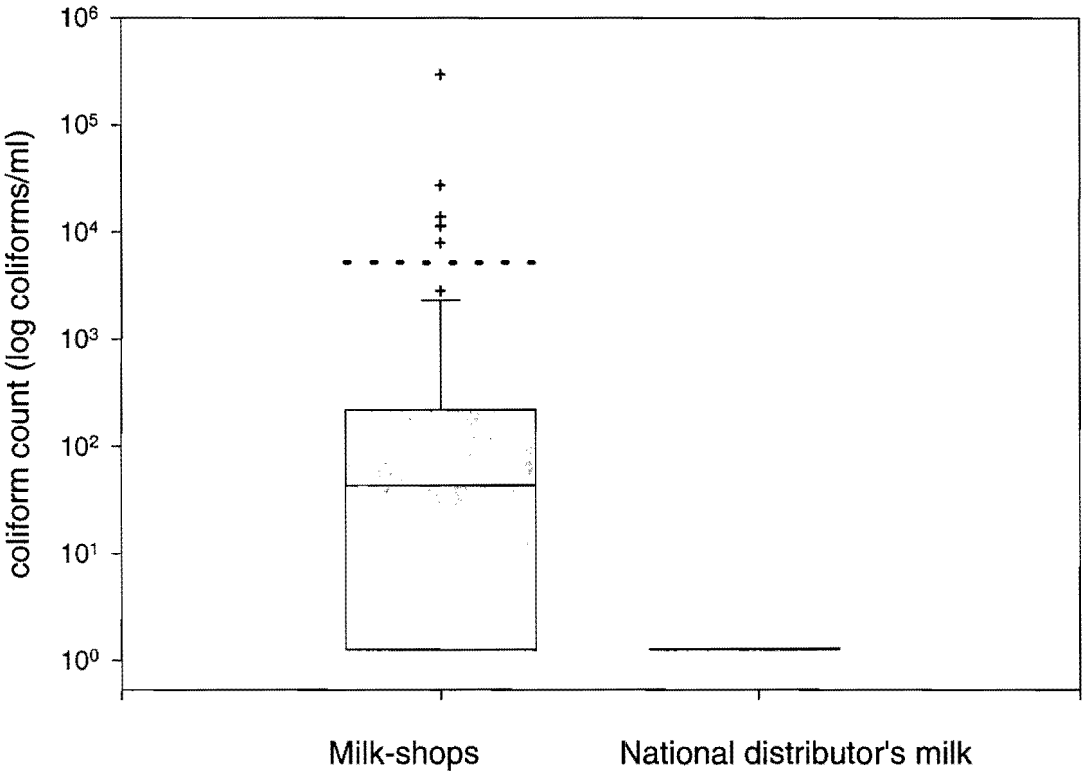


Figure 8: Coliform counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor

Escherichia coli (E. coli)

Table 10: Presence of *E. coli* in the milk purchased over a six-week sampling period.

Origin	Number tested	*E. coli* positive in 1 mℓ	% positive
Milk-shop 1	27	21	77.8
Milk-shop 2	27	1	3.7
Milk-shop 3	27	0	0
Milk-shop 4	27	2 (11)[a]	7.4 (40.7)[a]
Milk-shop 5	27	0	0
Supermarket 1* **National distributor X**	25	0	0
Supermarket 2* **National distributor X**	27	0	0
Supermarket 3* **National distributor X**	27	0	0

* milk from the same national distributor, purchased at three different outlets
[a] indicates suspect *E. coli*.

Out of 135 milk-shop milk samples tested for *E. coli*, 24 (17.7%) were positive in 1 mℓ, and a further 11 (8.1%) were suspect for the organism (Tables 10 and 16). Over 95% of isolates 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. Milk-shop 4 sold 14 (51.9%) samples which were *E. coli* negative. The remaining thirteen (48.1%) samples were either positive or suspect positive for *E. coli*. Excluding the suspect samples, only 7.4% of Milk-shop 4's milk was *E. coli* positive, but if one includes the suspect samples then 40.7% were positive for *E. coli*. Milk-shop 2 sold one sample which was positive for *E. coli* (Table 10). The milk originating from Milk-shops 3 and 5 never contained any *E. coli* organisms.

The national distributor's milk was always negative for *E. coli* in 1 mℓ (Tables 10 and 16).

Staphylococcus aureus (*S. aureus*) and *S. aureus* Enterotoxins

Table 11: Presence of *S. aureus* in the milk purchased over a six-week sampling period

Origin	Number tested	*S. aureus* positive in 1 mℓ
Milk-shop 1	27	26 (96.3)[a]
Milk-shop 2	27	4 (14.8)
Milk-shop 3	27	5 (18.5)
Milk-shop 4	27	8 (29.6)
Milk-shop 5	27	11(40.7)
Supermarket 1* **National distributor X**	25	0 (0)
Supermarket 2* **National distributor X**	27	0 (0)
Supermarket 3* **National distributor X**	27	0 (0)

* milk from the same national distributor, purchased at three different outlets
[a] indicates percentage of total

Fifty-four (40%) of all milk-shop milk samples purchased contained the organism *S. aureus* in 1 mℓ (Tables 11 and 16, and Figure 9). One third of these organisms was found in correctly pasteurised milk and the other two thirds in milk which was not correctly pasteurised. Analysing the two milk-shops which sold milk which was alkaline phosphatase positive, *S. aureus* was found in 96% of Milk-shop 1's, and 30% of Milk-shop 4's milk.

Of the 51 *S. aureus* positive cultures which were tested for the production of enterotoxins, four (7.83%) produced heat stable staphylococcal enterotoxins A (SEA), B (SEB), D (SED) or a combination of them (Table 16). All the toxins isolated originated from Milk-shop 1. SEA/SEB was produced by two *S. aureus* strains and SEA/SEB/SED by the other two strains.

The national distributor's milk did not contain any *S. aureus* or enterotoxin in 15 milk samples tested (Table 16 and Figure 9).

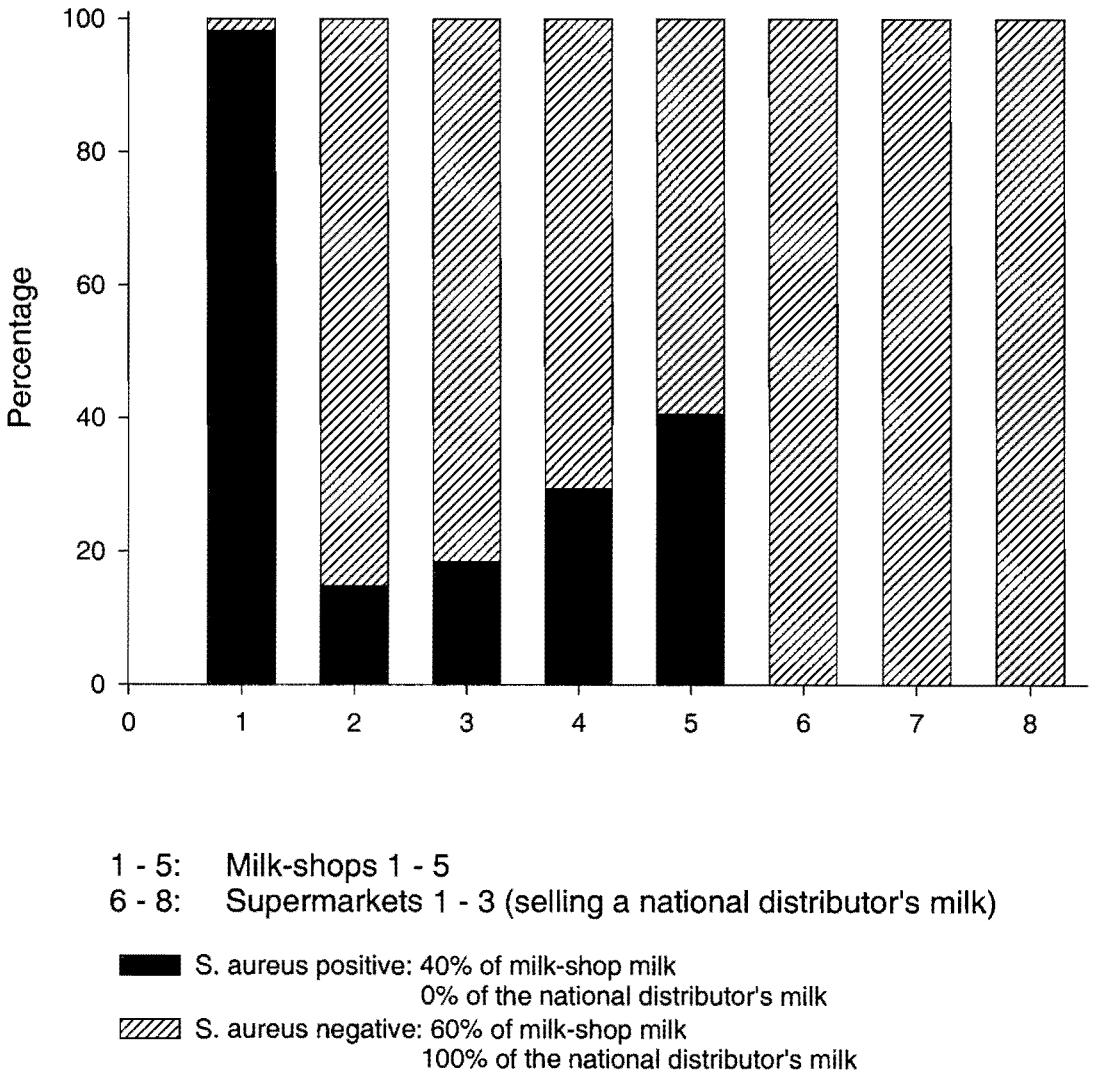


Figure 9: Presence or absence of *S. aureus* in the milk

Thermo-resistant Inhibitory Substances

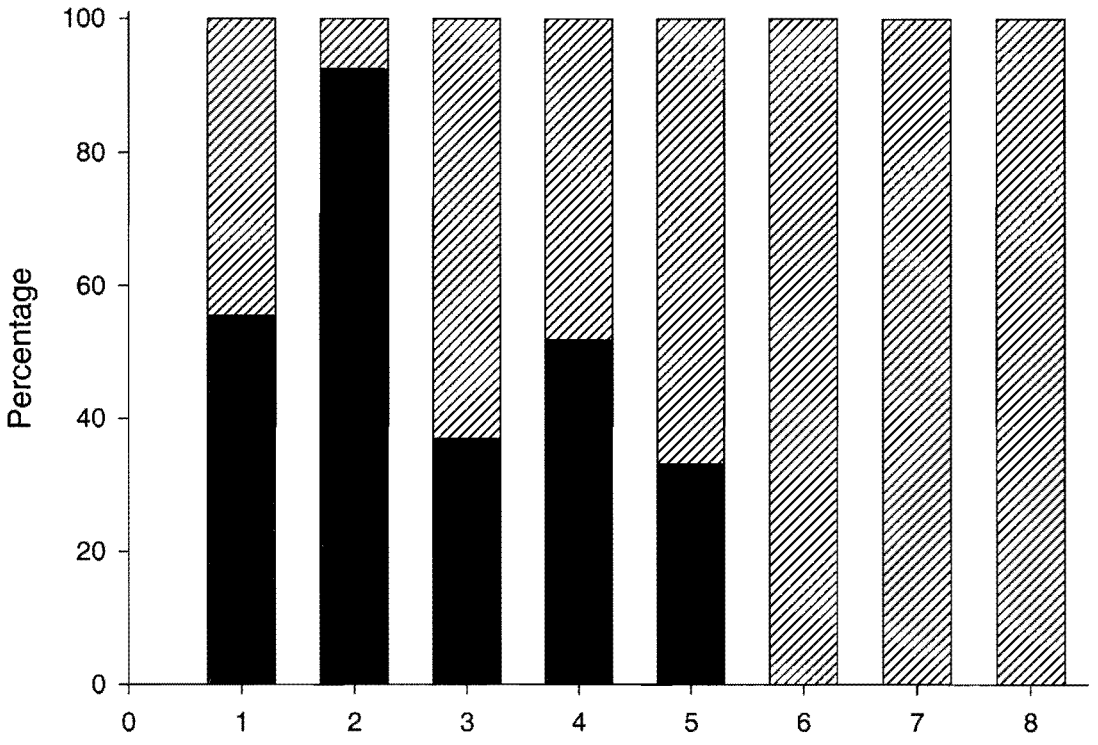
Table 12: The presence of inhibitory substances in the milk purchased over a six-week sampling period

Origin	Number tested	Inhibitory substances present
Milk-shop 1	27	15 (55.6)[a]
Milk-shop 2	27	25 (92.6)
Milk-shop 3	27	10 (37.0)
Milk-shop 4	27	14 (51.9)
Milk-shop 5	27	9 (33.3)
Supermarket 1* **National distributor X**	25	0 (0)
Supermarket 2* **National distributor X**	27	0 (0)
Supermarket 3* **National distributor X**	27	0 (0)

* milk from the same national distributor, purchased at three different outlets
[a] indicates percentage of total

The occurrence of inhibitory substances in milk-shop milk was high, with 73 out of 135 (54%) samples containing some sort of inhibitory substance (Tables 12 and 16, and Figure 10). Inhibitory substances present in the milk ranged from 33.3% in milk-shop 5 to 92.6% in milk-shop 2. The milk was not analysed further to determine which substances were present.

The results showed that the national distributor's milk never contained any inhibitory substances (Table 16 and Figure 10).



1 - 5: Milk-shops 1 - 5
6 - 8: Supermarkets 1 - 3 (selling a national distributor's milk)

Positive for inhibitory substances: 54% of milk-shop milk
0% of the national distributor's milk

Negative for inhibitory substances: 46% of milk-shop milk
100 % of the national distributor's milk

Figure 10: Presence or absence of inhibitory substances in the milk

Somatic Cell Count

Table 13: The somatic cell counts over the six-week sampling period

Origin	Number tested	Median count (cells/mℓ)	Geometric mean (cells/mℓ)	Range (cells/mℓ)
Milk-shop 1	27	500 000	564 000	372 000 - 961 000
Milk-shop 2	27	423 000	371 000	32 000 - 582 000
Milk-shop 3	27	425 000	503 000	324 000 - 778 000
Milk-shop 4	27	192 000	155 000	12 000 - 502 000
Milk-shop 5	27	455 000	429 000	80 000 - 1 623 000
Supermarket 1* **National distributor X**	25	120 000	116 000	64 000 - 137 000
Supermarket 2* **National distributor X**	27	117 000	111 000	63 000 - 138 000
Supermarket 3* **National distributor X**	27	127 000	123 000	80 000 - 145 000

* milk from the same national distributor, purchased at three different outlets

Milk-shop milk somatic cell counts varied between 1.2×10^4 and 1.6×10^6 cells per mℓ, with a median count of 4.2×10^5 cells (Tables 13 and 14, and Figure 11). Only 18.7% (25 out of 135 samples) of somatic cell counts were above the legal limit of 500 000 cells/mℓ.

The national distributor's milk always had somatic cell counts of less than 150 000 cells per mℓ (Tables 13 and 15, and Figure 11) and differed significantly ($p < 0.05$) from all the milk-shops except for Milk-shop 4 (Figure 12). The national distributor's milk purchased at the three different supermarkets did not differ significantly ($p > 0.05$) from each other with respect to the somatic cell count over the trial period.

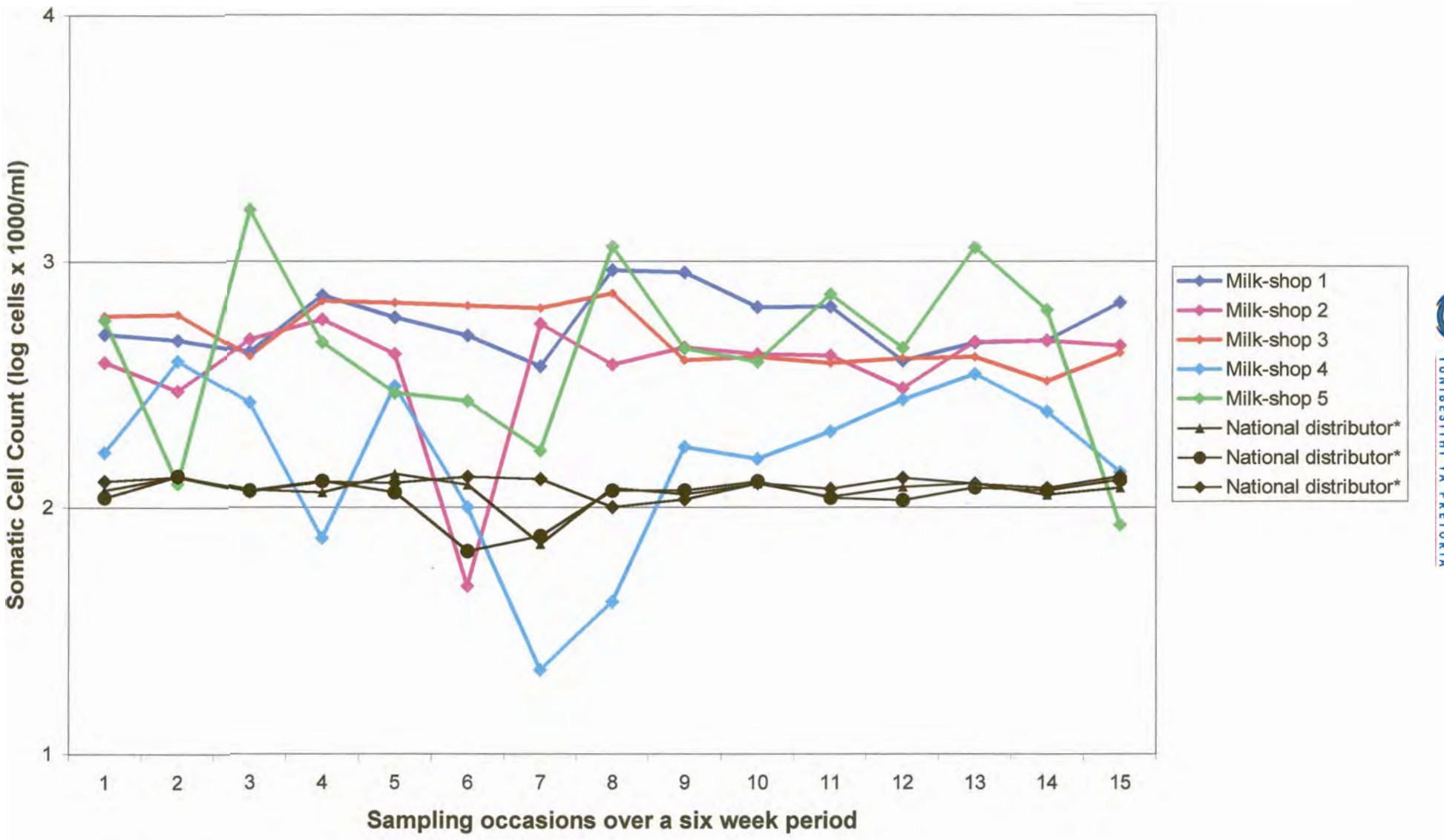


Figure 11: Somatic Cell Counts over the sampling period

* the same brand of a national distributor's milk was purchased at three different supermarkets

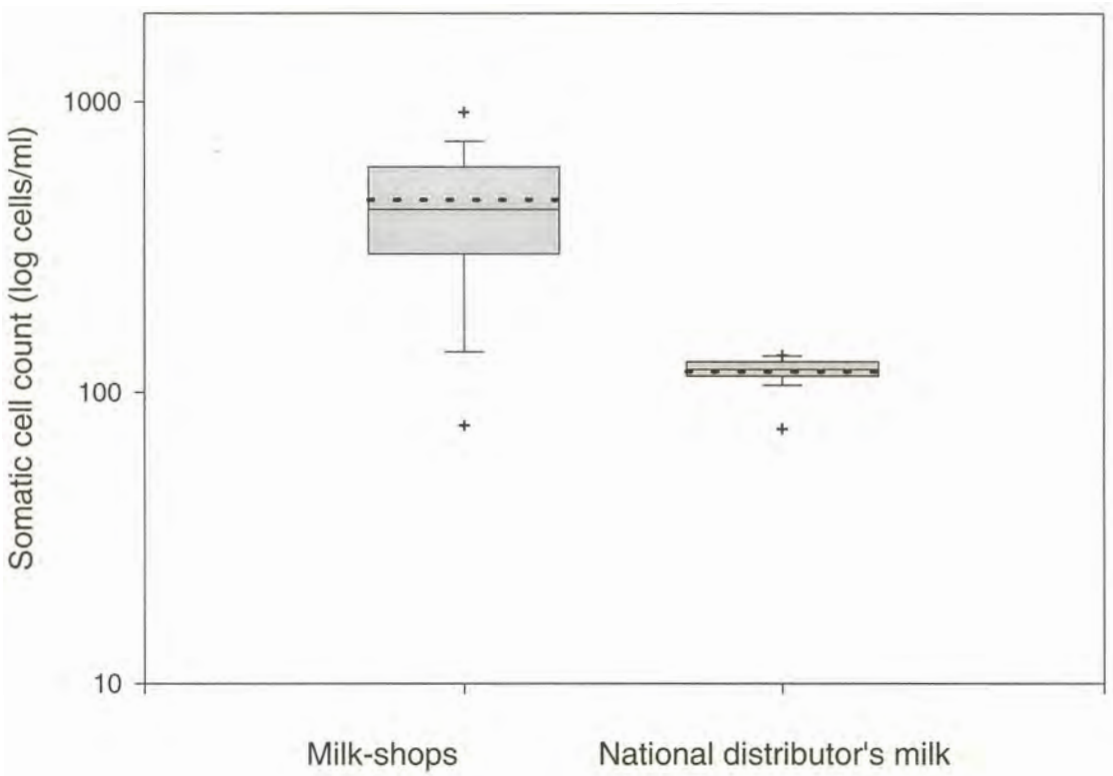


Figure 12: Somatic cell counts (mean, median, 10th, 25th, 75th and 90th percentile and values exceeding this range, see Figure 3) of milk-shop milk and that of the national distributor

Table 14: Summary of the bacterial and somatic cell counts of pasteurised milk obtained from milk-shops

	Legal limit	**Milk-shop milk**		
		Median	**Geometric mean**	**Range**
Standard plate count (CFU/mℓ)	< 50 000	41 000	55 961	100 - 26 600 000
Coliform count/mℓ	< 20	30	93	0 - 344 000
Psychrotrophic bacterial count (CFU/mℓ)	EU limit: < 100 000	24 000 000	27 254 324	300 000 - 221 000 000
Somatic cell count/mℓ	< 500 000	424 000	404 400	12 000 - 1 623 000

Table 15: Summary of the bacterial and somatic cell counts of pasteurised milk obtained from a large national distributor

	Legal limit	**National distributor's milk**		
		Median	**Geometric mean**	**Range**
Standard plate count (CFU/mℓ)	<50 000	2 200	2 428	700 - 8 700
Coliform count/mℓ	<20	0	-	0
Psychrotrophic bacterial count (CFU/mℓ)	EU limit: <100 000	9 200	13 051	1 600 - 480 000
Somatic cell count/mℓ	<500 000	120 000	116 668	63 000 - 145 000

Brucella abortus

All samples tested were negative for antibodies to *B. abortus* (Table 16).

Salmonella spp.

Seventeen *E. coli* positive samples were further tested for the presence of *Salmonella* spp. in 1 mℓ, but these samples were all negative for the organism (Table 16).

Table 16: Potential pathogens or hazards found in pasteurised milk samples over the six-week sampling period

Potential Pathogen or hazard	**Milk-shop milk**			**National distributor's milk**		
	Nº tested	**Nº pos**	**% pos**	**Nº tested**	**Nº pos**	**% pos**
E. coli	135	24 (35)[a]	17.7 (25.9)[a]	79	0	0
S. aureus	135	54	40	79	0	0
S. aureus enterotoxin	51	4	7.8	15	0	0
B. abortus	135	0	0	79	0	0
Salmonella spp.	17	0	0	not tested	----	----
Inhibitory substances	135	73	54.1	79	0	0
Alkaline phosphatase	135	52	38.5	79	0	0

[a] includes suspect *E. coli* cases

pH of the Milk

Table 17: The mean pH at sale, and after incubation at 21 °C for 18 hours, over the six-week sampling period

Origin	Number tested	Mean pH at sale	Mean pH after incubation
Milk-shop 1	27	6.79 (±0.06)	6.48 (±0.31)[b]
Milk-shop 2	27	6.78 (±0.06)	6.70 (±0.13)
Milk-shop 3	27	6.76 (±0.08)	6.73 (±0.08)
Milk-shop 4	27	6.70 (±0.10)[a]	6.56 (±0.30)[b]
Milk-shop 5	27	6.77 (±0.07)	6.65 (±0.18)
Supermarket 1* **National distributor X**	25	6.77 (±0.04)	6.78 (±0.06)
Supermarket 2* **National distributor X**	27	6.75 (±0.06)	6.78 (±0.05)
Supermarket 3* **National distributor X**	27	6.75 (±0.07)	6.76 (±0.05)

* milk from the same national distributor, purchased at three different outlets
[a] differed significantly from all other outlets
[b] differed significantly from all other milk-shops

The mean pH value at sale for the milk-shop milk and for the national distributor's milk was 6.76. There was no significant ($p > 0.05$) difference in the pH of the milk at sale except for Milk-shop 4 which differed significantly ($p < 0.05$) from the other shops (Table 17).

The mean pH value after incubating the milk at 21 °C for 18 hours for milk-shop milk samples was 6.62 and for the national distributor's milk was 6.77. The pH of the national distributor's milk taken after incubating the milk for 18 hours at 21 °C differed significantly ($p < 0.05$) from the pH of the milk-shops. All milk-shop milk was visibly thicker than the national distributor's milk after incubation. Milk-shops 1 and 4 differed significantly ($p < 0.05$) from the rest of the milk-shops with respect to the pH after incubation (Table 17).

Fitness for Human Consumption

Table 18: Indication as to whether or not pasteurised milk samples passed all the criteria laid down by the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972) over a six-week sampling period

Origin	Number tested	Number of samples passed
Milk-shop 1	27	0 (0) [a]
Milk-shop 2	27	9 (33)
Milk-shop 3	27	4 (15)
Milk-shop 4	27	1 (4)
Milk-shop 5	27	4 (15)
National distributor's milk	79	79 (100)

[a] Indicates percentage of total

Table 18 and Figure 13 show that 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 (Act 54 of 1972). Milk-shop 1 never sold milk which was fit for human consumption, whereas the remaining four milk-shops, only complied with the Act between 4% and 33% of the time.

One hundred percent of 79 samples which were purchased from a large national distributor passed all the criteria laid down in the Act (Table 4 and Figure 13).

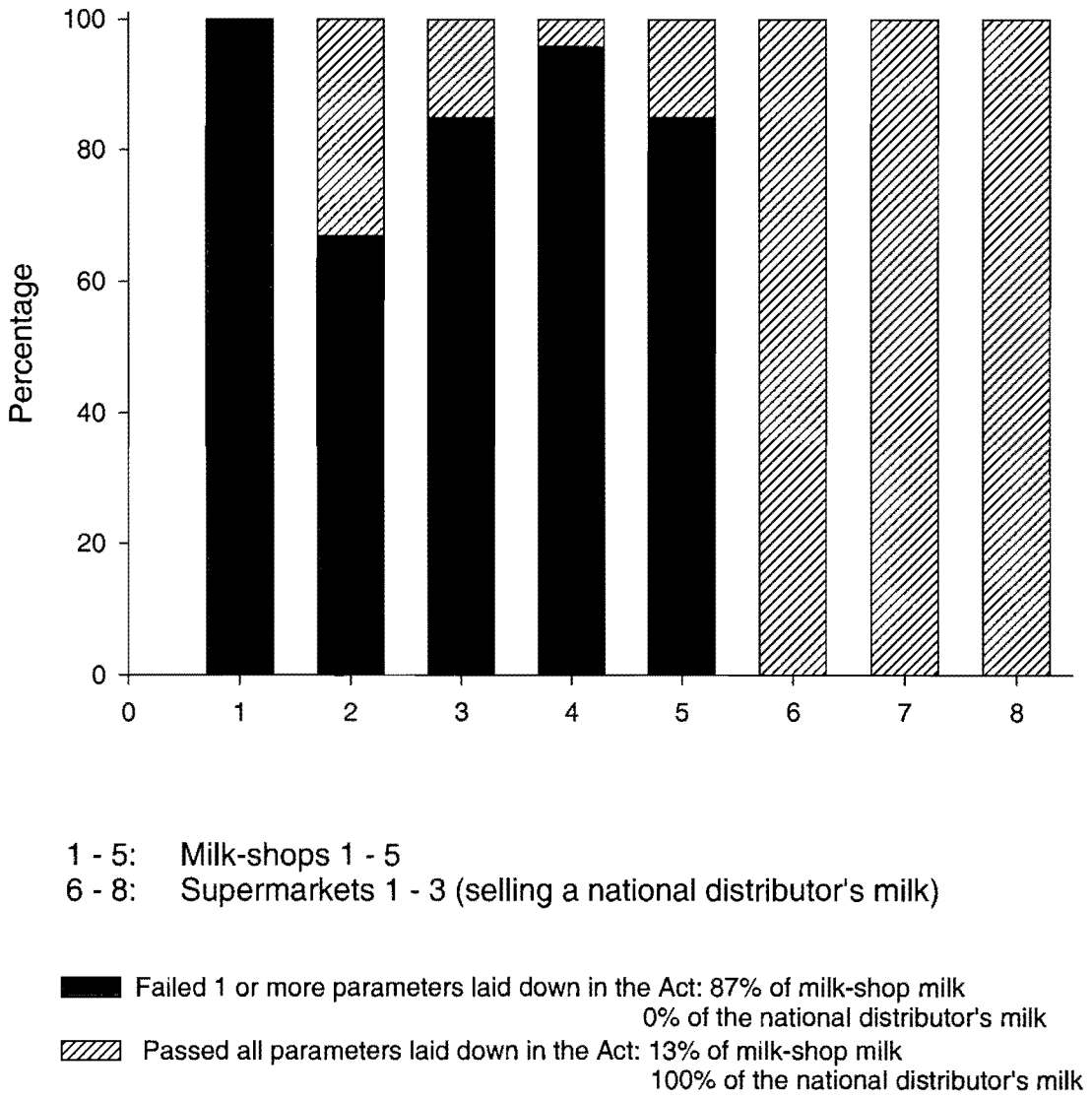


Figure 13: Indication of whether or not milk complied with all the criteria laid down in the Foodstuffs, Cosmetics and Disinfectants Act

CHAPTER 4

DISCUSSION AND CONCLUSIONS

As a result of the deregulation of the milk industry, milk-shops have become a common retail outlet for the sale of fresh milk over the past few years. This study aimed to determine the quality of milk available to the consumer, and to look at whether or not there was a difference between milk sold from milk-shops and milk sold by a large national distributor. In addition, the milk bought from both the milk-shops and the national distributor was also analysed to see whether it contained selected public health hazards.

The study showed that milk-shop milk quality differed significantly ($p < 0.05$) from the milk which originated from the national distributor and varied greatly between milk-shops and between sampling days over the six-week period. Milk from the national distributor showed minimal variation and was always well within the parameters laid down by law.

Price of the Milk

One of the deciding factors for the consumer on whether to purchase milk from a milk-shop or not, is the price of milk. The price of milk-shop milk should be lower than milk from other sources as the cost of packaging is excluded when consumers use their own containers.

The price of the pre-packaged milk-shop milk varied from R2.80 to R3.40 per litre (a 20% difference in the price), which was more expensive than the sachets from the national distributor, which varied between R2.85 and R2.90 per litre. Had the milk however, been purchased directly from the bulk tank, it would have been on average about R0.60 to R0.80 cheaper, as the cost of the container would then be eliminated. This would work out at about a 25% to 33% reduction in cost, and would make it cheaper than the national distributor's

milk. For the consumer this would be one of the benefits of purchasing milk from milk-shops, as a lot of money could be saved over time, providing they used their own containers.

Temperature of the Milk at Purchase

The storage temperature and maintenance of the cold chain is an important factor that influences the safety and keeping quality of milk, especially in a country with warm climatic conditions like South Africa. To delay the growth of micro-organisms, it is recommended to hold the milk at ≤5°C (Lück 1986). Lück *et al.* (1977) report that studies have shown that when the storage temperature is increased to 7°C the standard plate count of a milk sample after 7 days may be as much as 1 000 times higher than on a comparable sample stored at 4°C to 5°C. In another article, Gruetzmacher & Bradley (1999) cite several authors who found that a 3°C rise in temperature decreases the shelf-life of milk by one half. Even at 0°C to 2°C the total bacterial count of milk can increase significantly after 2 days, especially when the initial bacterial count is high (Lück 1986). The normal cold chain can, however, only contribute to a limited improvement of the shelf-life of pasteurised milk (or any other perishable dairy product) when the products contain large numbers of post-processing contaminants which grow at cold chain temperatures (Lück 1986). At elevated temperatures the growth of pathogenic organisms such as *S. aureus, Bacillus* spp., enterotoxin-producing *E. coli* and others is increased and therefore these can cause health hazards (Lück 1986).

Samples taken by Lück (1986) in South Africa showed that the temperature of pasteurised milk in display cabinets in supermarkets in different towns ranged from 0°C to 12°C and varied by 0.3°C to 6.4°C within the display cabinets. Seventy-one percent of 76 samples of pasteurised milk collected from different supermarkets exceeded the bacteriological standards laid down by the regulations at that time, one of the main reasons probably being failure to maintain the cold chain (Lück 1986). In this study, it was not possible to take temperatures inside the shop as this would have created suspicion. Therefore the temperature was taken as soon as possible after purchase, and at least within five minutes of purchase, to prevent a rise in the temperature due to environmental conditions. If there was a slight increase in

temperature in the time between purchase and measurement, it would have been a systematic error throughout all the samples. To overcome the possibility of contaminating the milk, 100 mℓ was decanted and used to take the temperature. The results showed that 73.4% of milk samples were kept at a temperature above 5°C. These high temperatures undoubtably influenced the aerobic standard plate count, the psychrotrophic bacterial count and the coliform count (see later). The highest aerobic standard plate counts were found in Milk-shop 4 whose temperatures varied from 6.5°C to 11°C with none of the milk ever being below 5°C. Temperatures also varied in the display cabinet or refrigerators as two samples were purchased from each shop on a particular day, and 50% of these did not always have the same temperature. Taking into account the literature on the effect of temperature on bacterial counts, one can conclude that the temperatures observed in this study undoubtably played a role in the high bacterial counts seen. The cold chain was not maintained as it should have been. It must also be remembered that this study was done in the winter months, and that had it been done in the warmer summer months, even higher temperatures may have been expected.

Aschaffenburg and Mullen Phosphatase Test

Milk contains at least 20 enzymes, and one of these, alkaline phosphatase, has a thermal resistance greater than that of the most heat-resistant of the non-spore-forming pathogens commonly found in milk (Holsinger *et al.* 1997). This property provided the basis for a negative test for alkaline phosphatase to indicate proper pasteurisation of milk. The alkaline phosphatase test is a rapid test which has been used to determine the efficiency of pasteurisation. The determination of the adequacy of pasteurisation is vital to ensure the safety of pasteurised milk, since individual tests for the detection of pathogens in pasteurised milk require time consuming procedures.

Raw milk with low aerobic plate counts or low somatic cell counts may or may not contain pathogenic bacteria capable of causing illness. Conversely, an elevated total bacterial count may or may not coexist with the presence of human pathogens. Pasteurisation is designed to

destroy all bacterial pathogens common to raw milk, excluding spore-forming bacteria (Steel *et al.* 1997).

The Foodstuffs, Cosmetics and Disinfectants Act provides for two methods for the pasteurisation of milk. The first method is the batch method whereby milk is heated to a temperature of at least 63°C and kept at that temperature for at least 30 minutes, after which it must be cooled within 30 minutes to a temperature lower than 5°C. The second method is the "high-temperature short-time (HTST) method" whereby milk is heated to 72°C for 15 seconds, after which it is rapidly cooled to a temperature of 5°C or less.

Of the 135 milk-shop milk samples tested, 52 (38.5%) were alkaline phosphatase positive indicating inadequate pasteurisation. In a national survey done in 1995 (Department of Health 1995), 14% of all "pasteurised" milk samples failed the phosphatase test. This result was lower than that of this research project, but nonetheless shows that significant percentages of "pasteurised" milk enter the market without being able to pass the alkaline phosphatase test. The alkaline phosphatase positive samples in this study all originated from Milk-shop 1 and Milk-shop 4, who were in effect repeatedly selling incorrectly pasteurised, or raw milk, to the public. Milk-shop 1 never obtained a negative alkaline phosphatase result. This could pose a serious health hazard to the consumer as correct pasteurisation is necessary to kill all the pathogens in the milk. Of significance was the fact that there were "Pasteurised milk" signs at all the milk-shops which misled the consumer into thinking that the milk was safe. Both Milk-shops 1 and 4 even displayed signs outside their shops, advertising the sale of pasteurised milk.

Every milk-shop in the study had a visible HTST pasteuriser in the milk-shop. Whether or not the milk went through the pasteuriser is unknown. Milk-shops 1 and 4 either did not pasteurise at all or the pasteuriser did not work efficiently. Some of the smaller pasteurisers do not have a return valve on the pasteuriser so that milk which was not heated correctly is not diverted back into the pasteuriser. The Foodstuffs, Cosmetics and Disinfectants Act states that if pasteurisation is carried out according to the high-temperature short-time method, then the process should be mechanically controlled with regard to the temperature range of the

milk and the period for which the milk is kept at the prescribed temperature. The apparatus used must be calibrated monthly to ensure the correctness of the pasteurisation process. Thermographic recordings of pasteurisation temperatures must be made and kept for at least four weeks. 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. Since the same milk-shop was tested over a six-week period, the fault in pasteurisation was an ongoing problem and not an isolated case. The owners themselves should also have been aware of the regulations and seen to it that thermographic recordings were done. These recordings would have pointed out that the temperatures of pasteurisation were never achieved. A suggestion might be that people who work with perishable foods such as milk or meat which 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 on the processes involved. Public health aspects should also be part of the training.

A positive alkaline phosphatase result may also indicate the possible addition of raw milk to pasteurised milk. During processing, care must be taken to minimise the risk of contamination of the pasteurised product or the cooling medium. In pasteurisation by the HTST procedure, the pressure on the pasteurised product during the pasteurisation process is greater than that on the unpasteurised product (Ledford 1998). Any leakage will therefore occur in the direction from the pasteurised product to the unpasteurised product or cooling medium, and it is therefore unlikely that unpasteurised milk contaminated the pasteurised product in the pasteuriser. The possibility of this occurring on a daily basis in Milk-shops 1 and 4 is not possible. Once milk has been pasteurised it must be stored in a clean bulk tank and no raw milk should be added to the pasteurised product. Again, the author feels that it is highly unlikely that raw milk would have inadvertently been added to the pasteurised product on a daily basis, unless the milk-shop owners had no idea about the importance of separation of the two products. The local authority should also have picked this up and acted upon this fact, especially since they do monitor milk on a weekly basis.

Reactivation of the phosphatase enzyme by high bacterial numbers in the milk may also lead to a positive alkaline phosphatase test result. In this study, alkaline phosphatase levels in Milk-shops 1 and 4 were always positive over the six-week period, regardless of whether or not the bacterial counts were high. In the authors' opinion it is doubtful that reactivation of the phosphatase enzyme by high bacterial numbers was the cause of a positive alkaline phosphatase test, especially since it occurred on a daily basis. Microbial and reactivated phosphatase tests were not performed in this study, but may have been helpful in determining whether or not the phosphatase present was as a result of postpasteurisation contamination with microorganisms, or due to the raw milk containing spores which survived and germinated to produce heat-stable phosphatases.

If unpasteurised or raw milk is sold it should be labelled as such either on the bottle or sachet, or in the case of a bulk tank, on the tank itself, in accordance with the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972). By not informing the consumer that the milk was not correctly pasteurised, the consumer could unknowingly be placed at risk of picking up potential pathogens, especially if that consumer had specifically chosen the milk because it was stated that it was pasteurised.

The milk sold from Milk-shops 1 and 4 was not fit for human consumption on the basis of the phosphatase test. As pasteurisation was not carried out correctly, it could pose a health hazard for the consumer, especially in immunocompromised people, such as those with AIDS, young children and the elderly (Farber & Hughes 1995).

Bacterial Counts

At the beginning of the trial insufficient serial dilutions were made for assessment of some of the milk-shop samples which had very high bacterial counts (aerobic plate counts, psychrotrophic counts and coliform counts). The bacterial growth from these shops was such that colonies were too numerous to count and as a result of this, no figures could be entered on the data sheet. Therefore, there are some discrepancies in the total number of samples

evaluated. Initially, the statisticians recommended a trial period of four weeks, but because values were missing due to the high counts, the trial was extended by two weeks so that there would be at least 20 samples taken from every milk-shop and from each of the supermarkets. There would also be four samples taken on each day of the week (Monday to Friday) over the period.

Standard Plate Count

Standard plate counts or total aerobic colony counts are used to estimate viable bacterial populations in the milk and reflect the hygienic practices used in the production and handling of the milk (Houghtby *et al.* 1994). They give a crude indication of the shelf-life of the milk. High microbial levels in raw milk may be attributed to factors such as poor hygiene during milk handling either on the farm or in the shop. High counts in pasteurised milk could be due to initial high bacterial counts in raw milk, post-pasteurisation contamination of the milk or failure to maintain the cold chain after initial on-farm primary chilling. Pasteurised milk may not contain more than 50 000 CFU/mℓ of milk (Government Printer 1997 Foodstuffs, Cosmetics and Disinfectants Act).

Standard plate counts for milk-shop milk ranged from 1.0×10^2 to 2.7×10^7 CFU/mℓ, with a median value of 41 000 CFU/mℓ. Using the arithmetic mean was not a good reflection of central tendency with this data, as the data contained some extreme values of bacterial counts which skewed the mean to a very high number. Therefore the median was used as it is less affected by extreme values. This can clearly be seen when one compares the mean and median values of the standard plate count. The data shows an arithmetic mean of 909 132 CFU/mℓ whereas in actual fact 74% of samples had counts lower than 50 000 CFU/mℓ and were therefore within the limits laid down by law (Foodstuffs, Cosmetics and Disinfectants Act). The geometric means were also computed and showed good correlations with the medians, although they were slightly higher. It is disturbing to note that there was such a large percentage of high counts. Results of a national survey done in 1995 by the Department of Health (Department of Health 1995) showed that 48% of pasteurised milk samples did not

comply with the standard plate count. This is higher than the results obtained in this study, but shows that the results of this study are not an isolated occurrence.

High counts were found in both correctly pasteurised milk, and in milk which was not correctly pasteurised. Looking at the shops which did not pasteurise their milk correctly, it was found that 33.3% of Milk-shop 1's and 70.4% of Milk-shop 4 's milk showed counts higher than 50 000 CFU/mℓ. Milk-shop 3 and Milk-shop 5 which did pasteurise their milk correctly showed counts of over 50 000 CFU/mℓ in 51.9% and 70.4% of cases respectively. Statistically, there was no significant ($p > 0.05$) difference in the standard plate count between those milk-shops which pasteurised and those that did not, and this was probably because the standard plate counts varied so much in each of the shops and each shop contained both high and low counts. This can also be seen when looking at the median values of the milk-shops. Milk-shops 1 and 4 (both alkaline phosphatase positive) had median values of 15 600 and 303 500 CFU/mℓ respectively, whereas Milk-shops 2, 3 and 5 (alkaline phosphatase negative) had medians of 900, 52 000 and 103 000 CFU/mℓ (Table 6). It cannot, however, be concluded from the above statement that pasteurisation has no effect on the standard plate count as many other factors, such as the maintenance of the cold chain and post pasteurisation contamination, also play a very important role in the total bacterial count (see discussion on bacterial counts).

There was no significant ($p > 0.05$) difference with respect to the standard plate count between the milk of Milk-shop 2 and that of the national distributor. Milk-shop 2's milk always had counts of less than 43 000 CFU/mℓ with a mean and median count of 6 939 and 900 CFU/mℓ respectively. This shop also differed significantly ($p < 0.05$) from the other milk-shops, indicating that it is possible for a milk-shop to have low aerobic bacterial counts. However, when one looks at the presence of inhibitory substances in the milk, the milk of Milk-shop 2 contained some form of inhibitory substance in 92.6% of samples. These inhibitory substances may well have inhibited the growth of the aerobic bacteria in the milk. Statistically it was not possible to ascertain whether or not the inhibitory substances played a role in the aerobic standard plate count of Milk-shop 2. Combining the data of all the milk-shops, no significant ($p < 0.05$) difference in the aerobic standard plate count could be found between milk which contained inhibitory substances and that which did not.

Over the trial period of six weeks, milk purchased on a Monday had a significantly ($p < 0.05$) higher standard plate count than milk purchased on a Friday. This could be due to the fact that leftover milk from the previous week was sold on a Monday. Bacterial counts would have increased over the weekend, especially if the cold chain was not maintained correctly. If one looks at individual weeks this did not always happen on each Monday at each milk-shop, but the combined data showed this trend.

The standard plate counts of milk from Milk-shop 3 and from Supermarket 3 differed significantly ($p< 0.05$) from each other, even though they were being sold from the same shop. This shows that even though both were sold from the same shop, the origin of the milk is important in determining its quality.

Standard plate counts for the national distributor's milk were always below 9 000 CFU/mℓ, indicating good quality milk from the farm to begin with as well as hygienic processing in the factory, and maintenance of the cold chain in distribution to the point of sale.

Psychrotrophic Bacterial Count

In developed countries, there is general agreement that the psychrotrophic bacterial count is the most reliable method of indicating conditions of production on the farm (Bishop & Juan 1988). As a result of the refrigeration of milk, psychrotrophic bacteria dominate over the mesophiles, and many argue that the standard plate count therefore cannot reflect the true conditions of production practices on the farm. In fact, spoilage of milk is accomplished by smaller numbers of psychrotrophic bacteria than by mesophilic bacteria. This is the reason why the standard plate count (mesophilic count) of milk will not give a reliable indication of the possible keeping quality of refrigerated milk (Bester *et al.* 1986). In Europe and the United States of America therefore, all milk is also subjected to the psychrotrophic bacterial count, which may not be greater than 100 000 CFU/mℓ (cited by Phillips & Griffiths 1990, Suhren & Heeschen 1990).

Psychrotrophic bacteria are ubiquitous in nature and are common contaminants of milk. They originate from equipment, milk stone deposits, air, water, and the people working with the milk (via the air) and give an indication of the potential shelf-life of the milk as they are able to grow under refrigerated conditions. Psychrotrophs are not part of the normal udder microflora, so the number present in raw milk is related to sanitary conditions during production and to length and temperature of storage before pasteurisation (Frank *et al.* 1992). The growth of psychrotrophs in milk result in off flavours such as stale, bitter, putrid and rancid tastes (Bester *et al.* 1986, Shah 1994). Defects in the milk such as coagulation and thickening which result from heat resistant lipases and proteinases degrading the casein also occur (Bramley & McKinnon 1990, Frank *et al.* 1992). These defects occur as a result of the extracellular enzymes produced by the psychrotrophs which survive heat treatment. Although psychrotrophs may account for less than 10% of the initial raw milk flora, they grow rapidly and dominate the flora during refrigeration (Shah 1994).

Internationally, the standard psychrotrophic bacterial count requires incubation of the plate for 10 days at 7°C (Marshall & Peeler 1992). This length of time is commercially unacceptable to determine the psychrotrophic population of raw or pasteurised milk. It does not allow for corrective action to be taken should a problem arise. In order to increase the numbers of gram negative psychrotrophic bacteria in milk, preliminary incubation may be used (Byrne *et al.* 1989). Various elevated incubation temperatures, e.g. 18°C or 21°C, have been recommended to give a more rapid and accurate estimate of the psychrotrophic population. The modified Petrifilm method with a pre-incubation temperature of 21°C for 18 hours, and a plate incubation period of 48 hours, was chosen for this study since it gave results within 66 hours and was highly correlated with the psychrotrophic bacterial count done at 7°C for 10 days. This method provides an estimate of the growth potential of psychrotrophic bacteria that may be present in the sample (White 1998). This selective preliminary incubation, followed by a rapid plating technique, could become a rapid test for potential shelf-life evaluation.

The rate of multiplication of psychrotrophic bacteria in refrigerated raw milk is often increased by the presence of high initial numbers. The growth of psychrotrophs in farm bulk

tank milk is also stimulated when cooling to 4°C is slow or delayed. Cooling from 35°C to < 5°C should be achieved within 2 hours (Thomas *et al.* 1971). In South Africa the regulations allow for a 3 hour time period to cool the milk down to < 5°C (Health Act, No 63 of 1977: regulations relating to milking sheds and the transport of milk, as amended). Farm bulk tank milk produced under reasonably hygienic conditions can be safely held at 4°C or less for 2 to 3 days before processing, but storage at 7.2°C or higher encourages the multiplication of psychrotrophic bacteria and the development of slightly unclean or rancid flavours within 48 hours (Thomas *et al.* 1971).

Only the milk obtained from the national distributor fell within the parameters laid down for psychrotrophs in Europe on all occasions except one. On this occasion, the national distributor's milk which was purchased at Supermarket 3, had a psychrotrophic bacterial count of 480 000 CFU/mℓ which was above the European legal limit and would have been rejected there. The milk purchased on that day had a temperature of 7.5°C which could partially explain the high psychrotrophic count as the storage temperature of milk has a significant effect on the lag phase of psychrotrophic bacteria, and a decrease in temperature results in an increased lag phase (Bester *et al.* 1986). During the rest of the trial period the national distributor's psychrotrophic median count ranged from 8 000 to 13 600 CFU/mℓ.

The psychrotrophic counts in milk-shop milk were extremely high, and even though the standard plate counts of 74% of milk-shop samples were within the legal limit of less than 50 000 CFU/mℓ, none of them would have passed the psychrotrophic count. Lag phases of psychrotrophic bacteria are generally shorter than those of mesophilic bacteria (Bester *et al.* 1986), which might be a reason why the psychrotrophic counts are so much higher than the mesophylic counts.

The presence of psychrotrophic bacteria in pasteurised milk is a result of post-pasteurisation contamination since most of these organisms are unable to survive heat treatment (Shah 1994, Gruetzmacher & Bradley 1999). These results clearly show that production practices in the milk-shops were not desirable, and that perhaps we should look at whether or not we should also use psychrotrophic counts in South Africa to evaluate our milk in terms of the keeping

quality of the milk. The shorter the shelf-life of the milk, the quicker it will deteriorate, even if refrigerated. However, many milk-shops are situated in the poorer socio-economic areas, where consumers might not have adequate refrigeration facilities. Many people may end up discarding milk as a result of the short shelf-life it has, in effect increasing the price they pay for a litre of milk.

Unlike the aerobic plate count, there was no significant ($p > 0.05$) difference in the psychrotrophic bacterial count between the different days of the week. This may have been due to the fact that the counts were high throughout the trial period in each one of the milk-shops. The storage temperature undoubtably played a role here too since the storage temperatures of the milk were on average always above 5°C.

The results showed that there was a significant ($p < 0.05$) difference in psychrotrophic bacterial counts between milk containing inhibitory substances and milk not containing any, which suggests that antimicrobial residues had an impact on the psychrotrophic numbers. Had the milk not contained any residues, it is possible that the psychrotrophic counts may have been even higher.

The results also showed that there was a significant ($p < 0.05$) difference in psychrotrophic bacterial counts between milk which was pasteurised correctly (alkaline phosphatase negative) and milk which was not pasteurised correctly (alkaline phosphatase positive), with counts lower in correctly pasteurised milk. Psychrotrophs are inactivated during pasteurisation and therefore the bacteria found in the pasteurised milk were as a result of post pasteurisation contamination indicating inadequate hygiene procedures in the shop. Failure to maintain the cold chain is another possible reason. The very high psychrotrophic counts in the unpasteurised milk suggest that contamination may have taken place both on the farm as well as in the shop and that high storage temperature may have also played a role.

There was a correlation between the aerobic plate counts and the psychrotrophic bacterial counts in Milk-shops 2 and 4, as well as in the national distributor's milk purchased from Supermarket 1 (Table 8). No correlation could be found in any of the remaining shops. Other

researchers who have looked for a correlation have found that the aerobic standard plate count cannot be used to predict the psychrotrophic count and is therefore also not a very good indicator of the shelf-life of the milk (Bester *et al.* 1986).

Coliform Counts

The presence of large numbers of coliform bacteria in milk are suggestive of unsanitary conditions or practices during production, processing, distribution or storage. Coliforms are destroyed by pasteurisation, and therefore their presence after correct pasteurisation is indicative of bacterial recontamination post-pasteurisation (White 1998).

Coliform counts in milk-shop milk varied tremendously between milk-shops over the six-week period ranging from 0 to 3.4 x 10^5 coliforms per mℓ. Even though 68% of samples had 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 and the geometric mean was 93 coliforms per mℓ. Both are unacceptably high and show that contamination took place post-pasteurisation. However, if one excludes the two milk-shops which sold raw milk, the median coliform count in the remaining milk-shops was below the 20 coliforms per mℓ limit allowed for in the Foodstuffs, Cosmetics and Disinfectants Act (Table 9). Nevertheless, milk-shop owners need to be made more aware of basic hygiene measures when handling the milk. Coliform counts for the national distributor's milk were always zero, again indicating hygienic processing of the milk.

It is possible for milk-shops to keep post-pasteurisation contamination to a minimum, as Milk-shop 3 did not differ from the national distributor's milk. In the national survey carried out in 1995 by the Department of Health (Department of Health 1995), 48% of pasteurised milk samples contained coliforms at levels above the legal limit. This was higher than the result obtained in this study, re-emphasising the fact that the results obtained in this study are plausible. It therefore shows that the standard of pasteurised milk sold in this country is, on the whole, not up to the standards laid down in the Foodstuffs, Cosmetics and Disinfectants

Act, and is in fact being sold illegally. Nevertheless, it must also be remembered that the figures obtained by the Department of Health included milk from both large and small distributors and does not necessarily mean that the results are a reflection of both. In this study, the national distributor's milk never contained any coliforms and therefore always conformed with the standards prescribed in South Africa.

As with the standard plate count, there was a significant ($p < 0.05$) increase in coliforms in Monday milk samples compared with Wednesday and Friday samples. This once again suggests that milk not sold was stored over the weekend, and used for sale on Monday. Post-pasteurisation contamination with coliforms must have taken place, and with holding temperatures above 5 °C, these coliforms multiplied over the weekend, giving high counts on Mondays.

It is difficult to produce milk on the farm without any coliforms being present since coliforms are ubiquitous in the environment. Pasteurisation is therefore a processing step that ensures the destruction of coliforms acquired on the farm. Milk-shops 1 and 4 differed significantly ($p < 0.05$) from the other shops with respect to the coliform count, in that they were much higher. They were also the only two shops which did not pasteurise correctly, and the results clearly showed that there was a significant ($p < 0.05$) difference in coliform count between those shops which pasteurised and those which did not, indicating that pasteurisation is an important measure in decreasing coliform counts.

Antibiotics or other inhibitory substances in the milk suppressed coliform bacteria in the milk since there was a significant ($p < 0.05$) difference in coliform count between milk samples containing inhibitory substances and those which did not.

Bacterial Counts in General

Standard plate counts, coliform counts and psychrotrophic counts in milk-shop milk were often above legal limits. It is not known how long milk was stored in the display cabinets or

bulk tank prior to purchase, although results showed that milk on a Monday often had higher bacterial counts than milk purchased later in the week. This suggests that milk, which was often contaminated by spoilage organisms, was not discarded but kept until it was sold. Milk no longer fresh should have been discarded by shop owners and not kept on the shelf until it was ultimately purchased. Sampling took place every second day except for the time period over a weekend when it took place four days later due to the design of the study. As bacterial counts were highest on a Monday, it shows that the milk-shops purchased milk at least once a week, if not more. Milk-shops buy milk direct from the farm and can process immediately, unlike the bigger distributors who lose an average of four days from the time it is pumped into the tanker on the farm, until it is finally purchased by the public in a retail store. Hygienically produced milk ought to have a shelf-life of at least 10 days. Bishop (1993) showed that the estimated shelf-life of milk with a total bacterial count of < 1 000 CFU/mℓ was > 14 days. Milk with a total bacterial count of between 1 000 and 200 000 CFU/mℓ had an estimated shelf-life of 10 to 14 days. Milk with a total bacterial count of > 200 000 CFU/mℓ had an estimated shelf-life of < 10 days. These estimates show that the shelf-life of milk-shop milk was most probably 7 days or less, which is very short.

Inadequately washed and sterilized milking and milk handling equipment constitute the main source of bacteria in farm milk supplies (Thomas *et al.* 1971). It has been reported that good procedures of cleaning and sterilizing milking equipment resulted in milk with lower numbers of total and of psychrotrophic bacteria than in milk produced on farms with poor procedures (cited by Thomas *et al.* 1971). Milkstone deposits are caused by inadequate milking machine cleaning and/or poor quality (hard) water in the dairy. Milkstone is a combination of mineral and protein deposits on stainless steel and other surfaces. It can protect bacteria from hot water, detergents and the sanitisers used to clean the milking machine. It provides nutrients for the rapid growth of bacteria in the milk. When the milkstone deposits break down or are dislodged from the stainless steel surface, large numbers of bacteria can be released into the milk.

The temperature of the cleaning agent is also of utmost importance. Only 1% of the rinses of pipeline plants had unsatisfactory colony counts when the initial temperature of the detergent

wash solution circulated was >77°C, whereas nearly 40% had unsatisfactory counts and 30% of the rinses contained coli-aerogenes organisms when the initial circulation temperature was < 65°C (Thomas *et al.* 1971). It was further found that poorly cleansed pipeline milking plants contributed to exceedingly large numbers of bacteria in the milk, especially in the presence of milkstone or milky residues. The correct concentrations of the detergent used is essential, as is the time the detergent is in contact with the equipment (contact time) and the circulation temperature.

Thomas *et al.* (1971) also found that the bacterial content (including the psychrotrophs) of milk was much higher in summer than in winter, particularly when production practices were poor. This study was done in the winter months from June to August, and counts were therefore expected to be even higher in the summer months when daily temperatures are much higher than in winter.

High bacterial counts could also be due to farmers not chilling the milk fast enough on the farm or not adequately maintaining the cold chain after primary chilling, such as when the milk is transported or after bottling in the shop. At sale, Milk-shop 1 had temperatures varying from 3.5°C to 10°C with 48% of samples less than 5°C. Milk-shop 4's temperatures varied from 6.5°C to 11°C with none of the milk ever being below 5°C. Temperature abuse will influence bacterial counts.

Another reason for high bacterial counts could be poor quality water on the farm. Water in the dairy should be potable as not only can poor quality water have an effect on bacterial counts, but hard water has a negative effect on cleaning agents, binding to them so that higher concentrations have to be used in the cleaning of equipment (Clark 1962). If the water in the dairy is contaminated with bacteria it must be chlorinated (2 - 3 ppm)(Giesecke *et al.* 1994). The water used in the milk-shop for cleaning out the storage tanks and pasteuriser must also be potable. As all the milk-shops were situated in developed urban areas, it is most likely that the water used in these milk-shops was chlorinated municipal water, and therefore the water should have been potable.

Farmers who sell milk to milk-shops are usually small-scale farmers who cannot afford to distribute their own milk. They often also buy in milk from other small farmers to make up volumes. This can have detrimental effects on milk quality as the more milk is handled and the cold chain is broken, the higher bacterial counts become.

Supervision in the parlour and milk-room is often lacking on South African dairy farms. The milkers are usually left on their own. Workers are also not trained appropriately on the correct milking procedure or in the hygienic handling of milk once it leaves the cow. They are often ignorant of basic hygiene measures.

Transport of milk to the milk-shop is another area where bacterial counts can be increased. Milk is often transported in small bulk tanks on the back of trailers or bakkies. These are not refrigerated and therefore the cold chain is broken, especially when the weather is hot. Some milk-shop owners have to travel to various farms to pick up milk supplies to make up a large enough volume. Pipes used to pump the milk from the bulk tank on the farm into the tank on the vehicle are often not cleaned in between farms and the milk remaining in these pipes during transport to the next farm become an ideal medium for bacteria to multiply in at ambient temperatures.

In the milk-shop, the effectiveness of cleaning and sanitising practices greatly influences the level of contamination, and the pasteuriser can be a source of contamination if it is inadequately cleaned or maintained (Ledford 1998, Gruetzmacher & Bradley 1999). Micro-organisms may originate from pipes and valves. Air and condensates may also be significant sources of contamination, as can be the bulk tank where milk is stored after pasteurisation. It was shown that aseptically sampled milk from the outlet of a clean pasteuriser could achieve a shelf-life of more than 30 days (Gruetzmacher & Bradley 1999).

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 employ hygiene programmes on the farm and in the shop which should

consist of good manufacturing processes, quality control, hazard analysis and critical control point principles.

Escherichia coli

E. 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. *E. coli* is readily isolated from the intestinal tract of warm blooded animals, including dairy cattle. Raw milk is contaminated through contact with faecal material. *E. coli* may also be isolated from the milk of mastitic animals. *E. coli* is destroyed by pasteurisation with a wide margin of safety (Holsinger *et al.* 1997, Ryser 1998).

Nearly 18% of milk-shop milk samples were *E. coli* positive, 95% of which originated from the two milk-shops which sold inadequately pasteurised milk. Unfortunately, on many of the plates containing 1 mℓ of undiluted milk from Milk-shop 4, it was impossible to accurately say whether or not *E. coli* was present since the plate contained so many coliforms that all one could see was one enormous gas bubble under the film. These were labelled as suspect samples. This would have to be a drawback of using the dry rehydrated film method for the coliform and *E. coli* count, since high coliform numbers obliterate *E. coli* organisms. Other methods such as the Modified Eijkman Test for *E. coli* might be more useful in such cases, but this method is far more labourious to perform and takes far more time to get results. If one were to add in the suspect samples from Milk-shop 4 then nearly 26% of all milk-shop samples purchased were positive for *E. coli*. In fact then, 78% of Milk-shop 1's milk and 41% of Milk-shop 4's milk contained *E. coli*, indicating gross contamination of the milk at farm level. This high prevalence is possible since the milk from these two milk-shops was not pasteurised correctly, and was therefore raw. Such milk is very easily contaminated with *E. coli* since the organism is prevalent in the environment. These results again correspond well with the results obtained by the Department of Health in 1995 (Department of Health 1995), where it was found that 26% of all pasteurised milk samples contained *E. coli*.

As *E. coli* is destroyed by pasteurisation, the presence of this organism in pasteurised milk samples would indicate human contamination after pasteurisation by handlers who practice poor personal hygiene or by contact with water containing human sewage. People handling milk should be educated in safe food handling techniques and proper personal hygiene practices including hand washing after using the lavatory.

The national distributor's milk was always negative for *E. coli* organisms indicating effective pasteurisation of raw milk and no recontamination afterwards.

An attempt was made to determine the presence of heat-stable *E. coli* enterotoxins in the milk using the *E. coli* ST EIA test kit (Oxoid), which is a competitive enzyme immunoassay used to detect *E. coli* enterotoxins. Centrifuged milk samples were used as it was not possible to isolate *E. coli* organisms from the petrifilm so as to culture them. The method however, clearly stipulates that a culture filtrate must be used. The results, after using the whole milk, had to be dismissed as most of them showed a (false) positive result. Future research might look at the presence of these toxins in South African milk supplies.

Staphylococcus aureus and *S. aureus* Enterotoxins

Staphylococcus aureus is ubiquitous within the farm environment and carried by approximately half of the human population, and therefore many dairy products contain low levels of enterotoxigenic staphylococci (Ryser 1998). Forty percent of all milk-shop milk contained the organism *S. aureus*. Organisms in this study were found in incorrectly pasteurised milk, which indicates that they may have originated from animals with subclinical mastitis. *S. aureus* is the dominant mastitis organism in South Africa, being prevalent in at least 75% of South African herds (Giesecke *et al.* 1994, Swartz *et al.* 1984). *S. aureus* in raw milk may also have originated from human carriers.

The organism was also isolated from milk which had been correctly pasteurised, indicating that it must have originated from the people who handle the milk, since this organism is

destroyed by pasteurisation (Asperger 1994). 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 (Asperger 1994).

S. aureus is a poor competitor and is readily outgrown by lactic acid-producing micro-organisms, so growth is limited in raw milk (Holsinger *et al.* 1997, Asperger 1994). Notwithstanding this fact, 96.3% of milk from Milk-shop 1 and 29.6 % of milk from Milk-shop 4 contained the organism. If the milk is not refrigerated, the enterotoxigenic strains can grow and produce enterotoxin. Of the 51 *S. aureus* cultures which were tested, four (7.83%) produced heat stable staphylococcal enterotoxins A (SEA), B (SEB), D (SED) or a combination of them. All toxin producing strains isolated originated from Milk-shop 1. Four out of 19 (21%) *S. aureus* strains from this particular milk-shop produced toxins, and were thus enterotoxigenic.

S. aureus enterotoxins can survive the pasteurisation process and may cause food poisoning in man, thereby posing a health risk (Flowers *et al.* 1992). When ingested they cause nausea, vomiting and diarrhoea. Even if this milk had subsequently been pasteurised correctly, the toxin remains a health risk since it is heat stable. The occurrence of the enterotoxigenic strains in milk calls for improved udder health, milking hygiene and milk handling.

SEA/SEB and SEA/SEB/SED were the most frequently produced enterotoxins. Bolstridge & Roth (1985) 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 the majority producing enterotoxins A or C or a combination of A and C. A study done in Kenya by Ombui *et al.* (1992) found enterotoxin C to be the most frequently encountered toxin in that country. 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 (Asperger 1994).

The production of enterotoxin by staphylococci can be completely managed by temperature control. Toxin production is favoured if the milk is cooled slowly after milking, if it is inadequately stored, and if storage before use is too long. Multiplication of the bacteria and

toxin formation are almost completely inhibited below 7°C (Asperger 1994). In this temperature range only psychrotrophic spoilage bacteria will grow which have a distinct effect on staphylococci. Temperature abuse above 10°C and poor starter culture activity during fermentation are the most often cited contributing factors in dairy-related outbreaks of staphylococcal poisoning (Ryser 1998).

The national distributor's milk did not contain any *S. aureus*, or toxins in the 15 samples tested.

Thermo-resistant Inhibitory Substances

Of public health importance was the fact that 54% of milk-shop milk samples purchased contained some type of inhibitory substance. These 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.

Antibiotics are administered to control diseases such as mastitis in lactating animals, and antibiotics applied either by infusion, injection or orally, may enter the milk supply. The main source of antibiotic contamination in the milk is through the application of intramammary products. Untreated quarters may be contaminated via the blood circulation or by diffusion. Other ways of contamination are the percutaneous, intrauterine, subcutaneous, intramuscular and intravenous application of antibiotics.

Antibiotics can enter the milk supply:

i) if the correct withholding period is not adhered to by the farmer after administering antibiotics to lactating cows

ii) through extra-label use of antibiotics (ie. increased dose, increased frequency of treatment, unproven route of administration) which is shown to be associated with an increased risk that antibiotic levels in milk will persist beyond the milk-withholding time period (Angelidis *et al.* 1999)

iii) through accidental or intentional transfer of a batch of milk that is contaminated with antibiotics in the bulk tank.

Milking equipment which is not rinsed adequately may also contain residues of disinfectants which are used in the cleaning process.

The prevalence of inhibitory substances in milk-shop milk was high, ranging from 33.3% in Milk-shop 5 to 92.6% in Milk-shop 2. Residues are illegal in terms of the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972). The fact that there are antibiotics in the milk may mean that the milk originated from cows which were treated for mastitis, and therefore the milk may also contain large numbers of potentially dangerous pathogens such as *S. aureus* which could pose another threat to public health. There may also be biologically active metabolites or unchanged drugs in the milk which may result in problems such as allergies in man or may lead to increased resistance of micro-organisms (Bishop *et al.* 1994). Bacterial resistance can affect therapy by reducing the ability of an antibiotic to eliminate or control infection. Conditions favouring the development and selection of bacteria carrying resistance factors are thought to be associated with repeated or prolonged use (Sundlof & Cooper 1996). In the worst case, infection can overcome the victim before appropriate therapy can be instituted. In some cases, resistance renders an infection immune to virtually every antibiotic available. More often bacterial resistance increases therapeutic costs because inappropriate drug choices prolong diseases.

Nearly all reports of adverse reactions from food-borne residues implicate penicillin as the offending agent, and the source of penicillin residues is most often milk or dairy products (Sundlof & Cooper 1996). These milk residues most likely originated from intramammary infusion of penicillin used in the treatment of mastitis. Although a substantial number of farm milk samples have been found to contain small amounts of penicillin, there have been relatively few published reports of adverse reactions from milk residues (Sundlof & Cooper 1996). Symptoms varied in intensity from mild skin rashes to exfoliative dermatitis (Sundlof & Cooper 1996). Both epidemiologic and experimental data indicate that food-borne residues

of penicillin as low as 5 to 10 international units (IU) are capable of producing allergic reactions in previously sensitized persons (Sundlof & Cooper 1996).

It cannot be excluded that antibiotics, e.g. chloramphenicol, may have a direct toxic effect on the consumer. In addition to acute incidents related to residues, there is also concern about the long term effects on public health from chronic exposure to illegal residues. Milk is an important part of the diet of infants and young children and unfortunately, on a body weight basis, they consume greater quantities of milk than adults. Residues, such as those found in the milk-shop milk, are therefore of even greater concern in this segment of the population.

Intramammary infusions are readily available to dairy farmers who can buy many of them over the counter. There is also no monitoring programme in South Africa to ensure that farmers adhere to the correct withdrawal period. Previously it was stated that 40% of all milk-shop milk contained the organism *S. aureus*, a major cause of mastitis. This may very well be the reason why the farmers are treating their animals with antibiotics. These findings may reflect misuse or abuse of antibiotics by farmers.

Inhibitory substances may have influenced the test results of psychrotrophic and coliform bacterial counts, giving lower values due to inhibition of bacterial growth, as there was a significant difference in these counts between milk containing inhibitory substances, and milk not containing any. Suhren (1995) demonstrated that the activity of bacteria when the total bacterial count exceeded about log 6.5/mℓ (3 million/mℓ) could result in false negatives. She concluded that this was probably due to the ß-lactamase producing micro-organisms within the flora. Only five samples, all originating from Milk-shop 4, had counts in excess of 3 million CFU/mℓ on the standard plate count. They were all, however, positive for inhibitory substances.

The brilliant black reduction test is a screening test and does not specify what inhibitory substance was used. Positive samples would need to be further analysed by more sophisticated methods which allow for the identification and quantification of the antimicrobial substance used. Penicillinase may be added to the positive milk sample and

reincubated to see whether or not the substance present was a beta lactam antibiotic (penicillins, cephalosporins) or not.

The major non-specific antibacterial factors in milk, namely lactoferrin and lysozyme, whose content is increased in colostrum and mastitic milk, have been given as reasons for "false" positives in microbial inhibitor tests. Studies have shown that the concentration of single components needed to achieve an inhibition was unphysiologically high, whereas the combination of both substances showed a synergistic effect and therefore showed positive results in physiological concentrations (Suhren 1995, Suhren & Heeschen 1996).

False positive assay outcomes are most common on individual animal samples, but less so in bulk tank samples, and are rarely found in tanker milk or milk obtained from more than one supplier (Cullor 1996). Milk-shop milk usually originates from more than one supplier (personal communication with shop owners).

Bacillus stearothermophilus is insensitive towards practically applied sanitisers and is not markedly influenced by concentrations to be expected in milk samples (Suhren 1995, Suhren & Heeschen 1996).

Somatic Cell Count

High somatic cell counts are found in cows in very early or very late lactation, as well as in cows with udder infections, and consist mainly of white blood cells (pus cells) and some epithelial cells. Somatic cells affect the soundness of milk and high counts make the milk aesthetically unacceptable. Eighty-one percent of milk-shop milk samples had somatic cell counts below the legal limit of 500 000 cells/mℓ. From an udder health point of view, a count of 500 000 cells/mℓ is high and indicates that a large percentage of the dairy herd has subclinical mastitis. Only bulk tank (herd) counts of less than 200 000 cells/mℓ suggest that mastitis is under control. Even though the somatic cell counts were below legal limits, in 83% of the milk-shop samples they were above 200 000 cells/mℓ and therefore high from an udder

health point of view. This is substantiated by the high prevalence of *S. aureus* in milk-shop milk. *S. aureus* was present in 96% and 41% of Milk-shops 1 and 5 respectively, and both these shops had mean somatic cell counts above 400 000 cells/mℓ.

The national distributor's milk always had counts of less than 150 000 cells/mℓ. 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 was so constant and so low over the six-week period.

Brucella abortus

Brucella abortus is a zoonosis which causes undulant fever in man and has not yet been eradicated from cattle in South Africa. Milk-borne brucellosis continues to be a problem worldwide although vaccinations and milk pasteurisation have drastically decreased the incidence of brucellosis transmitted via milk (Flowers *et al.* 1992). Once infected with brucellosis, cattle shed the organisms intermittently in the milk for as long as five months, thereby infecting those who drink the milk (Ryser 1998). When naturally contaminated raw milk is held at 25°C to 37°C, *Brucella* populations typically decrease to non-detectable levels within 2 to 3 days. However, brucellae survive at least 42 and 800 days if such milk is stored at plus 4°C and minus 40°C respectively (Ryser 1998). Commercial pasteurisation effectively kills *B. abortus* with a large margin of safety (Garin-Bastuji & Verger 1994, Ryser 1998).

There is no single test by which a bacterium can be identified as *Brucella* (Garin-Bastuji & Verger 1994). A combination of growth characteristics, serological and bacteriological methods is usually needed for identification. *Brucella* are usually present in low numbers in bulk tank samples and isolation from such milk specimens is very unlikely (Garin-Bastuji & Verger 1994). As a result of this, the diagnosis of *Brucella* infection is easier if based on serological methods. The brucella milk ring test (BMRT), which detects anti-*Brucella* antibodies in the milk, is routinely used as a screening test for the detection of brucellosis.

All samples in this trial tested by means of the brucella milk ring test were negative for antibodies to the organism. In the field the sensitivity is increased when the test is repeated each month. It is therefore unlikely that there could have been false negatives as all milk samples were tested at least 2 to 3 times per week. Due to the fact that the brucella milk ring test is highly sensitive, it is far more likely to get false positive results and these have to be interpreted with care. In a national survey done in South Africa in 1995, a total of 11 out of 918 (1.2%) samples tested were found to be positive for the presence of *Brucella* antibodies (Department of Health 1995). The prevalence of the disease is low in South Africa, although unconfirmed reports have indicated that the incidence of the disease is on the increase due to the fact that the Brucella Eradication Scheme is no longer administered by the Government, and therefore no compensation is paid out to positive herds which are slaughtered.

Salmonella

Salmonella spp. are food-borne zoonotic pathogens which can contaminate milk and other foods as a result of poor hygiene practices. Salmonellae are widespread in the environment and can therefore enter the milk from various sources including insects, birds, rodents, pets, cattle, water and humans (El-Gazzar & Marth 1992). Salmonellae normally grow at 35°C to 37°C, but can grow at much lower temperatures, provided that the incubation time is suitably extended (El-Gazzar & Marth 1992). Standard vat and high-temperature, short-time pasteurisation destroy salmonellae with a wide margin of safety (Marth 1969, Ryser 1998, Holsinger *et al.* 1997). *Salmonella* decreases in milk during extended storage at less than or equal to 7°C, and to minimize problems, milk or any other foods should be held at or below 2°C to 5°C at all times (El-Gazzar & Marth 1992).

The gastrointestinal illness which develops from the ingestion of *Salmonella* spp. can be treated successfully with antibiotics, but there is a segment of the population (immunocompromised people) who will develop serious complications and may even die, if infected (Mossel 1987, Holsinger *et al.* 1997).

All *E. coli* positive samples tested for the presence of *Salmonella* were negative for the latter group of organisms. Nevertheless, this does not preclude the fact that other faecal organisms such as *Campylobacter* or *Yersinia* may well be present in such samples. The low incidence of this pathogen in raw milk samples could possibly have been due to the high aerobic plate counts found in the incorrectly pasteurised milk. As with pathogens such as *S. aureus*, lactic acid producing bacteria may compete with *Salmonella* and limit their growth.

If salmonellae had been present in milk-shop milk, numbers could have increased as temperatures were often above 5°C. At 12°C and 20°C, *Salmonella* populations double every 8.8 and 20 hours respectively, which reinforces the need for constant refrigeration (Ryser 1998).

The organism *Campylobacter* was not looked for in *E. coli* positive samples as the survival of many strains of *C. jejuni* in raw milk is poor (Flowers *et al.* 1992). In general, *C. jejuni* grows poorly in food and dies rapidly when exposed to ambient temperature and atmospheres. This could explain why low incidences, and difficulty in recovery of the organism from suspect milk samples, have been reported (Flowers *et al.* 1992). However, only low numbers of *C. jejuni* (2 to 3 cells per millilitre) are needed to produce symptoms of gastroenteritis in humans (Robinson & Jones 1981). Further research might be needed in this area, perhaps looking at the incidence of *C. jejuni* in bovine faecal material where it might be isolated more easily.

pH

The average pH of normal cow's milk is considered to be 6.6. The mean pH value at sale for the milk-shop milk and for the national distributor's milk was 6.76, and they did not differ from each other. The mean pH value after incubating the milk at 21°C for 18 hours for milk-shop milk samples was 6.62 and for the national distributor's milk was 6.77, and this was a significant ($p < 0.05$) difference. The milk-shop milk was also visibly thicker than the national

distributor's milk after incubation. At this stage, the national distributor's milk still showed a normal consistency.

By incubating the milk, significant numbers of bacteria were able to multiply and form lactic acid which resulted in a small decrease in pH. This corresponds to the fact that there was also a significant difference between milk-shops and the national distributor's milk in respect of the psychrotrophic count which was also done after incubating the milk at 21 °C for 18 hours.

Incubating the milk, to determine the consistency of the milk may give a crude indication of the psychrotrophic count of the milk, and may also possibly give an indirect indication of the shelf-life of the milk.

GENERAL CONCLUSIONS

The results showed that milk-shop milk differed significantly ($p < 0.05$) from the milk which originated from the national distributor, and that 87% of the milk samples purchased at milk-shops were not fit for human consumption on the basis of the Foodstuffs, Cosmetics and Disinfectants Act. They further showed that the milk purchased from milk-shop outlets is of a poor bacteriological quality and that many samples contained pathogens, residues of inhibitory substances and toxins which may affect the health of the consumer. Consumers are therefore unwittingly exposed to unnecessary health risks, by drinking unsafe milk. These findings are similar to those found after a survey done throughout South Africa in 1995 by the Department of Health which concluded that 73% of pasteurised milk samples did not comply with all the regulations (Department of Health 1995). Their results included the milk of national distributors. In this study it was found that all the samples purchased from the national distributor passed all the criteria laid down in the Act, and therefore samples which 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 incorrectly pasteurised, and the high prevalence of *E. coli* and *S. aureus* in these raw milk samples proves the greater risk of raw milk. People might mistakenly believe that if they have been drinking raw milk for a long time, they will not become ill from it. However, if there are new pathogenic organisms in the milk to which the consumers have not been exposed, or normally occurring bacteria are present in very high numbers, then illness can occur. However simple exposure to pathogens does not necessarily lead to human infection or disease. The health impact of exposure is influenced by the volume of milk consumed, the concentration of the pathogens within that milk, the total number of organisms to which a person is exposed through various sources and the dose-response of an individual to such exposure (Steele *et al.* 1997). These factors will vary between situations and individuals. 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. These would include pregnant women, alcoholics, diabetics, transplant recipients, AIDS and cancer patients, very young infants, steroid users, and patients with chronic renal disease and iron storage disorders (Farber & Hughes 1995).

Not only can unsafe milk affect the health of the consumer, but it may also have economic implications such as medical and hospitalization 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 chain, effective pasteurisation and prevention of post-pasteurisation contamination. People handling milk should be educated in safe food handling techniques and proper personal hygiene practices.

There is a need for more stringent control over milk-shops by the relevant authorities. However, public education is also needed as legislation alone is insufficient.

The results of this study have shown that we can reject the null hypothesis and accept the alternative hypothesis which stated that there was a statistically significant difference in quality at point of sale between milk sold from "milk-shops" and milk which originated from a commercial national distributor.

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Addendum I

DRY REHYDRATED FILM METHOD FOR STANDARD COLONY COUNT

(Petrifilm 3M)

(Method according to Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972: Regulations relating to milk and milk products, No R.1555. *Government Gazette* No 18439, 21 November 1997, 4-29)

(1) Mix milk thoroughly before sampling from bulk milk

(i) Laboratory work must be performed at room temperature.

(ii) Prepare a 1:10 dilution by adding 1 mℓ of milk to 9 mℓ of sterile phosphate buffer. Mix well. Prepare a 1:100 dilution by adding 1 mℓ of the 1:10 dilution to 9 mℓ of sterile phosphate buffer. Mix well. Prepare a 1: 1000 dilution by adding 1 mℓ of the 1:100 dilution to 9 mℓ of sterile phosphate buffer. The final pH should be between 6.6 and 7.4.

(2) Place the films for aerobic bacterial counting on a flat surface and label them. Lift the top film and carefully transfer 1 mℓ of the 1:1000 dilution to the center of the bottom film by holding the pipette perpendicular to the film. Release the top film to drop onto the sample. Repeat the process with the 1:100 dilution of the sample.

(3) Distribute the sample evenly on the film by applying gentle downward pressure with a spreader. Remove the spreader and leave the film undisturbed for one minute to solidify.

(4) Stack the films in piles of not more than 20 and incubate the films, with the clear sides up, at 32° C ± 1° C for 48 ± 2 hours.

(5) Remove the films from the incubator at the end of the incubation period and count the colony forming units (CFU) with the aid of magnification under uniform artificial illumination.

(i) All the red colonies, regardless of their size and intensity, should be counted. Films with 25 - 250 CFU should be counted. Calculate the number of viable bacteria per mℓ milk.

(ii) An estimated count can be made on films where the CFU exceeds 250, by counting at least 4 squares or 20 percent of the growth area. Calculate the number of viable bacteria per mℓ milk and record as "estimated" count.

(iii) The presence of very high concentrations of colonies cause the entire growth area of the film to become red or pink in colour and/or numerous bacteria are growing on the edges of the growth zone. Report these as too numerous to count (TNTC).

Phosphate buffer

Potassium dihydrogen orthophosphate .....5. 08 g
Disodium hydrogen orthophosphate in 2 ℓ distilled water ..... 13.63 g
Sterilize for 15 minutes at 121° C

Dry rehydrated film for standard colony count

% solids on film

Cold water soluble gel .....1 - 10%
Tetrazolium indicator dye ..... < 1%
Standard method nutrients..... 1 - 5 %

Addendum II

DRY REHYDRATED FILM METHOD FOR COLIFORM AND *ESCHERICHIA COLI* COUNT (Petrifilm 3M)

(Method according to the Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972: Regulations relating to milk and milk products, No R.1555. *Government Gazette* No 18439, 21 November 1997, 4-29)

(1) Mix raw or pasteurized milk thoroughly before sampling from bulk. The pH should be between 6.6 and 7.4.

(2) Place the films for *E. coli* and coliform counting on a flat surface and label them. Lift the top film and carefully transfer 1 mℓ of the milk to the center of the bottom film by holding the pipette perpendicular to the film.

(3) Slowly roll the top film onto the sample to prevent air bubbles being trapped under the top film.

(4) Distribute the sample evenly on the film by applying gentle downward pressure with a spreader. Remove the spreader and leave the film undisturbed for one minute.

(5) Stack up to 20 films and incubate the films, with the clear side up, at 32°C for 24 (±2) hours.

(6) At the end of the incubation period remove the films from the incubator and count the colonies with the aid of magnification under uniform artificial illumination.

Re-incubate films for an additional 24 (±2) hours to detect any additional *E coli* growth.

(i) Blue colonies associated with gas are *E. coli* and red colonies associated with gas are coliform colonies. Colonies that are not associated with gas are not counted as coliforms colonies. All the red and blue colonies associated with gas represent the coliform colony count. Films with 15 - 150 colonies should be counted.

(ii) An estimated count can be made on films where the colonies exceed 150, by counting at least four squares and multiplying the obtained number of colonies by 5. Record as "estimated" coliform colony count.

(iii) The presence of very high concentrations of colonies cause the entire growth area on the film to become purple blue (*E. coli*) or reddish (coliforms) and /or many small colonies and/ or small gas bubbles are present. This must be recorded as too numerous to count (TNTC).

Dry rehydrated film for coliform and E.coli counts

	% of solid on plate
Violet red bile nutrients	1 - 5%
Cold water soluble gel	1 - 10%
Tetrazolium indicator dye	<1%
Glucuronidase indicator	<1%

Addendum III

ASCHAFFENBURG AND MULLEN PHOSPHATASE TEST

(Method according to the Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972: Regulations relating to milk and milk products, No R.1555. *Government Gazette* No 18439, 21 November 1997, 4-29)

1. All glassware and media must be sterile.

2. Milk samples to be examined can be stored at 4°C, but must not be frozen.
   Heat the samples to 20°C - 25°C before examination.

3. **Precautions:**

   3.1 Do not test a milk sample that shows signs of sourness.
   3.2 Pipettes must not be contaminated with saliva.
   3.3 The substrate (di-Sodium-p-nitrophenyl-phosphate) must be stored in a fridge (4°C).
   3.4 Do not perform the test in direct sunlight.
   3.5 Use only distilled water for the test.
   3.6 Glassware must be cleaned in a specific manner and stored separately from other glassware.

4. **Cleaning of glassware:**

   4.1 Rinse with water.
   4.2 Wash thoroughly with warm water containing soda.
   4.3 Rinse with distilled water and air dry.
   4.4 Sterilize and store for future use.

4.5 Glassware for the test shall not be used for any other purpose and shall be kept separate from all other operations in the laboratory.

5. Preparation of chemicals:

5.1 Buffer solution:

Weigh 0.175 g anhydrous sodium carbonate and 0.075 g sodium bicarbonate and place into a 50 mℓ volumetric flask. Fill with distilled water to the 50 mℓ mark. Dissolve the contents by gentle mixing. If the meniscus falls below the 50 mℓ mark, refill with buffer solution and again mix gently. Return the stopper and mark contents clearly.

5.2 Buffer substrate solution:

Weigh 0.03 g p-nitrophenyl phosphate and place into a 20 mℓ volumetric flask. Add above buffer substrate up to the 20 mℓ mark and mix gently. If the meniscus falls below the 20 mℓ mark, more buffer solution must be added and mixed gently. Stopper and store the solution in a fridge (4°C). Protect the solution against sunlight by storing in a brown bottle or wrapping the flask in foil to exclude all light. Label the flask clearly-date, the solution and initial. Under correct storing conditions the solution has a storage life of 7 days after which it must be disposed of.

5.3 Put 5 mℓ of the buffer substrate solution into a test tube (that will fit into the "Lovibond" apparatus) and stopper. Heat contents to 37°C in a water bath.

5.4 Add 1 mℓ of the milk sample, return the stopper and mix by shaking.

5.5 Incubate for exactly 2 hours at 37°C in a water bath.

5.6 Prepare a control sample consisting of 5 mℓ buffer substrate solution and 1 mℓ boiled milk of the same milk sample. Mix by shaking and incubate for exactly 2 hours in a water bath at 37°C.

5.7 Remove the milk sample and control and read the result with the "Lovibond". Use the Lovibond disk displaying A.P.T.W.5 or A.P.T.W.7.

5.8 Record the result.

Addendum IV

COUNTING OF SOMATIC CELLS IN MILK BY MEANS OF THE FOSSOMATIC (FSCC)

(Method as per the instruction manual of the Fossomatic)

1. **General**

Milk samples:

- Unpreserved milk samples should be stored in a refrigerator between 0°C and 5°C for not longer than 60 hours.
- Samples preserved with potassium dichromate can be stored for 7 days at room temperature.

Fossomatic:

- An approximate standardization is carried out by the manufacturers.

2. **Equipment and materials**

- Fossomatic;
- Water bath operating at 40°C.
- Pressure supply of at least 6 bar.
- Fixative: Potassium Dichromate.
- Reagents:
  * Ethidium Bromide;
  * Triton X-100;
  * Potassium Hydrogen Phthalate;
  * Potassium Hydroxide;
  * Ammonia solution (25%);
  * Deionized water;

3. **Preparation of solutions**

A) Basic solutions:

3.1 Ethidium Bromide - **DANGER CARCINOGENIC**

3.1.1 Weigh off 1.0 g Ethidium Bromide

3.1.2 Dissolve the 1.0 g Ethidium Bromide in 1 000 mℓ deionized water. The process can be speeded up by heating to 40-60°C.

3.1.3 Store in a lightproof and airtight bottle no longer than 60 days (1 litre will be enough for ± 10 000 samples).

3.2 Triton X - 100

3.2.1 Dissolve 10 mℓ Triton X - 100 in 1 000 mℓ deionized water heated to ± 60°C.

3.2.2 The solution can be stored airtight for maximum 25 days (1 litre will be enough for ± 5 000 samples).

3.3 **Buffer**

3.3.1 Dissolve 51.0 g Potassium Hydrogen Phthalate and 13.75 g Potassium Hydroxide in 10 litres of deionized water. Process can be speeded up by heating to 50°C.

3.3.2 Add 150 mℓ of the Triton X - 100 solution.

3.3.3 Store airtight no longer than 7 days (10 litres will be enough for ± 1000 samples).

B) **Working solutions:**

4. **Dye solution**

4.1 Add 26 mℓ Ethidium Bromide solution (3.1.3) to 2.5 litres of buffer (enough for ± 250 samples).

5. **Rinsing liquid**

5.1 Add 10 mℓ of Triton X-100 solution (3.2.2) and 25 mℓ of a 25 % ammonia solution to 10 litres of deionized water (enough for ± 350 samples).

6. Working solutions should not be used if more than 7 days old.

7. Procedure for fixing and counting of samples.

7.1 Milk samples must be taken in a sterile, particle-free container with a leak proof cap.

7.2 Add from 5mg to 10mg of potassium dichromate to a 5 mℓ sample and mix to a uniform colour.

7.3 Store for at least 18-24h at room temperature. If they have to be counted within 24 hours, they should be treated with potassium dichromate for at least 3 hours.

7.4 Prepare samples by warming it to 40°C in a water bath for 5 minutes.

7.5 Remove the sample from the bath and keep at room temperature until counted (within 15 minutes).

7.6 Use a clean pipette tip for each sample. Press the tip firmly on the pipette.

7.7 Mix sample prior to counting by inverting it gently at least 5 times.

7.8 Open the cap.

7.9 Draw up 0.2 mℓ sample by pressing the button down to the first stopping position only. Release the button of the pipette slowly while taking care that no air bubbles are drawn up.

7.10 Keep the pipette in a vertical position.

7.11 Wipe tip on the outside gently with a tissue to remove excess milk. Take care not to touch the bottom end of the tip with the tissue.

7.12 Inject the sample into the intake chamber of the Fossomatic by pressing the button on the pipette down firmly and completely and once only.

7.13 Results are displayed and printed and must be multiplied by 1000 to give the somatic cell count per mℓ milk. Identify the result with the sample number.

Addendum V

THE BRILLIANT BLACK REDUCTION TEST FOR DETECTING RESIDUES OF ALL ANTIBIOTICS AND SULFONAMIDES (BR TEST AS)

(Method according to Bishop *et al.* (1994) *In:* Marshall R.T. (ed.) Standard methods for the examination of dairy products. American Public Health Association, Washington, D.C.: 389-391)

The BR TEST AS combines the methods of agar diffusion and colour reduction. The BR test system contains endospores of *Bacillus stearothermophilus* var. *calidolactis*, strain C953, nutrients, the redox indicator "brilliant black," the antifolate "Tetroxoprim (TXP)," and agar-agar. The BR TEST AS detects the residues of all antibiotics and sulfonamides in one cycle. The detection limits of the various antibiotics and sulfonamides are compiled in Table 19. Drug residues exceeding the detection limits inhibit the metabolism of the indicator test organisms during incubation. Thus, reduction of the indicator is prevented and the blue colour of the BR test system is maintained. During incubation of inhibitor-free milk samples, the blue oxidation stage of the redox indicator is irreversibly converted to the yellow reduction stage. The test is useful with raw or pasteurised fluid milk products.

A. *Equipment and supplies*:

1. BR TEST AS 12x8 test strip kit: Contains three BR test plates consisting of 12 strips for 8 samples each and 36 adhesive tapes for sealing the test strips. The test plates, which are welded with an aluminium foil film, have a shelf life of 3 months when stored at 2° to 4°C.
2. Microliter pipet: 0.1 mℓ, with pipet tips.
3. Water bath: Without a mechanical agitator; alternatively a thermostat-controlled heating block (64° ± 1°C).
4. Floating stands made of Styropor: As supports for the BR test tubes in the water bath.

5. Inhibitor-free milk: When the negative result is available, this milk can be frozen in small portions and stored as a negative standard.

6. Positive milk standard:
   a. Lyophilised penicillin standard
   b. Lyophilised sulphonamide standard

   Dissolve each in 5 mℓ of MS (microbiologically suitable) water, producing positive milk standards containing 0.005 IU of penicillin G sodium (a) and 0.5 μg of sulfamethazine per millilitre (b).

B. ***Controls:***

Prepare a negative control to check the proper function and to determine the reduction time. Prepare one positive control from each of the penicillin and sulphonamide standards to check the test sensitivity.

1. Negative control: 0.1 mℓ of inhibitor-free bulk milk.
2. Positive control: Lyophilised positive standards dissolved in 5 mℓ of MS water.

Reduction time is over when the redox indicator in the negative control has changed from blue to yellow. Read the two positive controls at the same time.

Table 19: Minimum inhibitor concentrations (MIC)[a] of antibiotic and sulphonamide substances detectable by the BR TEST AS

Substance	**Minimum Inhibitor Concentrations**
Penicillin-G-Na[b]	0.0025-0.003
Procaine penicillin G	0.0020
Cloxacillin	0.0200-0.040
Ampicillin	0.0015
Cephapirin	0.0020
Dihydrostreptomycin	3.000-5.000
Neomycin	0.200-0.450
Gentamycin	0.350-0.400
Erythromycin	0.030-0.075
Tylosin	0.009-0.030
Chlortetracycline hydrochloride	0.350-0.650
Oxytetracycline hydrochloride	0.200-0.300
Tetracycline hydrochloride	0.200
Chloramphenicol	1.800-3.000
Bacitracin[b]	0.040-0.050
Virginiamycin	0.120-0.200
Sulfadimidine-Na (sulfamethazine)	0.075-0.300
Sulfanilamide	2.000
Sulfadoxine	0.030-0.100
Sulfaguanide	0.200
Sulfathiazole	0.005-0.010
Sulfadiazine	0.010-0.080
Sulfaphenazole	0.007-0.010
Dapsone	0.003-0.006

[a]Because the BR TEST AS functions with milk only, all MIC values have been determined in raw milk. The added inhibitors bind with the milk proteins. Only the free, not the protein-bound, portion has a direct antibacterial effect. Thus, the absolute test sensitivity for some of the drugs is considerably higher than that shown in the Table. The MIC values have been determined in the residue laboratory of the Tierarztliche Hochschule in Hanover, Germany, by Prof. Dr. Wenzel and Dr A Ebrecht.

[b]Values are in international units per millilitre; all other values are in micrograms per millilitre (ppm) of milk.

C. ***Procedure, screening test:***

1. Indelibly and clearly mark each tube, strip, or plate to identify the samples.
2. Remove the cap(s) from the tube(s), or the cover(s) from the strip(s) or plate(s).
3. Add 0.2 mℓ of the well-mixed sample to each tube or well, changing the pipet or pipet tip with each new sample.
4. Cover the tube(s) with the cap(s), or the strip(s) or plate(s) with adhesive foil.
5. Incubate at 64° ± 1°C for approximately 2.75 hours. Incubation time with a heating block is more variable than with a water bath because heat transfer is less efficient in the former. Tubes are placed in the Styropor floater for incubation in the water bath; strips and plates are floated in the bath without a support.
6. Incubate a negative control with each set of tests and each new lot of tubes, strips, or plates within a set. Stop the incubation when the colour in this control changes from blue to yellow. Check for the change in colour at 10-minute intervals after 2.5 hours of incubation.
7. Interpretation: Samples containing inhibitor residues above concentrations in Table 19 show no colour change; that is, they retain their blue to the end of incubation. They are considered positive.

Addendum VI

BRUCELLA RING TEST

(Method according to the standards written up by the South African Institute of Medical Research (S.A.I.M.R.) Code 0615)

A. Equipment and materials

Brucella abortus antigen (obtained from the Veterinary Research Institute, Onderstepoort).

4 x 30 $\mu\ell$ pipette tips

Pipettes

3 x 1 mℓ plastic pipettes

3 x sterile soda glass tubes (15x125 mm)

37°C ±0.5°C incubator

10 mℓ boiled milk (negative control)

30 $\mu\ell$ Brucella positive control (used for TMX controls)

B. Milk sampling

1. Milk must be thoroughly mixed before sampling.
2. The sample should be taken well below the milk surface.
3. Milk from 2-3 cans may be pooled. In the case of bulk tanks a single sample is satisfactory provided the test is done with a double volume of milk but a single volume of antigen.
4. Refrigerate the milk sample at 4°C for at least 12 hours before testing.
5. Milk may be stored for up to 2 weeks at 4°C without loss of titre but souring may be a problem.
6. Avoid excessive shaking of milk sample.
7. Excessive heating (45°C for more than 5 minutes) leads to destruction of Brucella antibody.
8. The test cannot be carried out on homogenized pasteurised milk.

9. The milk must have sufficient cream but too much cream can interfere with test readings.
10. Sour milk makes reading of the test impossible.

C. Method

1. Mix antigen thoroughly and pour sufficient for the day's testing into another bottle and keep at room temperature together with rest of samples for at least 1 hour before testing. Any antigen left after a day's testing must be discarded.
2. Shake antigen thoroughly and dispense 0.03 mℓ (30 $\mu\ell$) amounts into three tubes labeled "test" "negative control" and "positive control".
3. Mix the milk samples thoroughly but gently and dispense 1 mℓ into the tube labeled "test" and the "positive control" tube. Add 1 mℓ of boiled milk sample into the tube labeled "negative control".
4. Add 30 $\mu\ell$ Brucella positive control sera to the positive control tube.
5. Shake well to mix thoroughly within 1 minute after the milk has been added to the antigen.
6. Incubate at 37°C ±0.5°C for 1 hour.

D. Results:

Positive: Cream layer darker blue or same shade blue as milk column.

Negative: Cream layer white or lighter shade of blue than the milk column.

Negative control will have no blue ring.

Positive control will have a blue ring.

Information

Individual cows may be tested but tend to give more false positives. This may be overcome by serial dilutions of the milk with known negative milk. A reaction of 1:10 or higher is indicative of infection.

Addendum VII

BAIRD-PARKER AGAR BASE

(Method according to the manufacturer's instructions: Biolab Code C 41)

A highly selective medium for the isolation of coagulase-positive *Staphylococci*

COMPOSITION

Tryptone	10.0
Meat extract	5.0
Glycine	12.0
Yeast extract	1.0
Lithium chloride	5.0
Sodium pyruvate	10.0
Agar	20.0

pH = 6.8 (±0.2)

PREPARATION

Suspend 63 g in 1 litre of distilled water. Bring to the boil whilst stirring until dissolved. Dispense 90 mℓ volumes into bottles and sterilize in the autoclave at 121°C for 15 minutes. This medium can be stored for 1 month at 4°C.

To each 90 mℓ of the basal medium aseptically add at 50°C, 1 mℓ of 1% sterile Potassium Tellurite solution (BX15) and 5 mℓ of a 50% Egg Yolk Emulsion (BX14). Pour 15 mℓ volumes into petri dishes.

METHOD

Plates should be used within 24 hours and should be dry when inoculated.

Spread 0.1 to 1 mℓ of the dilutions on the surface of plates. Incubate at 35°C (or 37°C) for 24-26 hours and for a further 24 hours if no *Staphylococcus aureus* are seen.

EVALUATION

Staphylococcus aureus colonies are black, glossy and convex with a diameter of 1 to 1.5mm with a white margin surrounded by a clear zone 2 to 5mm broad. After 48 hours they may give an opaque zone extending into the clear medium. The medium tends to inhibit growth of coagulase-negative organisms, colonies which appear are irregular and usually have broad opaque zones.

Micrococci occasionally grow to form very small black or brown colonies. Yeasts may grow as white colonies, and *Bacillus* species as brown, matt colonies.

Addendum VIII

THE STAPHYLASE TEST

(Method according to the manufacturer's (Oxoid) instructions)

1. General

   1.1 Principle: To determine the presence of coagulase producing staphylococci through clumping of fibrinogen-sensitized sheep red blood cells. (*Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus hyicus* subsp. *hyicus* (11-89%)).

   1.2 Detection of coagulase ("clumping factor"). Easy reading because of colour reagents.

2. Materials

   The Staphylase Test Kit (Oxoid) consisting of:

   - Staphylase Test Reagent.
   - Staphylase Control Reagent.
   - Disposable Reaction Cards.

   Note: **BOTH REAGENTS CONTAIN 0.1% SODIUM AZIDE AS PRESERVATIVE.**

3. Procedure

   3.1 Store Staphylase Test kits at 2°C - 8°C in an upright position.

   3.2 Perform this test on all KOH(-), catalase(+) cultures.

   3.3 Shake the Test and Control Reagents vigorously to obtain a homogenous suspension. Reagent cells in the dispensing pipettes should be mixed in the suspension.

   3.4 Cut the reaction cards into strips, one strip with test and control circle for each culture.

3.5 Using a 1 μl plastic loop, smear colonies on the test and control strip of a reaction card for that colony.

3.6 Add one drop of test reagent to the test circle and one drop of control reagent to the control circle.

3.7 Mix the contents of the test circle with a 1 μl plastic inoculation loop. Observe for agglutination while mixing. Repeat the process with a new 1μl plastic inoculation loop for the control circle. The control circle should show no agglutination. If the control circle shows a positive reaction, contact your supervisor.

3.8 Do the test with the positive control culture available.

Note: *Staphylococcus sciuri* may show false positive results.

The use of high-salt media may show a weaker reaction than usual. The staphylase test has an extremely high correlation with the tube coagulation test.

3.9 Record results.

3.10 Clean up workplace and store materials.

Addendum IX

DETECTION OF STAPHYLOCOCCAL ENTEROTOXIN

(Method according to the manufacturer's (Oxoid) instructions)

PRINCIPLE OF ASSAY

Polystyrene latex particles are sensitized with purified antiserum taken from rabbits, immunized individually with purified staphylococcal enterotoxins A, B, C and D. These latex particles will agglutinate in the presence of the corresponding enterotoxin. A control reagent is provided which consists of latex particles sensitised with non-immune rabbit globulins. The test is performed in V-well micro titre plates. Dilutions of the food extract or culture filtrate are made in five rows of wells, a volume of the appropriate latex suspension is added to each well and the contents mixed. If staphylococcal enterotoxins A, B, C and D are present, agglutination occurs, which results in the formation of a lattice structure. Upon settling, this forms a diffuse layer on the base of the well. If staphylococcal enterotoxins are absent or at a concentration below the assay detection level, no such lattice structure can be formed and, therefore, a tight button will be observed.

The diluent provided contains sodium hexametaphosphate, which has been shown to reduce the incidence of non-specific reactions with components of food matrices.

PRECAUTIONS

This product is for *in vitro* diagnostic use only.
Do not freeze.
Reagents with different lot numbers should not be interchanged.

Reagents and diluent contain 0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which are explosive by contact

detonation. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after waste disposal.

STORAGE

The SET-RPLA Kit must be stored at 2°C to 8°C. Under these conditions the reagents will retain their reactivity until the date shown on the kit box. After reconstitution, the enterotoxin controls should be stored at 2°C to 8°C. Under these conditions, the reconstituted enterotoxin controls will retain their reactivity for 3 months, or until the date shown on the kit box, whichever is the sooner.

METHOD OF USE

1. **Materials required but not provided**.

   Blender or homogeniser
   Micro titre plates (V-well) and lids
   Fixed or variable pipette and tips (25$\mu\ell$)
   Centrifuge capable of generating 900g (typically 3 000rpm in a small bench top centrifuge)
   Membrane filtration unit using low protein-binding disposable filters with a porosity of 0.2μm-0.45μm (such as Millipore SLGV)
   Sodium chloride solution (0.85%)
   Sodium hypochlorite solution (>1.3% w/w)
   25$\mu\ell$ dropper (optional)
   25$\mu\ell$ diluter (optional)
   Micro mixer (optional)
   Moisture box (optional)

2. Components of the kit

TD901 **Latex sensitised with anti-enterotoxin A.** Latex suspension sensitised with specific antibodies (rabbit lgG) against staphylococcal enterotoxin A.

TD902 **Latex sensitised with anti-enterotoxin B.** Latex suspension sensitised with specific antibodies (rabbit lgG) against staphylococcal enterotoxin B.

TD903 **Latex sensitised with anti-enterotoxin C.** Latex suspension sensitised with specific antibodies (rabbit lgG) against staphylococcal enterotoxin C.

TD904 **Latex sensitised with anti-enterotoxin D.** Latex suspension sensitised with specific antibodies (rabbit lgG) against staphylococcal enterotoxin D.

TD905 **Latex control.** Latex suspension sensitised with non-immune rabbit globulins.

TD906 **Staphylococcal enterotoxin A control.**

TD907 **Staphylococcal enterotoxin B control.**

TD908 **Staphylococcal enterotoxin C control.**

TD909 **Staphylococcal enterotoxin D control.**

TD910 **Diluent**. Phosphate buffered saline containing bovine serum albumin and sodium hexametaphosphate.

3. Toxin Extraction or Production

3.1 Blend 10g of sample with 10 mℓ of sodium chloride solution (0.85%) in a blender or homogeniser.

3.2 Centrifuge the blended sample at 900g at 4°C for 30 minutes.

NOTE: If refrigerated centrifuge is not available, cool the sample to 4°C before centrifugation.

3.3 Filter the supernatant through a 0.2μm-0.45μm low protein-binding membrane filter. **Retain the filtrate for assay of toxin content**.

4. Control

Each reconstituted toxin control will cause agglutination with its respective sensitised latex. The use of the toxin controls will provide references for the positive patterns illustrated below (see Interpretation of Test Results). The controls should be used from time to time only to confirm the correct working of the test latex. The toxin controls are not provided at a specified level and therefore must not be used as a means of quantifying the level of toxin detected in the test sample.

5. Assay method

5.1 *Working reagents*

The latex reagents and diluent are ready for use. The latex reagents should be thoroughly shaken before use to ensure a homogeneous suspension. To reconstitute the control reagents, add 0.5 mℓ of diluent (TD910) to each vial. Shake gently until the contents are dissolved.

5.2 Arrange the plate so that each row consists of 8 wells. Each sample needs the use of 5 such rows.

5.3 Using a pipette or dropper, dispense 25$\mu\ell$ of diluent in each well of the 5 rows.

5.4 Add 25$\mu\ell$ of test sample to the first well of each of the 5 rows.

5.5 Using a pipette or diluter and starting at the **first well** of each row, pick up 25$\mu\ell$ and perform doubling dilutions along each of the 5 rows. **Stop at the 7th well** to leave the last well containing diluent only.

5.6 To each well in the first row, add 25$\mu\ell$ of latex sensitised with anti-enterotoxin A.

5.7 To each well in the second row, add 25$\mu\ell$ of latex sensitised with anti-enterotoxin B.

5.8 To each well in the third row, add 25$\mu\ell$ of latex sensitised with anti-enterotoxin C.

5.9 To each well in the fourth row, add 25$\mu\ell$ of latex sensitised with anti-enterotoxin D.

5.10 To each well in the fifth row, add 25$\mu\ell$ of latex control.

5.11 To mix the contents of each well, rotate the plate by micro mixer or agitate by hand. Take care that no spillage occurs from the wells.

5.12 To avoid evaporation, cover the plate with a lid. Placing the plate in a moisture box is an acceptable alternative. **Leave the plate undisturbed** on a vibration-free surface at room temperature for 20 to 24 hours. It will help subsequent reading of the test if the plate is placed on black paper for the duration of this incubation.

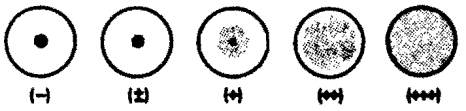
5.13 Examine each well in each row for agglutination, against a black background.

5.14 Centrifuge tubes, membrane filters, micro titre plates, lids and pipette tips should be sterilized by autoclaving at 121°C or disinfected before disposal in hypochlorite solutions (>1.3% w/w).

5.15 Dispose of culture extracts, food extracts, samples and toxin controls in hypochlorite solutions (>1.3% w/w).

INTERPRETATION OF TEST RESULTS

The agglutination pattern should be judged by comparison with the following illustration:



Results classified as (+), (++) and (+++) are considered to be positive

Results in the row of wells containing latex control should be negative. In some cases, non-specific agglutination may be observed. In such cases the results should be interpreted as positive, provided that the reaction with sensitised latex is positive to a higher dilution of test samples than that seen with the latex control. The last well in all rows should be negative. If positive patterns are observed in some of these wells, the reaction should be regarded as invalid.

NOTE: Certain staphylococcal strains are known to produce more than one enterotoxin.

LIMITATIONS OF THE TEST

The sensitivity of this test in detecting the enterotoxins has been reported to be 0.5ng/mℓ in the test extract. When a food extract is made with a dilution ration of 1:1 with diluent, the sensitivity is, therefore, 1ng/g of food matrix. The detection limit will vary according to any extra dilution conditions dictated by the type of food matrix. Concentrations of the enterotoxin in the food extract can be effected by a variety of methods, such as ultrafiltration. Production in culture of SETs depends on the growth conditions. A positive result obtained by the culture demonstrates the production of one or more SET under those circumstances; it does not imply the *in vivo* production of toxins to those levels.

Addendum X

Daily information sheet regarding the purchasing of milk

Date: **Day:** **Week:**

Sample no	Place of purchase	Time of purchase	Price	Temp	Pasteurised ?	Expiry date

Time at beginning of Sampling:

Addendum XI

Laboratory worksheet

Date: **Day:** **Week:**

Shop		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Temp																	
SPC	-2																
	-3																
	-4																
	-5																
	-6																
	-7																
PBC	-2																
	-3																
	-4																
	-5																
	-6																
	-7																
	-8																
	-9																

Shop		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E.coli																	
C o l i f m	1																
	-1																
	-2																
	-3																
Phosphat																	
SCC																	
BBRT																	
S. aureus																	
Staph toxin																	
BMRT																	
Salmonel																	
pH sale																	
pH incubate																	