# ANTIBIOTIC RESIDUES IN MILK

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the degree of Master of Science in the University
of Nairobi.'

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<u>DECLARATION</u>

(a) This thesis is my original work and has not been presented for a degree in any other University.

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(b) This thesis has been submitted for examination with my approval as University Supervisor.

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## ABSTRACT

The presence of antibiotic residues in foods represents a potential health hazard to man which at the present time is difficult to assess. Nevertheless, some problems have already been defined and legislature has been enacted to prevent or reduce the occurrence of antibiotic substances in food intended for human consumption. In addition, antibiotics in milk intended for the production of cheese or for the production of milk products requiring the use of bacterial (or yeast) cultures may result in the killing of these cultures with subsequent substantial losses to the dairy industry.

Only limited information on the incidence of antibiotic residues in milk in Kenya is available. A survey was therefore carried out on pooled milk samples obtained from various sources associated with the Kenya Cooperative Creameries. The agar diffusion method using Micrococcus luteus as the test organism was used for screening milk samples for inhibitory action on growth. Whenever inhibition of growth was observed, the milk sample was heated at 82$^{o}$C for 5 minutes to inactivate heat-labile inhibitory substances of a non-antibiotic nature occasionally found in milk. Furthermore, attempts were made to identify the antibiotic present by using penicillinase.

A total of 1,725 samples of raw milk were examined for the presence of heat-stable inhibitory substances to M. luteus. 89 samples (5.2%) were inhibitory, and 29 of these were shown

to contain penicillin, i.e. 1.7% of the total number of samples, or 33% of all inhibitory samples. The inhibitory substances in 67% of positive tests could not be identified. Quantitation of the penicillin concentration revealed a range from 0.02 to 0.03 iu per ml. milk.

Minimum inhibitory concentrations of penicillin and oxytetracycline on Streptococcus lactis and Lactobacillus bulgaricus were determined.

The results were as follows:

| | | |
|---|---|---|
| Strept. lactis | 0.26 unit/ml | (Penicillin) |
| | 0.60 μg/ml | (Oxytetracyline) |
| Lact. bulgaricus | 0.39 unit/ml | (Penicillin) |
| | 0.70 μg/ml | (Oxytetracycline) |

The above results show that low concentrations of antibiotics in milk can inhibit dairy "starter" cultures and cause economic losses to cheese and fermented milk industries.

Taking into account that milk from treated cows when added to the central milk supply is diluted, the amounts of antibiotic residues detected in the milk samples of the present investigation, however, were not likely to result in inhibition of starter cultures since they were far below the values demonstrated to have such effects.

Excretion of penicillin in milk of treated cows was also measured. Two routes of administration were used: the intramuscular and intramammary. A total of 12 milking cows were used (i.e. 6 cows per group) and the withholding periods for penicillin turned out as follows:

| Route of administration | Withholding period |
|---|---|
| 1. Intramuscular | 2 days |
| 2. Intramammary | 4 days (infused quarters) |
| | 1 day (non-infused quarters) |

The results of this study emphasize the importance of preventing antibiotics from entering milk supplies by strictly adhering to the appropriate withholding periods specified for the antibiotics used.

# INTRODUCTION

## IMPLICATIONS

Before the 1950's, mastitis was treated with various udder balms, ointments, and sulfur drugs. Then came the news of wonder-drugs such as penicillin and the prospect that mastitis at last would be conquered. But as so often happens when man overcomes one major problem, he finds that in his victory, he has created other problems. In this instance, the problem is the fact that penicillin-treated cows harbour antibiotic residues for several days depending upon the amount of penicillin injected into the udder and these residues are secreted with milk.

Antibiotic residues in milk are undesirable for public health and for technological reasons. If the necessary precautionary measures to prevent the delivery of polluted milk to the dairy industry are neglected, or are ineffective, the consumer will be faced with a potential hazard to his well-being in the shape of contaminated consumption milk and milk products. From the public health point of view, the officials have developed concern along three lines.

(a) The development of sensitivity reactions to antibiotics:

Sensitivity reactions occur after a sensitization period. The sensitized individual shows reactions to a dose completely harmless to a non-sensitized individual. The exposure frequency, mode of administration, chemical structure and, to a lesser extent, heredity, are important factors in drug allergy (Mol, 1975). The effects are completely out of proportion to the dose administered.

Of the antibiotics that may occur in milk, penicillin is the chief offender in stimulating sensitivity reactions according to medical authorities as reviewed by Albright *et al.*, (1961).

Medical evidence is not clear as to what level of penicillin in milk poses a danger to man. It has been suggested that the present recommended limit of 0.05 iu of penicillin per ml. of milk is too high and offers no guarantee of safety (Joint FAO/WHO Expert Committee on milk Hygiene Report, 1970). Recently the Joint FAO/WHO Expert Committee on Food additives recommended that penicillins "should not be allowed to give rise to detectable residues in human food." The Food and Drug Administration (FDA) have approved of two methods of analysis for use; however, specific conditions have been specified governing the use of each method. The first of these is the Disc Assay Method - A as described in standard Methods (1972); second is the *Sarcina lutea* cylinder cup method (1974). The table below lists several dairy products with indicated detection levels likely to be acceptable as based upon the sensitivity of the analytical method specified.

Table 1:

Acceptance criteria for penicillin residues in dairy products:

| Product | Sample dilution | Dilution factor | Method | Acceptance criteria (Unit/ml or gm of product) |
|---|---|---|---|---|
| 1. Raw milk (Indiv. producer) | None | 1 | Disc Assay | < 0.05 |
| 2. Raw milk (commingled) | None | 1 | Cylinder cup | < 0.01 |
| 3. Pasteurized milk | None | 1 | Cylinder cup | < 0.01 |
| 4. Butter milk | None | 1 | Cylinder cup | < 0.01 |
| 5. Cond., conc., evap. | 1 + 1 | 2 | Cylinder cup | < 0.02 |
| 6. Cheese, butter, ice cream | 1 + 4 | 5 | Cylinder cup | < 0.05 |
| 7. Dried milks | 1 + 3 | 4 | Cylinder cup | < 0.04 |

(Food and Drug Administration)

The empirical clinical use of penicillin during the past 30 years has resulted in a sensitized populations of unknown proportions. Stewart (1970) reviewed the partinent literature in 1965 and again in 1970 and concluded that it is not possible to determine a true figure of incidence but it lies between 1% and 10%. In 1973, he further stated that nothing had been found in the intervening period to contradict that estimate.

Penicillin hypersensitivity can be induced in two ways: (a) the immediate type involving reactions of humoral tissues with a specific antigen such as the penicilloyl-protein conjugates formed in tissues following intramuscular injection and the oral administration of the antibiotic and possibly the ingestion of certain foods such as milk and milk products, and (b) the delayed type which is a form of immunologic response that is mediated by sensitized lymphoid cells rather than by humoral tissue. This type of sensitization can be the consequence of long exposure to and contact with penicillin not therapeutically administered (Davis *et al.*, 1973), e.g., penicillin production plant workers, nurses, and pharmacists. Similarly, the dermatophyte *Trichophyton*, an etiologic agent of cutaneous mycosis, and other ubiquitous fungi produce penicillin-like molecules which may also sensitize an individual who never received penicillin therapeutically (Davis *et al.*, 1973). It is conceivable that long term ingestion of milk containing low levels of penicillin could also sensitize in this way (Olson and Sanders, 1975).

However, regardless, of the kind of exposure, reactions are varied: mild skin rashes, often urticarial to severe generalized urticaria, edema, anaphylactic reactions, and sudden death. The most severe and critical reactions are caused by the parenteral application of this antibiotic in therapeutic use.

Literature is sparse regarding reactions attributed to milk and milk products and those reported have been of the urticarial type. Generally these reactions occur in individuals who have been sensitized by therapeutic application. Zimmerman (1959) reported on 4 cases of chronic urticaria associated with the ingestion of dairy products. In each case the reactions cleared rapidly after the intramuscular injection of 800,000 units of neutrapen (penicillinase) and remained urticaria-free when dairy products were eliminated from the diet.

While it is clear that consumption of milk containing penicillin will elicit allergic reactions in the hypersensitive individual, there is no documental evidence that consumption of milk or milk products containing penicillin can alone induce the hypersensitive state (Olson and Sanders, 1975).

Of the oligo-saccharide antibiotics streptomycin is the most likely to create allergy (Mol, 1975). According to literature Becker (1976) reports that no case is described in which side-effects were produced after oral intake by sensitized humans.

A few anaphylactic type reactions and eczema after regular exposure of chloramphenicol have been reported.

Reports about sensitization against tetracyclines are so rare that suitable test people for research are lacking (Becker, 1976).

(b) The development of antibiotic resistant strains of microorganisms:

The chances for selection of resistant strains are generally best when the concentration of the antibiotic is near the minimum inhibitory concentration (MIC). Below this level there is no inhibition of sensitive members of the population and considerably above it only hyper-insensitive strains are not inhibited.

Resistance could be a result of molecular changes in the site of action of the antibiotic, production of an inactivating enzyme or a modification in the penetration of the antibiotic. Bacteria can also acquire resistance by spontaneous chromosomal mutation more commonly by transfer of a small genetic factor, resistance factor, or plasmid from resistant to a sensitive organism.

A matter of public concern is that certain infectious bacteria may develop resistance to antibiotics and become refractive to such treatment. Antibiotics kill large numbers of infectious bacteria but in so doing resistant variants that may not be killed are permitted to flourish into bacterial populations that are difficult to treat. Some have suggested that unintentional consumption of small amounts of antibiotics in foods might result in the development of resistant bacteria as reviewed by Albright *et al.*, (1961).

(c) Resistance transfer

The problems of resistance transfer outside the resistant species and cross or group resistance makes the residue problem a latent danger to public health (FAO/WHO Expert Committee on Food Additives, 1969).

Dairy foods made from milk containing antibiotics may sometimes contain antibiotic resistant-strains of infectious bacteria as reviewed by Albright *et al.*, (1961). These may serve as a vehicle for conveying them to the household of the consumer according to the findings of Thatcher and Simon (1955).

The indiscriminate use of antibiotics in the treatment of mastitis has favored the development of Staphylococci that are resistant to antibiotics (Daver and Davids, 1959; Marth and Ellickson, 1959). It has been suggested that milk may play a role in the dissemination of penicillin-resistant strains of Staphylococci (Brit. Med. J. as cited by Kaplan *et al*., 1962).

Starter failures

Dairy manufacturing plants which are more directly affected than others by the presence of antibiotics in milk are those that produce fermented milk products. Examples of these products are: acidophilus milk, cultured butter milk, Bulgarian milk, sour cream, cottage cheese, and other cheese varieties depending on lactate fermentation. All bacterial organisms which are involved in the production of fermented milk products show varying degrees of inhibition of growth in the presence of the different antibiotics.

Starter cultures appear to be more sensitive to the action of penicillin than to other antibiotics since Bradfield *et al*., (1952) demonstrated that mixed lactic acid starter cultures were not inhibited until 0.25 unit aureomycin per ml. milk was present.

*Streptococcus thermophilus* and *Strept*. *durans* are very sensitive to penicillin and so, to a lesser degree, are *Strept*. *lactis* and *Strept*. *cremoris*. The Lactobacilli are in general less sensitive to this antibiotic than Streptococci, but *Lactobacillus lactis* and *L*. *helveticus* are rather more sensitive than *L*. *bulgaricus* to penicillins. The Propioni bacteria are also fairly sensitive to penicillins (Mol, 1975).

Kosikowski and Mocquot (1958) compiled information of the relative sensitivity of cheese starter bacteria to penicillin (Table 2).

Jepsen (1962) gives a list of the inhibitory levels of some antibiotics against starter cultures in milk approximately (Table 3).

Table 2:

Critical penicillin levels in milk for bacteria

| Bacteria | Penicillin concentration significantly inhibiting growth (iu/ml) |
|---|---|
| Streptococcus cremoris | 0.05-0.10 |
| Strept. lactis | 0.10-0.30 |
| Strept. starter | 0.10 |
| Strept. thermophilus | 0.01-0.05 |
| Strept. faecalis | 0.30 |
| Lactobacillus bulgaricus | 0.30-0.60 |
| Lact. acidophilus | 0.30-0.60 |
| Lact. casei | 0.30-0.60 |
| Lact. lactis | 0.25-0.50 |
| Lact. helveticus | 0.25-0.50 |
| Lact. citrovorum | 0.05-0.10 |
| Propionibacterium shermanii | 0.05-0.10 |

Table 3 :

Inhibitory levels of certain antibiotics against starter cultures in milk*

| Antibiotic | Inhibitory level (per ml.) | Complete inhibition (per ml) |
|---|---|---|
| Penicillin (unit) | 0.05 | 0.1 |
| Chlortetracycline (μg) | 0.02 | 1.0 |
| Oxytetracycline (μg) | 0.01 | 2.0 |
| Chloramphenicol (μg) | 0.20 | 10 |
| Streptomycin (μg) | 0.40 | 20 |

* Overby (1952), cited by Jepsen (1962)

Cheese manufacture is dependent on the rate of acid development as well as the total amount produced. If either rate or total quantity of acid is reduced from the optimum, the quality of cheese suffers.

A concentration of 0.01 iu of penicillin per ml. of milk delays acid production. 0.10 iu per ml. accentuates this effect. The pH of the ripened cheese is then aberrant (approx. 5.0), the consistency pasty and the taste yeast-like. A concentration of 0.15 iu of penicillin per ml. of milk gives rise to the cheese which is totally aberrant. 0.50 iu/ml of milk prevents acid production altogether as reviewed by Mol (1975).

Hunter (1949) demonstrated that cheddar cheese made from milk containing 0.10-0.15 iu of penicillin per ml. of milk was critisized as having a fermented flavour and a weak, pasty body after 3 months of aging.

Bradfield (1950) found that when only 0.25-0.31% acidity developed in 7 hours due to the presence of antibiotics the cheese did not develop a normal flavour and the body was weak and pasty.

Jacquet (1953) reported that when Camembert cheese was made from milk containing 0.5-1.0 unit penicillin per ml. of milk the cheese was gassy. Harper (1960) studied the phenomena related to non-coagulation of casein in cottage cheese manufacture when the acidity was at or below the isoelectric point of casein. He showed that when small concentrations of tetracycline-type antibiotics were present in milk there occurred an interaction between casein, the antibiotics , and the calcium which prevented clotting of casein.

Mol (1975) reviewed the problems with production of sour milk products. Lesser concentrations of penicillin affect the production of the right acidity, flavour and consistency, hence lowering the quality of such products as buttermilk and yoghurts. Other antibiotics such as the tetracyclines and bacitracin, in higher concentrations, may produce similar effects. Streptomycins, neomycins and polymyxins have relatively milder effects and are consequently less feared by the dairy industry.

Presence of antibiotics in raw milk samples will also give false readings on bacterial counts either by destroying bacteria or by inhibiting bacteria growth thereby resulting in a higher grade for the milk than its quality deserves.

## LITERATURE REVIEW

### KENYA:

Milk production in Kenya is consumed locally to a large extent. The Kenya Co-operative Creameries Limited (K.C.C) produce a number of dairy products: Cream, butter, ghee, cheese, milk powder (skimmed milk powder, whole milk powder and "safariland" brand-spray wholemilk powder).

International trade in milk and milk products is quite a complex affair. In many of the overseas countries the residue problem has begun to attract attention and control measures and legislation have been or are being drawn up to deal with the problem.

The extent of the antibiotic residue problem in Kenya is unknown, and more information must be obtained before remedial measures can be undertaken. The Joint FAO/WHO Expert Committee on milk hygiene Report (1970) are of the feeling that this problem in developing countries is becoming more and more acute.

A survey of K.C.C. milk for antibiotic residues was carried out in the period 1977-1978 (See appendix 2). This was to investigate the incidence of antibiotic residues in milk in todays Kenya.

### OTHER COUNTRIES:

The presence of antibiotics in milk was a serious dairy industry problem two decades ago and it took a number of years to bring it under control. These aspects were reviewed by Cuthbert (1968).

Penicillin occurred more frequently in market milk than other antibiotics such as streptomycin, chlortetracycline, oxytetracycline, bacitracin, neomycin, chloramphenicol and polymyxin (Albright et al., 1961).

The occurrence of penicillin and other antibiotics in milk has been reduced in developed countries through wide spread testing, educational programmes and control. For instance, a few years ago 7-30% of fluid milk contained penicillin at levels of 0.05 iu per ml but the figure has now been reduced to 0.5-4.0% (Joint FAO/WHO Expert Committee on milk hygiene Report, 1970).

Surveys of market milk for antibiotic residues have been carried out in various developed countries. The figures are compiled in the tables (4-10) below:

Occurrence of antibiotic residues in milk

Table 4:

United States of America

(i) The results of 4 nationwide surveys ordered by the U.S. Food and Drug Administration (Delivery samples, assay sensitivity 0.05 iu of penicillin per ml. of milk, all "unnatural" inhibitors included)-compiled by Mol (1975).

Table 4 cont'd:

| Year | No. of samples | Positive (%) |
|---|---|---|
| 1954 | 94 | 3.2 |
| 1955 | 474 | 11.6 |
| 1956 | 1,706 | 5.9 |
| 1959 | 1,170 | 3.7 |

(ii) The National Incidence of Antibiotic Residues in producer milks (January 1 to October, 15, 1960) -compiled by Kosikowski (1960).

| Organisations Reporting | No. of samples analysed | No. positive for antibiotics | Incidence (%) |
|---|---|---|---|
| 17 dairies throughout the United States | 655,763 | 3,640 | 0.56 |
| 28 state health and State Agriculture Departments throughout the United States | 112,705 | 493 | 0.44 |
| 45 Total cooperating organisations | 768,468 | 4,133 | 0.54 |

(iii) Occurrence of Antibiotics in fluid milk (Joint FAO/WHO Expert Committee on Milk Hygiene Report, 1970)

| Period | Proportion of samples containing antibiotics (%) |
|---|---|
| 1960-1967 | 0.50 |

Table 5:

Great Britain

A. England and Wales

The results as ordered by the English Milk Marketing Board from October 1965 to April 1970 compared with the 1961 national survey. (Delivery samples, assay sensitivity 0.005-0.02 iu of Penicillin per ml. of milk, all "unnatural" inhibitors included). Approximately 975,000 samples were tested each year. compiled by Mol (1975).

| Year | Positive (%) |
| --- | --- |
| 1961 | 6.1 |
| 1965 | - |
| 1966 | 1.4 |
| 1967 | 1.1 |
| 1968 | 1.0 |
| 1969 | 0.9 |
| 1970 | - |

B. Scotland

The results as ordered by the Scottish Milk Marketing Board, the West of Scotland Agricultural College, the North of Scotland Milk Marketing Board and the Aberdeen and District Milk Marketing Board from 1964 to 1967, compared with the 1961 national survey and the West of Scotland Agricultural College survey of 1962. (Delivery samples, assay sensitivity 0.005-0.02

iu of penicillin per ml., all "unnatural" inhibitors included) - compiled by Mol (1975).

| Year | No. of samples | Positive (%) |
|---|---|---|
| 1956 | ? | 5.9 |
| 1961 | 2,700 | 9.9 |
| 1962 | 2,820 | 6.1 |
| 1964 | 36,300 | 3.7 |
| 1965 | 84,158 | 1.5 |
| 1966 | 90,833 | 1.6 |

C. Northern Ireland

The results as ordered by the Northern Ireland Milk Marketing Board from November 1964 to January 1967. (Delivery samples, assay sensitivity 0.01 iu of penicillin per ml.). Approximately 17,000 samples farm milk and 8,500 samples pasteurized milk were tested each year - compiled by Mol (1975).

| Year | Positive farm milk (%) | Positive - Pasteurized Milk (%) |
|---|---|---|
| 1964 | - | - |
| 1965 | 1.7 | 20 |
| 1966 | 1.3 | 18 |

Table 6:

Irish Republic

The results of a national survey made by the National Dairying Research Centre in 1964 and 1965. (Delivery samples, assay sensitivity 0.01 iu of penicillin/ml., all "unnatural" inhibitors included) - compiled by Mol (1975).

| Year | No. of samples | Positive Penicillin (%) | Other Inhibitors(%) |
|---|---|---|---|
| 1964 | 1, 114* | 4.6 | 13.6 |
| 1964-1965 | 2, 742** | 2.3 | 3.1 |

* - Random samples of 32 creameries (2,925 suppliers) June/Sept.

** - Fortnightly samples at one selected creamery (1964 June/Dec. to 1965 Jan./June).

Table 7:

Netherlands

(i) The results listed in the annual reports of the Food Inspection Services up to 1971 (assay sensitivity 0.01-0.0025 iu of penicillin per ml of milk) - compiled by Mol (1975).

| Year | No. of samples | Positive (%) |
|---|---|---|
| 1958 | 155 | 45.2 |
| 1959 | 418 | 23.9 |
| 1960 | 578 | 88.7 |
| 1961 | 550 | 9.1 |
| 1962 | 510 | 11.8 |
| 1963 | 2,152 | 6.5 |
| 1964 | 2,877 | 11.5 |
| 1965 | 5,974 | 6.5 |
| 1966 | 41,993 | 5.5 |
| 1967 | 90,934 | 3.7 |
| 1968 | 146,878 | 2.3 |
| 1969 | 194,538 | 1.4 |
| 1970 | 201,637 | 1.5 |
| 1971 | 215,241 | 1.1 |

(ii) The results listed in the annual reports of Animal Health Services and Milk Hygiene Authorities up to 1971 (assay sensitivity 0.01 -0.0025 iu of penicillin per ml of milk) - compiled by Mol (1975).

Table 7 (ii) cont'd.:

| Year | No. of samples | Positive (%) |
|---|---|---|
| 1960 | 14,078 | 11.1 |
| 1961 | 20,592 | 10.5 |
| 1962 | 19,113 | 6.8 |
| 1963 | 20,949 | 9.4 |
| 1964 | 77,410 | 7.0 |
| 1965 | 1,177,217 | 1.7 |
| 1966 | 1,611,687 | 2.6 |
| 1967 | 1,436,005 | 2.1 |
| 1968 | 716,087 | 1.3 |
| 1969 | 921,646 | 1.5 |
| 1970 | 1,677,863 | 1.4 |
| 1971 | 1,577,922 | 1.4 |

Table 8:

Denmark

The results of the regular quality control ordered by the Danish Veterinary Directorate from 1960 to 1976. (Delivery samples, assay sensitivity 0.02 iu of penicillin/ml, all "unnatural" inhibitors included) - compiled by Mol (1975).

Table 8 cont'd:

| Year | No. of samples | Positive (%) |
|---|---|---|
| 1960 | 9,175 | 0.28 |
| 1961 | 40,734 | 0.16 |
| 1962 | 113,184 | 0.036 |
| 1963 | 128,816 | 0.048 |
| 1964 | 163,051 | 0.045 |
| 1965 | 169,095 | 0.034 |
| 1966 | 143,808 | 0.040 |
| 1967 | 147,986 | 0.031 |
| 1968 | 185,078 | 0.053 |
| 1969 | 204,102 | 0.039 |
| 1970 | 179,450 | 0.026 |
| 1973* | 206,086 | 0.028 |
| 1975* | 173,777 | 0.039 |
| 1976* | 189,416 | 0.045 |

* Rasmussen (1978)

Table 9:

Australia

(i) Results of Penicillin Assay of milk samples taken at random from Factories. (Assay sensitivity by Keogh test 0.03 iu of penicillin per ml.) - Smith (1965)

Table 9 cont'd:

| Period | No. of samples | No. Positive | Incidence (%) | Range (iu/ml) |
|---|---|---|---|---|
| 1961-1962 | 1,523 | 55 | 3.6 | 0.03-0.75 |
| 1962-1963 | 1,883 | 37 | 2.0 | 0.03-0.40 |
| 1963-1964 | 2,127 | 43 | 2.0 | 0.03-0.10 |

(ii) Results of Penicillin Assay of City Milk Supply Samples (Assay sensitivity by Naylor's test 0.005 iu of penicillin per ml.) - Smith (1965)

| Period | No. of samples | No. Positive | Incidence (%) | Range (iu/ml) |
|---|---|---|---|---|
| 1961-1962 | 68 | 7 | 10.3 | 0.005-0.025 |
| 1962-1963 | 156 | 8 | 5.1 | 0.005-0.01 |
| 1963-1964 | 71 | 2 | 2.8 | 0.005-0.006 |

(iii) Occurrence of Antibiotics in fluid milk (Joint FAO/WHO Expert Committee on Milk hygiene Report, 1970).

| Period | Proportion of samples containing antibiotics (%) |
|---|---|
| 1960-1967 | 2.1 |

Table 10:

South Africa

Surveys of market milk for Antibiotic residues. Over 1,200 herd samples were collected in Johannesburg (Meare, as cited by Kaplan et al., 1962).

| Period | Positive Penicillin (%) |
|---|---|
| 1958-1959 | 3 |

From the U.S.A. (Table 4, i) and the Irish Republic figures giving a fair impression of the frequency of antibiotic residues in milk, show that the problem does, or did exist on a large scale (Mol, 1975).

From the U.S.A. (Table 4, ii), the surveys cover a 10-year period during which a total of 768,468 samples were tested for presence of antibiotics. In most cases, penicillin was the primary antibiotic found (Albright et al., 1961). The incidence reported by State Health and Agricultural Laboratories was 0.44% compared with that by Industrial Dairy Laboratory of 0.56%. This is a good check, giving the substance to the validity of these data. State Laboratories perhaps exercised care in guarding against false positives thus leading to lower incidence (Kosikowski, 1960). A nation wide average incidence of 6% has dropped to 0.54% incidence. This is a remarkable reduction made possible only through the full cooperation of all parts concerned (Kosikowski, 1960).

From Netherlands (Table 7), comparison of the figures is not easy (Mol, 1975). Some services use different sampling methods, can samples, mixed can samples and delivery samples were used. The assay techniques changed becoming more sensitive. Table 7 (i) shows that there has been a great improvement over the years as regards the incidence of antibiotic residues, especially when it is remembered that the test sensitivity for penicillin increased from 0.01 to 0.0025 iu/ml. The figures produced by the Animal Health Services (Table 7 (ii) were much more reliable because their sampling frequency, type of sample, and assay technique were uniform (Mol, 1975). At present, under a national uniform quality control scheme, there has been a decrease in the pollution frequency in farm milk from 1 to 0.7% after 1966 which is quite significant (Mol, 1975).

Denmark (Table 8) gives a better guarantee to the consumer of milk and milk products against contact with antibiotic residue in these products. This is due to the fact that in Denmark, antibiotic therapy must be carried out solely by qualified veterinarian and certified in writing. The veterinarian must instruct the farmer accordingly and also inform the dairy plant manager of the treatments performed (Jepsen, 1962).

From Australia (Table 9 ), the sales are controlled under two Acts, the Food and Drug Act and the Stock Medicines Act. Stocking and sale is restricted to pharmacists and holders of an appropriate wholesale dealers' licence. The latter includes dairy factories and stock agents. A veterinary surgeon who owns a shop, but may supply from consulting rooms to owners of animals about which he has been consulted. To protect those stockowners distant from a practioner a service is provided whereby an owner or a chemist on his behalf, may ring a practioner or a departmental veterinarian who is satisfied that an antibiotic is required may authorise the chemist to supply; then he will forward a prescription to cover the sale.

The lack of information received from other countries is due to lack of nation wide control schemes as reviewed by Mol (1975).

## METHODS FOR TESTING ANTIBIOTIC RESIDUES IN MILK

Several methods to detect antibiotic and chemotherapeutic residues in milk have been developed over the years. Still no completely acceptable method is available which can easily be applied under industry conditions. A rapid, simple test which will detect antibiotic concentrations in milk within minutes is needed.

Although such a test is not available, much has been accomplished in antibiotic testing since the discovery of penicillin. The incidence of inhibitory substances in milk reported for any particular survey depends, upon the method used to detect these substances and its sensitivity.

### Chemical (Physical) techniques

Colorimetric assay technique is used for determination of sulphonamides in milk and body fluids as developed by Bratton and Marshall (1939) with the sensitivity of 50 ppm of the different sulphonamides.

To increase sensitivity with a factor of 10 for sulphonamides chromatographic techniques have been used (Bican et al., 1963).

The colorimetric assay technique is also good for the assay of nitrofuran derivatives in plasma, as developed by Buzard et al., (1956). This technique can be adapted for the assay of other body fluids. It has a sensitivity of 1 ppm (Buzard et al., 1956).

Spectrophotometric assay is also possible in the assay of nitrofurans in milk and yields the same sensitivity of 1 ppm (Hawkins et al., 1961 and Henningson, 1961).

Chemical assay of several antibiotic compounds is in regular use e.g. colorimetric assay of Procaine penicillin and fluorometric assay of tetracyclines in cattle fodder as reviewed by Mol (1975). However, these chemical techniques are more laborious and less sensitive in general but not always compared to microbiological assay techniques. Comparison between the chemical and microbiological assay of procaine penicillin showed that the latter was perferable (Katz, 1963).

Microbiological techniques

These techniques are based on bacteriocidal, inhibitory or morphological effects of antibiotics on certain microorganisms as reviewed by Mol (1975). These include microscopic tests, tube tests and plate tests. Only the latter is widely used in the antibiotic residue testing.

Plate tests: There are a number of different application techniques in use:

(a) Direct application

This is suitable for solid samples e.g. tissues or cheese. Small pieces of the sample are placed directly on surface of the test plate, after which the plate is cultured.

Advantages: 1. Fairly simple and easy to apply.

2. Suitable for assay of solid samples.

Disadvantages: 1. Fairly large number of test failures due to non-specific inhibitory substances.

2. Relatively insensitive especially when assaying for traces.

3. Not suitable for liquid or powdered products.

(b) Application in cylinders

Florey *et al*. (1941) first described a cylinder plate method for the assay of penicillin. Their method underwent several modifications until Juncher *et al*. (1950) suggested using *Sarcina lutea* as the test organism. Subsequently, the Food and Drug Administration (FDA) adopted a modification of the cylinder plate method (Carter 1974) as described by Schmidt and Moyer (1944) as the official test to be used for penicillin assay in FDA laboratories.

The cylinders are small and of uniform size. These can be porcelain or steel made or fish spinal electrical insulating beads. They are normally placed on the test plate. Then they are filled with standard amounts of test sample.

Advantages: 1. Accurate

2. Great sensitivity especially to the residual levels of the penicillin family of drugs.

3. All factors influencing size of inhibition zone are kept under control.

Disadvantages: 1. More complex to perform and requires skilled analyst.

2. Not easily adopted for mass analysis (Abraham *et al.*, 1941).

3. Requires more specialised equipment

4. Requires 16-18 hr. of incubation, for longer incubation than desired for routine quality control work.

(c) Application in punch holes

Liquid samples are introduced into holes punched in the agar with the aid of a cork borer.

Advantages: 1. Easy

2. Sensitive

3. Inexpensive

4. Permits accurate assaying

5. Its adaptability for mass analysis is excellent especially when the residues are expected to be relatively high.

Disadvantages: 1. Time consuming

2. Chance of obtaining irregularly shaped inhibition zones is said to be high (Mol, 1975).

(d) Application in press holes

This variant of (c) was developed by Jaartsveld *et al* (1964). The holes in the test plate are made by placing a model in the medium just before it solidifies.

Advantages: 1. Size, shape and number of the holes can be accurately measured.

2. A large number of liquid samples can be applied to the test plate simultaneously using a mass pipette.

Disadvantages: 1. A fairly thick layer of the medium is necessary to obtain holes of sufficient size but this might have an adverse effect on the size of the inhibition zones.

(e) Application in paper discs

A disc assay method was described by Foster and Woodruff in 1943. Filter discs were used. Several researchers modified and evaluated this method and reported its reliability for detecting low concentrations of penicillin. Arret and Kirshbaum (1959) developed a rapid disc assay procedure (2.5 hr) which detected 0.05 iu/ml., and Marth et al. (1963) provided further modification to detect a concentration of as little as 0.03 iu/ml. within 3-4 hrs.

Standard paper discs are saturated with liquid sample and placed on surface of test plates.

Advantages: 1. Very simple and easy to apply.

2. Rapid screening method for detection of penicillin.

3. Good for routine laboratory analysis of a large number of samples.

4. Requires a minimum amount of equipment.

Disadvantages:
1. Accurate dosage of the liquid sample is impossible, there is an adverse effect on quantitative interpretation as reviewed by Mol (1975).
2. Problems of zone measurement e.g. if the incubation period is short, bacterial growth is very light and zones of inhibition are not well defined.

(f) "Reverse phase" technique

This variant of the paper disc technique was developed by Kosikowski and Ledford (1960). They mixed a suspension of spores of the test organism in a poor medium (physiological sodium chloride agar). The paper discs used were saturated in a rich medium and freeze-dried. These discs, once drenched in the sample liquid and placed on the test plate and culture, will show growth if the sample is negative and no growth or a "halo effect" if the sample is positive.

Advantages:
1. Quick reading especially with indicator colours or with the aid of a microscope.

Agar diffusion test (Delvotest - P)

This test was published by Van Os *et al.* (1975) using *Bacillus stearothermophilus* var. calidolactis as the sensitive organism.

Tablets containing nutrients and a pH indicator (bromcresol purple) are added to ampoules containing spores of the test organism which have been seeded in an agar growth medium. A milk sample is added and the ampoule is incubated for 2.5 hr. in water bath at 63-66$^{0}$C. The nutrients , pH indicator and antibiotic (if present in milk) diffuse into the agar medium. The colour of the medium is purple because of bromcresol purple. If no antibiotic present, the test organism grows, lowering the pH of the medium, and causing the colour of bromcresol purple to change to yellow in the agar medium. Antibiotics in concentrations sufficient to inhibit growth of the test organism cause the medium to remain purple indicating a positive antibiotic test.

Advantages:

1. Reliable and accurate
2. Very sensitive to penicillins
3. Sensitive to most antibiotics used in cattle .
4. Simple and easy to read
5. Quick and requiring only 2.3 hr. of incubation.

Disadvantages: 1. Expensive for large scale milk testing programmes.

Sensitivities of some of these methods with regard to penicillin

Disc assay method

(i) Using Bacillus subtilis as the test organism: The sensitivity of the American Public Health Association (1972) for penicillin in milk is about 0.05 unit/ml.

Greater sensitivities have been reported in the above method using the above test organism. Johns (1960) reported a sensitivity of 0.025 unit/ml. Parks and Doan (1959) could detect sodium penicillin G at 0.0129 unit/ml but only if the seeded agar was 24-72 hours old. Schiemann (1976) could detect concentration of penicillin between 0.00625 and 0.003125 unit/ml. sometimes. He determined with 100% reproducibility concentration of 0.0125 unit/ml.

(ii) Using Sarcina lutea (ATCC 9341) as the test organism: Naylor (1960) reported a sensitivity of 0.005 unit of penicillin per ml. of milk. Feagon (1964) indicated a sensitivity of 0.003-0.004 unit/ml.

(iii) Using Bacillus stearothermophilus var. calidolactis as the test organism:

The International Dairy Federation (IDF) in 1970 approved the Disc Assay Method for qualitative detection of penicillin in excess of 0.0025 unit/ml. Using modified IDF assay, Kaufmann (1977) could detect 0.004 unit of penicillin per ml. of milk using plates which have been stored for 8 days. Further, he could detect a level of 0.002 unit/ml when fresh plates and fresh standard solutions were employed.

Cylinder plate method

Using *Sarcina lutea* as the test organism: This method is suitable for concentrations of less than 0.025 unit per ml. as specified by the Association of Official Analytical Chemists (1975).

Ouderkirk (1976) could detect a level of 0.01 unit of penicillin per ml. of milk.

Delvotest - P (Agar diffusion method)

Using *Bacillus stearothermophilus* var. calidolactis as the test organism:

The manufacturers give the following statements: With a penicillin concentration of 0.003 iu or less per ml. of milk, the test result is nearly always negative (entirely yellow). With a penicillin concentration of 0.006 iu or more per ml. of milk, the test result is always positive (entirely purple). With in-between concentrations the results of the test will vary.i.e. there will be mainly yellow - purple and purple coloration.

False positives in analytical testing

False positives indicate vividly the danger of misinterpreting a natural inhibitory reaction for pharmaceutical antibiotic reaction upon testing.

Fresh cow's milk is known to contain several substances capable of bacteria inhibition (Berridge, 1955). These are believed to be "natural biological bacteriostats." Lactenin, lysozyme (also in saliva, tears, egg white) and other, still unidentified, "substances" have inhibitory properties and have been demonstrated in milk (Berridge, 1955 and Franc *et al.*, 1958).

Bacterial growth in milk may lead to production of certain antibiotic or antibiotic-like substances. Hurst (1972) reported that the best known inhibitor produced by *Streptococcus lactis* was nisin. Nisin or another family of naturally occurring antibiotics could be responsible for the zones of inhibitors observed in various experiments.

The natural inhibitory property of raw milk is generally considered to be heat-labile (Auclair and Hirsch 1953; Jones and Little, 1927, and Wolin and Kosikowski, 1958). Kosikowski and O'leary (1963) found that minimum pasteurization temperature was ineffective in eliminating natural inhibitory substances. Heat-treating of the milk to 82°C for 5 minutes removed all evidence of natural inhibitors from the raw milks.

The production of lactic acid in low pH milks invariably leads to a consideration of this compound as a factor in zoning. Pure lactic acid and commercial lactic acid cultures, have been tested for the purposes of finding out if they were responsible for inhibitory effects. Kosikowski (1963) showed that lactic acid was not a major contributing cause, inasmuch as a pH 4.2-3.7 was required before such zone formation was evident using B. subtilis as the test organim.

Duthie et al. (1976) showed that lactic acid was not responsible for inhibition at pH values 6.5 and 6.1 using Bacillus subtilis disc assay and Sarcina lutea cylinder cup method.

The mammary gland

The wall of the secretory ducts, the alveolar ducts, and the alveoli consists of a basement membrane, a layer of myoepithelial cells and on the internal surface a row of columnar glandular cells. The biological membrane separates the extracellular fluid from the secretion.

Biological membranes are lipoid in nature. For a drug molecule to cross a membrane, it may have to initially "dissolve" in the lipid areas of the cell membrane. The extent to which the dissolution in lipid areas can take place depends on the lipid solubility of the molecule. Lipid solubility is determined by the presence of lipophilic or non-polar groups in the structure of the drug molecule. When a molecule contains structural elements that allow hydrogen bonding with water, lipophilic properties of the molecule are decreased, and the hydrophilic or polar properties of molecule are increased. Polarity is high for ionized molecules including ionized form of dissociable drugs.

Most drugs are weak acids or weak bases and have one or more functioning groups capable of ionizing. The extent of ionization depends on whether a drug is an acid or a base, on pKa of the drug and the pH of the solution in which the drug is dissolved.

Biological membranes are permeable to the un-ionized form of a drug molecule which is more lipid soluble than the ionized form of the drug. When equilibrium is reached between the two sides of a cell membrane, the unionized form of a drug will be in equal concentration on either side of the biological membrane.

The pH values of the fluid on either side of the mammary gland epithelium are 7.4-7.6 (plasma) and 6.5-6.8 (milk) respectively. Therefore drugs will be ionized to a different extent in both media.

A weak acid becomes more ionized as the pH increases and a weak base becomes more ionized as the pH decreases.

Bases become more ionized in milk than in plasma. Acids become less ionized in milk than in plasma. Accordingly, the unionized fraction of a base which can diffuse from milk to the blood is comparatively small and the unionized fraction of an acid is comparatively large. On the overall, basic drugs will occur in milk in higher concentrations than in blood. Acid drugs will occur in milk in lower concentrations than in blood.

Examples:

| Acids | pKa | Concentration in milk / Concentration in blood |
|---|---|---|
| Benzyl Penicillin | 2.7 | 0.2 |
| Sulphadimidine | 7.4 | 0.6 |
| Sulphathiazole | 7.1 | 0.3 |
| Sulphanilamide | 11 | 1 |
| **Bases** | | |
| Erythromycin | 8.8 | 8.7 |
| Spiramycin | - | 8 |
| Penethamate | 8.5 | 6 |
| Trimethoprim | 7.6 | 7 |

## The mechanism of passage of drugs through the mammary gland epithelium

The distribution of a weak acid and a weak base across a lipid membrane was discussed by Brodie and Hogben (1957), Schanker (1962) and Brodie (1964). Rasmussen (1966) has applied this concept to the mammary gland epithelium as illustrated in figure 1 below:

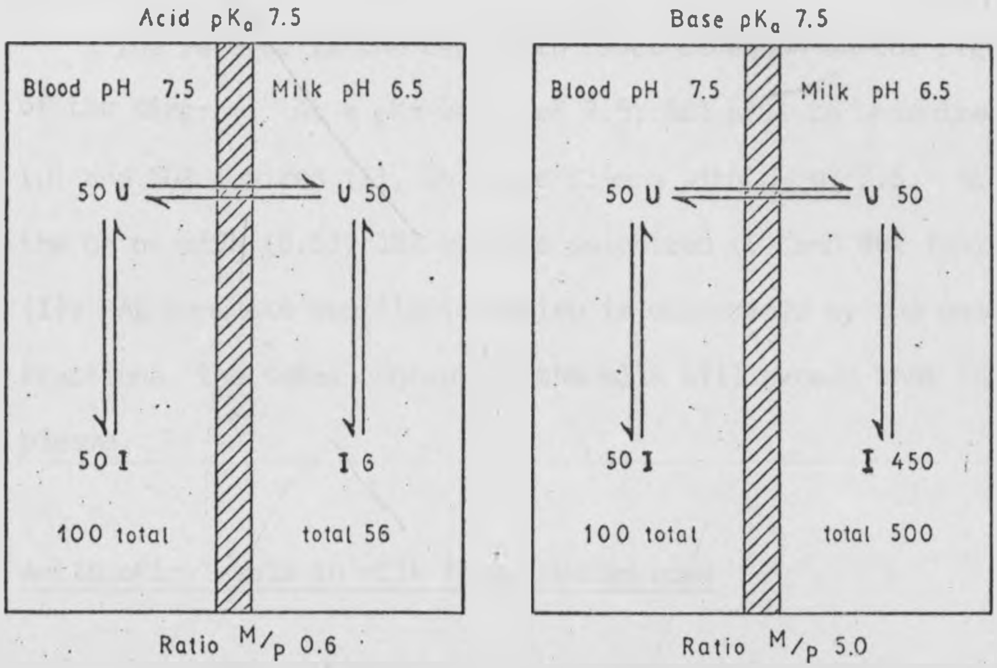


**Fig. 1.** Schematic representation of the distributions of a weak acid and a weak base across the mammary gland epithelium.

The shaded partitions represent the membrane being permeable to the unionized fractions (U) of the weak acid or of the weak base.

To simplify the calculations, the pH values of blood plasma and of milk have been set at 7.5 and 6.5 respectively.

Considering the weak acid with a pKa value of 7.5, in plasma at pH 7.5, 50% of it is unionized (U) and 50%) is ionized (I). The total concentration in plasma is thus twice as high as that of unionized acid (U). On the other side of the membrane, at pH 6.5 of milk, 90% of the weak acid is in unionized form (U) and 10% is in ionized form (I). Therefore, the total concentration in milk is lower than in plasma.

The reverse is the case with bases as shown on the right of the diagram. At a pKa value of 7.5, 50% will be unionized (U) and 50% ionized (I), in blood plasma with pH of 7.5. At the pH of milk (6.5), 10% will be unionized (U) and 90% ionized (I). At complete equilibrium which is determined by the unionized fractions, the total content in the milk will exceed that in plasma.

Antibiotic levels in milk from treated cows

The level of concentration and the total amount recovered in the milk vary widely between individual cows, even when identical schemes of therapy are followed. Variations of from about 8% to 80% in the amount recovered have been recorded.

Earlier work by Hollister et al. (1955/57/59), Murphy and Stuart (1954), and Sadek (1954) indicated that the concentration of antibiotics and the duration of time they persist in milk following treatment is dependent upon the dosage, stage of lactation, type of suspension vehicle, type of antibiotic and the physical condition of the udder.

Extensive research has been conducted on the duration of excretion of antibiotics in milk following intramammary infusion (Albright et al., 1961 and Plastridge, 1958), upon which extensive educational programs have been based to insure an antibiotic-free milk supply.

Until recent studies (Blobel and Burch, 1960, a, b & c; Cannon et al., 1962; Ormiston et al., 1960; Vaid et al., 1961, and Wright and Harold, 1960), information following intramuscular injections of antimicrobial drugs has been meager.

Intramammary therapy: With regards to intramammary therapy, Jackson and Bryan (1950 ) reported that the amount of milk produced bythe cow influences the amount of penicillin found in the milk following treatment. After injection of 300,000 units of procaine penicillin G in oil into quarters (during the middle of lactation period) milk levels of 0.50 iu or more resulted for 144 hours and at 216 hours 0.06 unit of penicillin per ml. of milk was still present. Using similar dosage level of penicillin to cows in their full flow of milk, they exhibited levels of 0.06 unit of penicillin per ml. of milk at 72 hours post-treatment. This implied that the longest persistence of penicillin was attained near the middle or end of the lactation period.

Foley et al. (1949) showed that the length of time penicillin or other antibiotics remained in the udder was directly influenced by its vehicle. To one group of animals they treated with penicillin (100,000 units/ml.) incorporated into a combination of mineral oil, lanolin derivatives, water, propylene glycol, and non-ionic wetting agents and concentrations of 0.014 to 4 units of penicillin per ml. milk could be detected 72 hours post-treatment. To another group, they used penicillin (100,000 units/ml.) with propylene glycol, a non-ionic wetting agent and no concentration of the antibiotic could be detected after a lapse of 48 hours. To a third group they infused penicillin (100,000 units/ml.) in water into the mammary glands and no detectable quantities of pencillin were found 24 hours post-treatment.

Streptomycin has been reported in milk for as long as 72 hours following treatment (Overby, 1952). Chlortetracycline can persist in milk of treated cows for as long as 5 weeks (Randall, et al., 1954), with large variation existing between cows in the fraction of the antibiotic excreted.

There are numerous intramammary infusion products containing certifiable antibiotics intended for use in treating mastitis in milk-producing animals. For each there is specified appropriate dosage and condition of use (i.e. for lactating or dry cow therapy or both). Currently the following products are used by the Kenya Veterinarians in the fields.

Intramammary infusion products

1. Terramycin* brand of oxytetracycline intramammary solution

Each tube of 14.2 gm contains

| | |
|---|---|
| Oxytetracycline hydrochloride B.P. | 426 mg. |
| Magnesium complex | 30 mg./g. |
| in a propylene glycol base | |

2. Mastalone

Each syringe (10 c.c.) contains

| | |
|---|---|
| Oxytetracycline hydrochloride | 200 mg. |
| Oleandomycin (as phosphate) | 100 mg. |
| Neomycin (as sulphate) | 100 mg. |
| Prednisolone | 5 mg. |
| in a special base | |

3. Vetramycin® - suspension

Each injector of 4.2. c.c. contains

| | |
|---|---|
| Penicillin G sodium | 600,000 iu |
| Dihydro-streptomycin $SO_4$ | 600,000 iu |
| Vitamin A palmitate | 10,000 iu |

in a non-irritant suspension base

4. Leo Yellow intramammary injector

Each injector of 5 c.c. contains

| | |
|---|---|
| Penethamate hydriodide (Leocillin®) | 150 mg. |
| Dihydrostreptomycin (as sulphate) | 150 mg. |
| Framycetin sulphate | 50 mg. |
| Prednisolone | 5 mg |

in vegetable oily suspension

5. Vagifurin

Each tube contains a single dose of

| | |
|---|---|
| Neomycin sulphate B.P. | 250 mgm |
| Nitrofurazone B.P.C. | 125 mgm |
| Polymixin B sulphate B.P. | 10,000 units |

6. Benestermycin Leo Dry Cow

Each injector of 5 c.c. contains

| | |
|---|---|
| Penethamate hydriodide (Leocillin®) | 100 mg. |
| Penethamine penicillin | 280 mg. |
| Framycetin sulphate | 100 mg. |

in slow release base

7. Orbenin

Orbenin cloxacillin sodium salt 200 mg.

Monohydrate

Antibiotics can also reach milk after oral or parenteral administration in cows for treatment of numerous infections.

Parenteral therapy: Parenteral administration of antibiotics in the milk-producing animals is generally the most preferable mode since it enables the blood serum concentration to be controlled properly.

The first reports on the presence of penicillin residues after systemic treatment in dairy cattle are from Welsh et al. in 1948.

After subcutaneous or intramuscular injections of potassium penicillin G in aqueous suspension to a cow at the rate of 5,000 units per pound of body weight, penicillin was found in the milk at the level of 0.032 unit/ml for 24 hours (Sadek, 1954).

With a dosage averaging 3,400 units/lb. penicillin (Procaine Penicillin in aqueous suspension) has been found in the milk for only 24 hours (Hollister et al., 1957).

Penicillin has been found to persist in the milk for 48 hours after injection of 5,000 units/lb. of Procaine Penicillin G in aqueous suspension (Randall et al., 1953).

Doses of 3,000 and 6,000 units/lb. of Procaine Penicillin in oil (PAM) have produced detectable concentrations of penicillin for up to 5½ days in some cows and for only 1½ or 2 days in others (Blobel and Burch 1960a).

Oral therapy: The oral application of antibiotics and chemotherapeutics to the ruminants results in fairly high concentrations in the rumen content and may create serious disturbances of the flora. Antibiotic residues in milk of the cows have been demonstrated by some investigators:

Skaggs and Miller (1959) reported detectable levels of penicillin in milk following oral administration of approximately 173,000 and 270,000 units of procaine penicillin.

Cannon et al., (1962) fed 12 cows with penicillin in oil at the rate of 10,000,000 units per cow in a single dose. The maximum duration of a detectable level of penicillin in milk was 86 hours with the peak mean level of penicillin in milk occurring at the 14-hour post-feeding interval.

Wright and Harold (1960) did not detect penicillin in milk of cows fed 1,000,000 units daily or 2,500,000 units twice daily of buffered potassium pencillin.

From these three studies, it would seem that the type, rather than the amount of penicillin preparation fed may have been involved in the differences in the findings of the three studies.

Intrauterine therapy: The intrauterine treatment with antibiotics may result in residues in the milk, but some investigators have not been able to demonstrate this:

Intrauterine infusion in each of 5 cows with 1,000,000 units of penicillin and 1 gm. of dihydrostreptomycin in 20 c.c. of sterile water did not result in residues of these antibiotics in milk samples taken 12, 24, 48 and 72 hours after infusion (Kendrick, 1960). Identical results were produced after infusing 500,000 units of penicillin and 0.50 gm of dihydrostreptomycin in 20 c.c. of sterile water in each of 4 cows, and after infusing 100 mg. of oxytetracycline in each of 5 other cows respectively (Kendrick, 1960).

Following intrauterine infusion of penicillin-streptomycin and furacin ® and vaginal deposition of furacin ®, Henningson et al. (1963) could not detect any chemical residues in any of the milk samples from the animals subjected to the three types of treatment.

Milk from cows that received intrauterine infusions of 1,000,000 units of potassium penicillin in aqueous suspension contained a detectable level of penicillin during the first three post infusion milkings (up to 27 to 31 hours) but penicillin was not detected in the milk at subsequent milkings (Cannon et al., 1962).

Transfer of antibiotics from treated to non-treated quarters of dairy cows

Conflicting views exist on the mechanism of transfer of antibiotics from treated to non-treated quarters of dairy cows

(Albright *et al.*, 1961). Statements were often made that after benzylpenicillin was injected, transfer of the drug was by direct diffusion from the treated to the untreated quarters (Hawkins *et al.*, 1962; Rollins *et al.*, 1970). The assumptions were based on the observation that antibiotics were usually found in different concentrations in milk from non-treated quarters of the udder (Albright, *et al.*, 1961) Rollins *et al.*, 1970). Furthermore it has been reported (Hawkins *et al.*, 1962) that benzyl penicillin was most frequently found and appeared in higher concentrations in milk from the adjacent and parallel non-treated quarters, whereas the drug was detected less frequently and at lower concentrations in milk from the diagonal quarters.

Others (Blobel, 1960) have postulated that the transfer of antibiotics from treated to non-treated quarters was through the blood stream, with some degree of diffusion occurring between the two quarters of one side.

Rasmussen (1972) examined the mechanism of excretion of a antipyrine, sulphanilamide, and sulphadimidine into the milk from non-treated glands after intramammary injection. He observed that the unionized antipyrine and sulphanilamide were equally distributed in serum and in milk from the non-treated quarters. However, the more ionized acidic sulphadimidine appeared in milk from the non-treated quarters at lower concentration than in serum. Non-ionic passive diffusion via the blood stream was therefore suggested as the principal mechanism involved in the transfer of these drugs.

Penicillin has been reported to occur in milk from non-infused quarters of cows in which one or more quarters were infused (Blobel, 1960; Evans and Stern 1960; and Ormiston et al., 1960).

The observation by Blobel (1960) that penicillin diffused from the treated to the non-treated quarters, appears to contradict findings of the classical studies referred to by Smith (1959) in which dye and radioactive barium were employed. Both of these studies and others which have been reviewed by Albright et al. (1961) indicated that no direct diffusion occurs between quarters of the udder of cows.

The transfer of chlortetracycline alone or in combination with other antibiotics in an ointment carrier has been reported (Fincher et al., 1962; Randall et al., 1953-54).

Blobel and Burch (1960c) found that a transfer of oxytetracycline from treated to untreated quarters occurred following intramammary infusion of 426 mg. oxytetracycline per quarter. Oxytetracycline levels in milk samples from untreated quarters of infused cows did not exceed 0.08 unit/ml. of milk and were consistently lower than the corresponding blood serum levels. Using 852 mg. of tetracycline under similar experimental conditions the respective levels in untreated quarter milk samples varied between 0.05 and 0.10 unit/ml. of milk.

Preservation:

There is a possibility that antibiotics may be added deliberately to milk and milk products as a preservative measure. For the preservation of milk and milk products admixture, dipping and/or antibiotic coating of application are generally combined with other more conventional preservation methods because the application of antibiotics only delays spoilage and cannot guarentee sterility or prevent contamination.

The preservative effects of the admixture of penicillin, streptomycin, chlortetracycline and oxytetracycline in milk are reported. The addition of nisin to starters for cheese was once a popular field of research as is the addition of pimaricin to cheese coatings today (Mol, 1975).

# MATERIALS AND METHODS

## A. For testing of antibiotic residues in milk:

### Requirements:

Milk samples

Cooler box, freezer packs

Sterile universal bottles

Test organism - *Micrococcus luteus*

Assay medium - Mueller Hinton Agar

Bacterial suspension in Dextrose broth

Antibiotic stock solutions - Procaine penicillin G
Benzyl penicillin sodium salt

Petri dishes - glass with glass covers

Pasteur pipettes (sterile), rubber teats

Plate reading device - a pair of geometrical divider and a ruler

Penicillinase discs, a pair of forceps

Graph paper

### Assay procedures:

#### (i) Milk sampling:

In the period 1977-1978, on several occasions, a total of 1,725 raw milk samples were collected. Pooled milk samples were collected, in sterile universal bottles (10-20 ml) at the receiving platforms of the creameries. These were kept in a cooler box and maintained cold with frozen freezer packs.

On arrival to the laboratory, they were stored in a cold room at +4$^{o}$C until analysed.

(ii) Preparation of the test organism

The test organism used throughout the analysis was *Micrococcus luteus*. The original stock culture was maintained in nutrient agar slants and kept at 4$^{o}$C. When it was needed for use, a loopful of the stock culture was inoculated in 10 ml. of sterile Dextrose broth, mixed well, then incubated overnight at 37$^{o}$C. A loopful or two of well shaken broth culture was streaked on blood agar and the plates were incubated for two days at 37$^{o}$C. The plates were later transferred to the refrigerator until the day of use. Subculturing was done at weekly intervals.

(iii) Preparation of broth culture

The following regimen was follwed to prepare the broth culture of *M. luteus*. First a set of test tubes containing 10 ml. of sterilized Dextrose broth each, were assembled in a test tube rack. Using a sterilized wire loop two isolated single colonies of *M. luteus* from the blood agar plates were inoculated into each tube. The contents were mixed well, then incubated for 24 hours at 37$^{o}$C.

(iv) Preparation of standard antibiotic solution

The antibiotic stock solution was Procaine Penicillin G in aqueous suspension (300,000 iu/ml). On the day of use, 1 ml. of it was diluted with distilled water to give a concentration of 10 iu/ml. From this, further dilutions were made to give concentrations of 0.06 and 0.04 iu/ml. The latter concentrations were used immediately or stored at +4$^{0}$C for not more than 24 hours.

(v) Punch hole technique

The punch hole technique was applied as outlined by Kampelmacher *et al.* (1962) with slight modifications. Mueller Hinton agar plates were stored at +4$^{0}$C for 2 to 10 days before using. Approximately 3 ml. of well shaken broth culture was flooded in each plate. The plates were rocked gently to ensure that the whole surface of the media was covered. Then by tilting each plate, the excess was drained off using a sterile pasteur pipette. The plates were left to dry for a while. Holes were cut out in the agar (10 per plate) by means of a sterile cork borer (thus giving holes 7 mm diameter). Eight holes per plate were then labelled with a marker to correspond with the milk sample numbers. Sterile pasteur pipettes were used to fill the holes with the test milk samples. Penicillin at concentrations 0.06 and 0.04 iu/ml were tested in one area of each plate, along with the milks under examination to assure proper daily functioning of assay and to compare the inhibitory effects of raw milk against reference point.

The set up was left for 15-20 minutes at room temperature to allow pre-diffusion to take place. The plates were then incubated overnight at $37^{0}$C.

After incubation, a pair of geometrical divider was used to measure the sizes of detectable zones of inhibition. Diameters of the zones were estimated to the nearest 0.5 mm. Inhibitory zones larger than or equal to 8.0 mm. were considered as positive tests.

Those milk samples that showed detectable zones of inhibition by the overnight test were heated at $82^{0}$C for five minutes in a thermostatically controlled waterbath, then cooled at $+4^{0}$C, before retesting in the similar technique as with the raw milk samples. In addition, the test was run in pairs (Pair I - heated cooled milk only and Pair II - heated cooled milk tested against penicillinase impregnated discs).

To determine if the zones of inhibition resulted from penicillin, penicillinase impregnated discs were placed near the wells (of Pair II) containing the test milk samples. The wells in both pairs (I and II) were observed for any reaction after incubation. Absence of zone of inhibition around the heated cooled test sample (of Pair I) which was initially observed in raw test sample was considered as total elimination of the "natural" inhibitor. However, persistence of the zone of inhibition around the test sample indicated the "unnatural" inhibitory substance was present. If the zone of inhibition around the sample was decreased near the penicillinase impregnated disc, penicillin was considered present and vice versa.

## Preparation of standard curve for penicillin

Benzyl penicillin sodium salt was used as the standard for penicillin. Penicillin was first diluted in antibiotic-free whole milk and tested over a wide range of concentrations. The test organism was *M. luteus*. Then on subsequent runs, dilutions were made over increasingly narrower ranges and were still tested by punch hole technique using the same technique as with the test milk samples. The idea was to establish more exact sensitivities. Zone diameters were measured and recorded as with the test milk samples. The test was run in four trials. The standard concentrations of penicillin (unit/ml) used to establish the standard response line for punch hole technique were: 1, 0.8, 0.6, 0.4, 0.2, 0.08, 0.06, 0.04, 0.02, 0.008 and 0.006. Data for the standard curve were collected by averaging the diameters of the zones of inhibition produced on all plates by the various standard concentrations. These averages were plotted on the logarithmic scale of the abscissa against the zone diameters in centimeters on the ordinate of semilogarithmic graph paper and gave a straight line curve (see Appendix 1).

The measurement of the diameter of the zone formed by the test milk sample was applied to the scale to find the units per ml. of penicillin.

B. For determination of minimum inhibitory concentrations of antibiotics on bacterial starter cultures:

Requirements:

Bacterial starter cultures (lyophilised form):

*Lactobacillus bulgaricus*

*Streptococcus lactis*

Assay media:

(i) M.R.S. (de Man Rogosa Sharpe) agar/broth
(Selective for lactobacilli)

(ii) Blood agar/Dextrose broth
(to grow *Streptococcus lactis*)

Antibiotic stock solution: Oxytetracycline (50 mg./ml.)
Procaine penicillin G (300,000 iu/ml)
Benzyl penicillin sodium salt
(1,000,000 units/ml)

Pipettes (sterile) - 1 ml. or 2 ml.
Pasteur pipettes (sterile) and rubber teats
Petri-dishes glass with glass covers
Plate reading device - a divider and a ruler

Preparation of the cultures:

Initially the above starter cultures were mixed well in their respective broths and then incubated overnight at 37°C. To obtain isolated single colonies of each, the culture broths were streaked on the respective agar media, incubated overnight at 37$^{o}$C. Slide smears were made from the suspected colonies of both cultures, then were stained with Gram stain and examined microscopically. Both cultures were Gram positive and the shapes were oval for Strept. lactis while rod and long for Lact. bulgaricus. The plates were transferred to the refrigerator and subculturing was done at weekly interval.

The test was run in two stages:

Stage I - Plate agar diffusion test

The preparation of the broth cultures for the two starter cultures in their respective broths was similar to that described for M. luteus.

A tenth dilution series of each antibiotic stock solution (oxytetracycline and procaine pencillin G) was made in distilled water to give a range of concentrations to be tested (see Appendix 4: 1A, 2A).

For the assay, the punch hole technique was adopted. The preparation of the plates was as with the testing of milk samples. The holes were numbered to correspond with the antibiotic concentrations made. The rest of the set up was similar to that of testing raw milk samples. The experiment for each starter bacterium was run in triplicate and the average for each was considered.

Stage II - Broth test:

Test tubes containing the respective sterile broth (10 ml each) were assembled in racks, then labelled according to the various range of concentrations of the antibiotic to be tested.

Taking the concentration of antibiotic that gave the least zone of inhibition in Appendix 4: 1A and 2A) as reference point concentration, fresh dilutions of the antibiotic were made as before. Then for oxytetracycline, a concentration of 50 μg/ml was made and was used to obtain the different concentration ranges. For penicillin, 10 unit/ml was made and used to obtain series of different concentration ranges (see Appendix 4: 1B and 2B). The use of benzyl penicillin sodium salt was to counter check penicillin G.

The test tubes were then inoculated with one drop each of the respective broth culture by means of sterile pasteur pipettes. The contents were mixed well, incubated for 16-17 hours at $37^{o}C$.

After incubation, the tubes were shaken, and the readings were taken and recorded. The lowest concentration of the antibiotic preventing the growth of the bacteria as distinguished by the turbidity (growth) or clearness of the tube contents was considered as the minimum inhibitory concentration (MIC) for that particular bacteria. The test was done in duplicates at four trials. The controls in each trial included (i) test tube with 10 ml. of respective broth alone, (ii) test tube with respective broth + bacteria.

C. For elimination of penicillin in milk

Requirement:

Animals - 12 lactating cows

Antibiotics - Procaine penicillin G and Vetramycin ®-suspension

Disposable syringes (10 ml.) and needles (18 gauge)

Universal bottles (sterile)

Assay Medium - Mueller Hinton agar

Test organism - *Micrococcus luteus*

Bacterial suspension in Dextrose broth

Petri dishes - glass with glass covers

Pasteur pipettes (sterile), rubber teats

Plate reading device - a divider and a ruler

Cotton

Disinfectant - 70% Ethyl alcohol

Routes of administration

(a) Intramuscular injection

Six milking cows were injected intramuscularly with procaine penicillin G in aqueous suspension at the rate of 10,000 units per kg. of body weight in a single dose. The injections were made after the morning milking. Treatment by intramuscular injection consisted of 20-ml penicillin per cow. (Each ml. contains 300,000 units of procaine penicillin G with 0.103% methylparaben and 0.011% propylparaben as preservatives).

(b) Intramammary infusion

Another additional six lactating cows received intramammary infusions of vetramycin ®. The teats were cleaned with cotton soaked in 70% ethanol and later dried with clean dry cotton. Treatment by intramammary infusion consisted of infusing contents of one 4.2 ml. disposable syringe into the left fore quarter and one into the right hind quarter of each cow immediately after morning milking. Following administration, the teat and the udder were briefly massaged with an upward motion to encourage dispersion of the drug through the quarter.

The formulation of the intramammary infusion contained the following ingredients per 4.2 ml. disposable syringe: Penicillin G sodium 600,000 i.u., Dihydrostreptomycin sulfate 600,000 i.u., and Vitamin A 10,000 i.u.

Collection of samples and penicillin assay:

Five strips of milk per quarter were let out before any milk sample taking throughout the exercise. Quarter milk samples were collected before treatment was initiated (approximately 15-20 ml.).

(a) From intramuscular treatment: On the first day of post-treatment, quarter milk samples of each cow were collected at 1, 2, 4, 6 and 8-hr. interval. Collection of quarter milk samples continued in the mornings and evenings until two consecutive samples were found to contain no detectable penicillin by the overnight punch hole plate method using *Micrococcus luteus* as the test organism.

Pooled quarter milk samples for each cow was done in the laboratory and tested in similar manner as with quarter milk samples.

(b) From intramammary infusion: As a routine procedure, milk from the non-treated quarters of each cow were collected first. Quarter milk samples were collected in the evening on the day of treatment (15-20 ml.). Quarter milk samples for the assay were collected immediately prior to the regular twice-daily milking until two consecutive samples were found to contain no detectable penicillin by the overnight punch hole plate method. In all tests, penicillin was identified by use of penicillinase.

## Statistical analysis

To anticipate the variation in penicillin levels at each post-treatment time after the intramuscular and intramammary routes of administration, the respective standard deviations were calculated and represented by means of vertical bars equidistant from the corresponding average readings (fig. 2 and 5).

The calculation of standard deviation at each time interval is obtained by applying the formula below (Bailey, 1973):

$$s.d. = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

where s.d. = standard deviation

x = Penicillin concentration (units/ml.) in milk tabulated in Appendices 5 and 7

n = Total number of observations at each time interval

## RESULTS

Table 11 shows the layout of the inhibitory substances in milk collected from K.C.C. centres in the period 1977-1978. A total of 1,725 milk samples were collected and tested for the presence of antibiotic residues. From this study, 89 (5.2%) milk samples contained substances inhibitory to Micrococcus luteus and 29 of these were shown to contain penicillin, i.e. 1.7% of the total number of samples.

Table 12 shows heating influence on zoning of penicillin. Two concentrations of penicillin (1.0 and 0.6 units/ml) were selected for this study. Penicillin samples were divided into 10 sets for each concentration above, to correspond with the heating time (1-10 minutes). The temperature remained constant (82$^{o}$C).

Table 13 shows the pH influence on zone size using M. luteus as the test organism. Various pH values of 0.2 M acetate buffer were tested.

Table 14 shows the minimum inhibitory concentrations (MIC) of penicillin and oxytetracycline (Terramycin Q-50) on Streptococcus lactis and Lactobacillus bulgaricus. From this study, the MIC of the two antibiotics on both cultures were:

| | | |
|---|---|---|
| Strept. lactis | 0.26 unit/ml | (Penicillin) |
| | 0.60 μg/ml | (Oxytetracycline) |
| Lact. bulgaricus | 0.39 unit/ml | (Penicillin) |
| | 0.70 μg/ml | (Oxytetracycline) |

Figures 2-5 show the penicillin levels in the milk of treated cows. Figs. 2-4 illustrate the penicillin levels after intramuscular administration and fig. 5 shows penicillin levels following intramammary therapy.

(a) Intramuscular administration

Penicillin could be detected in milk of the treated cows upto 46 hours on the average (fig. 2). The milk of some of these cows gave negative tests 46 hours after injection (Figs. 3 and 4).

The initial detection of penicillin in milk following treatment was 2 hours for 5 cows and 4 hours for 1 cow.

The penicillin content in the milk was highest at 8 hours after treatment. The penicillin concentration at this peak ranged from 0.100 to 0.178 units/ml., with an average of 0.136 units/ml. (fig. 2, 3 and 4).

(b) Intramammary therapy

At post-infusion hour 106, milk from treated quarters was negative for the presence of penicillin. For the first 34 hours post-infusion, penicillin concentration in the milk of treated cows was greater than 1 unit/ml. At 48 hours post-infusion the concentration dropped to 0.779 units/ml. on the average (fig. 5).

Milk from untreated quarters was also examined for the presence of penicillin and it was detected in the milk from 6 quarters out of 11. (N.B. One cow had only 3 quarters functioning). Penicillin was detected only between 10- and 24-hours post-infusion time. Penicillin concentrations in the milk from these quarters ranged from 0.019 to 0.224 units/ml (see appendix 8).

Table 11: Inhibitory substances in milk collected from K.C.C. centres:

| K.C.C. | No. of samples analysed | Total No. Positive | No. Positive for Penicillin (%) | No. positive for other inhibitors(%) |
|---|---|---|---|---|
| 1. Industrial Area (Nairobi) | 283 | 17 | 8 (2.83%) | 9 (3.17%) |
| 2. Meru | 90 | 11 | 3 (3.33%) | 8 (8.89%) |
| 3. Kiganjo (Nyeri) | 68 | 9 | 5 (7.35%) | 4 (5.88%) |
| 4. Nakuru | 342 | 9 | 4 (1.17%) | 5 (1.46%) |
| 5. Naivasha | 240 | 20 | 5 (2.08%) | 15 (6.25%) |
| 6. Nyahururu | 272 | 12 | 1 (0.37%) | 11 (4.04%) |
| 7. Kitale | 298 | 9 | 3 (1.00%) | 6 (2.01%) |
| 8. Eldoret | 132 | 2 | 0 | 2 (1.52%) |
| TOTAL | 1,725 | 89 | 29 (1.68%) | 60 (3.48%) |

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| TOTAL | 1,725 | 89 | 29 (1.68%) | 60 (3.48%) |

Table 12: Heating influence on zoning of penicillin

| Penicillin (units/ml) | Zone presence and size (dia. - cm) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unheated | Heated to 82°C at different times (Min) | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1.0 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.2 | 3.1 | 3.0 | 3.0 |
| 0.6 | 2.9 | 2.9 | 2.9 | 2.9 | 2.9 | 2.9 | 2.9 | 2.8 | 2.8 | 2.8 | 2.7 |

dia. - cm.. = diameter in centimeters

Table 13: pH influence on zone size

Acetate buffer

| pH | Zone presence and size (dia. -cm.) | | |
|---|---|---|---|
| | Trials 1 | 2 | 3 |
| 7.0 | Neg. | Neg. | Neg. |
| 6.5 | Neg. | Neg. | Neg. |
| 6.0 | Neg. | Neg. | Neg. |
| 5.5 | Neg. | Neg. | Neg. |
| 5.0 | Neg. | Neg. | Neg. |
| 4.5 | 0.80 | Neg. | Neg. |
| 4.0 | 1.5 | 1.4 | 1.3 |
| 3.5 | 1.7 | 1.6 | 1.5 |
| 3.0 | 1.9 | 1.8 | 1.8 |

Neg. = no zone appearing

Acetate buffer was prepared from two solutions

Solution A: Acetic acid 0.2M (11.55 ml. of glacial acetic acid into 1 litre $H_2O$).

Solution B: Na acetate 0.2M (anhydrous) - (16.4474 gm. into 1 litre $H_2O$).

Table 14: Minimum inhibitory concentrations of penicillin and oxytetracycline on *Strept. lactis* and *Lact. bulgaricus*

| Culture | Minimum inhibitory concentration | |
|---|---|---|
| | Penicillin (units/ml) | Oxytetracycline (μg/ml) |
| *Streptococcus lactis* | 0.26 | 0.60 |
| *Lactobacillus bulgaricus* | 0.39 | 0.70 |

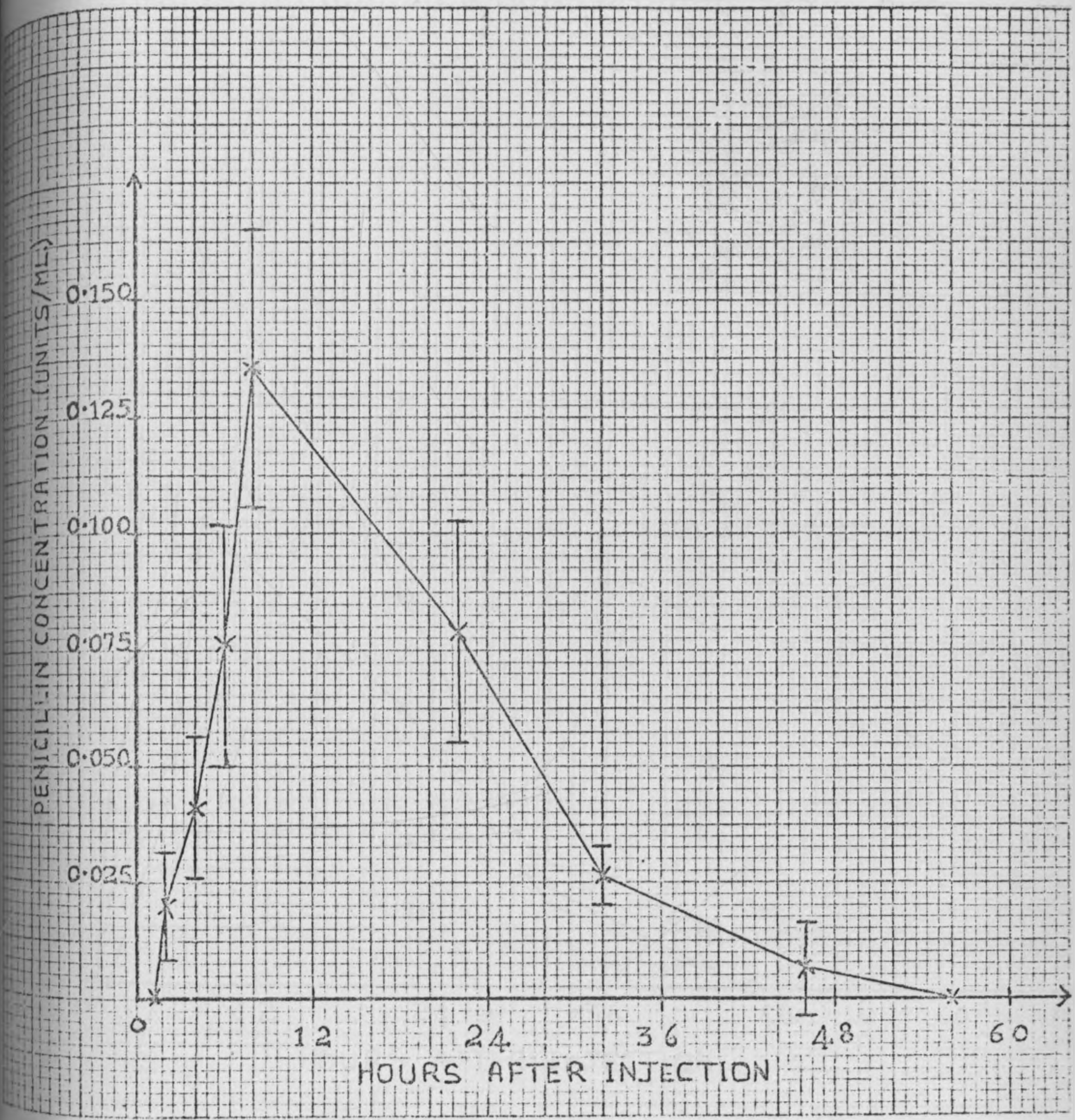


Fig. 2: Penicillin concentrations in milk of cows injected intramuscularly with procaine penicillin G in aqueous suspension (the results given are average readings for 6 cows)

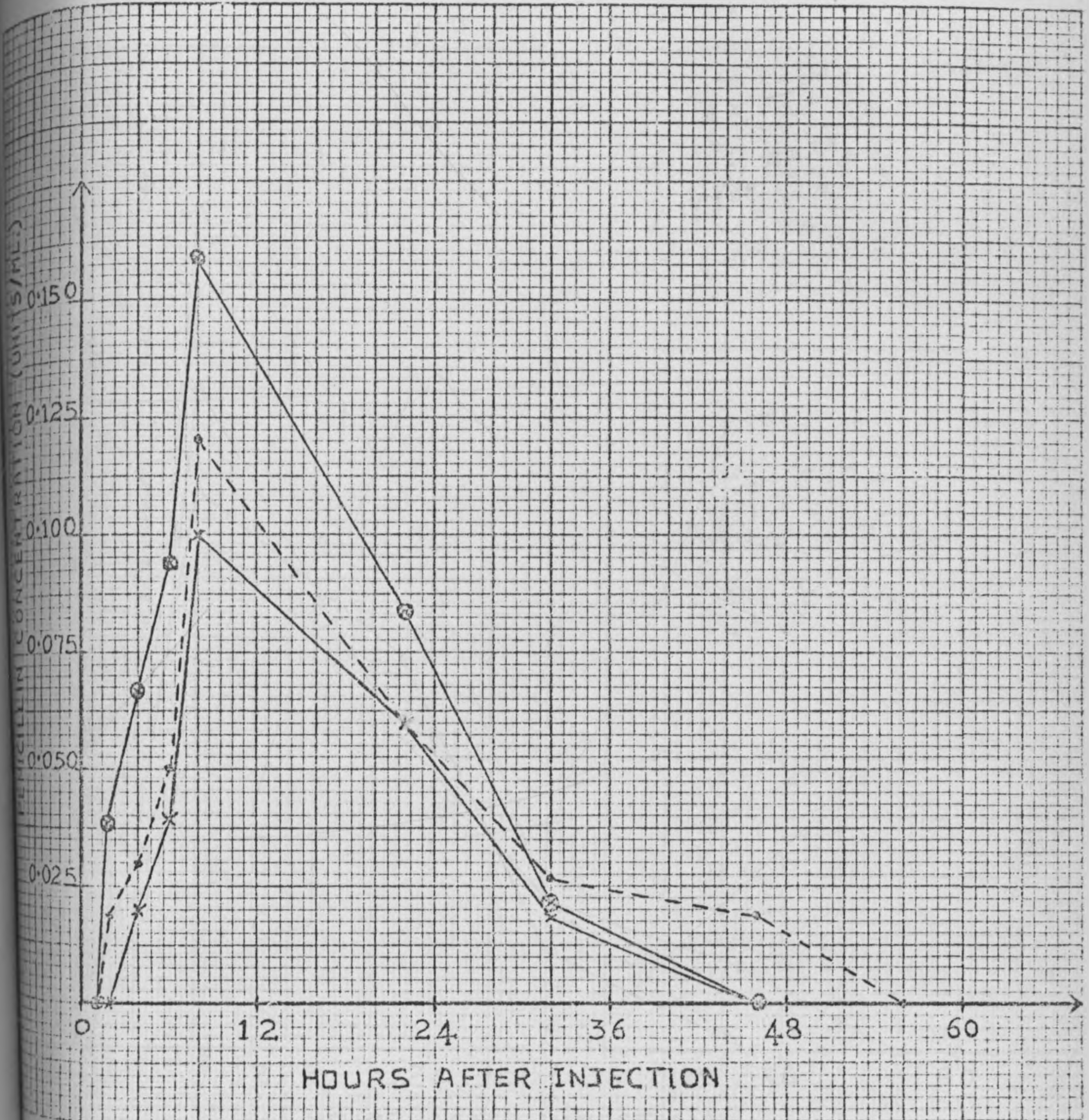


Fig. 3: Penicillin concentrations in milk of cows injected intramuscularly with procaine penicillin G in aqueous suspension (the results given are for pooled quarter milk samples ⊗——⊗—— cow 1, ----·----- cow 2, —x——x—— cow 3).

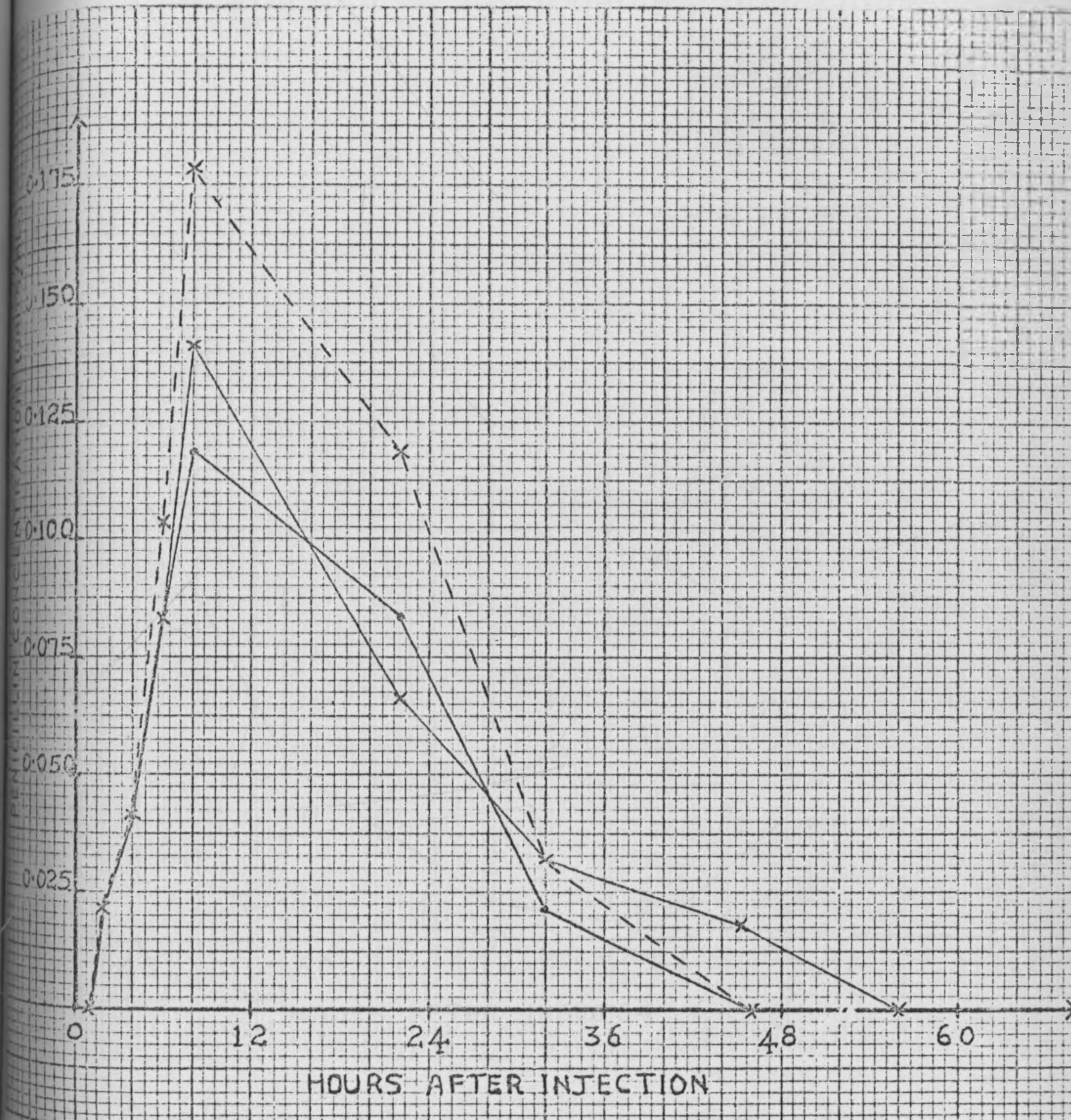


Fig. 4: Penicillin concentrations in milk of cows injected intramuscularly with procaine penicillin G in aqueous suspension (the results given are pooled quarter milk samples ——x——x—— cow 4, --x --x -- cow 5, —.——.—— cow 6).

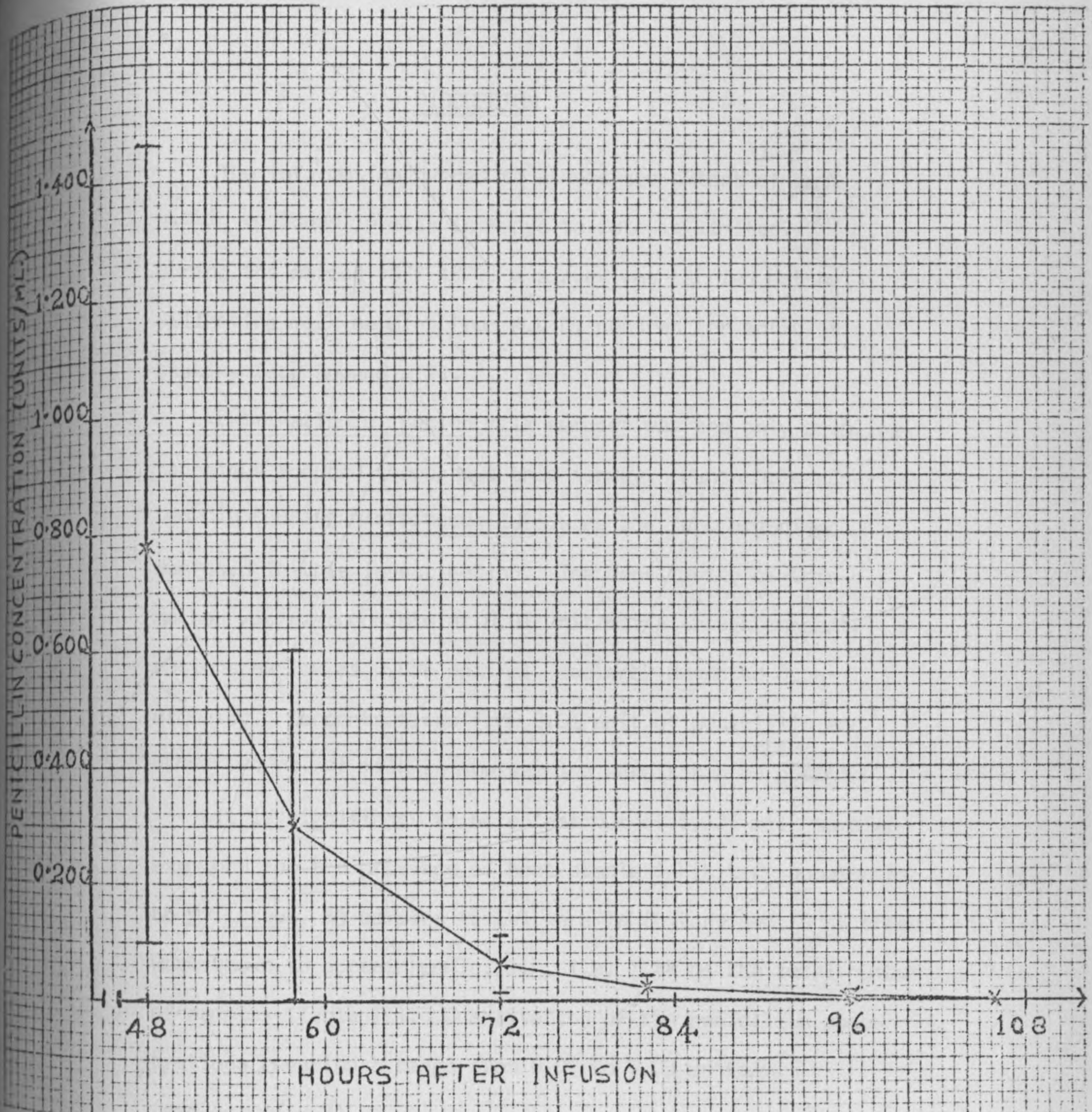


Fig. 5: Excretion of penicillin in the milk after infusion of Vetramycin ® - suspension (One injector of 1.2 million iu per udder quarter. The results given are average readings for 6 cows).

## DISCUSSION

The information needed to assess the extent of antibiotic residues in milk in Kenya to date is limited. During the period 1977-1978, a survey was carried out by collecting milk samples from K.C.C. centres for antibiotic testing (see Appendix 2).

Examination of pooled milk samples was the only sampling technique adopted throughout this survey. Punch hole assay technique using Micrococcus luteus as the test organism was used in this investigation. The sensitivity of this method was reproducible at 0.01 unit/ml. although concentrations as low as 0.006 unit/ml. could be detected occasionally. Concentrations from 0.01 unit of penicillin and above per ml. of milk in the samples were taken as positive.

It is significant to note that out of 1,725 milk samples examined for the presence of heat-stable inhibitory substances to M. luteus, 89 samples (5.2%) were inhibitory and 29 of these were shown to contain penicillin, i.e. 1.7% of the total number of samples (Table II). Quantitation of the penicillin concentrations revealed a range from 0.02 to 0.03 units/ml. (see appendix 3).

Because of the limited information on the incidence of antibiotic residues in milk in Kenya, comparison of the above figures is not possible.

The inhibitory substances were detected in milk collected from various K.C.C. centres listed in Table II. The number of positives (penicillin and other "unnatural" inhibitors) varied from K.C.C. to K.C.C.

On the overall, the incidence of penicillin (1.7%) is less than that of other "unnatural" inhibitors (3.5%) in 1,725 milk samples that were examined in the period 1977-1978.

The figure obtained in this survey compared with those from a number of other countries is compiled in Appendix 9. It must be remembered that comparison of these figures is not quite easy. A number of factors like - sampling techniques, assay techniques and sensitivities, sampling frequencies, the manner of reporting the positive inhibitors (either recording samples with penicillin as positive or others including "unnatural" inhibitors in their figures) do affect the interpretation of the figures obtained in any survey.

In the testing of antibiotic residues in milk, greater exercise was guarded against false positives. This involved finding out the influence of heating on zoning of antibiotics (e.g. penicillin) and of pH values on zone sizes.

Heating influence on zoning of penicillin

Results of most laboratories recommend that during the testing of raw milks for antibiotics, the samples should be heated before reporting a positive test result for inhibitor (Duthie et al., 1976).

The heating of positive-zoning, fresh, raw milks to eliminate false positives in pharmaceutical antibiotic testing is a precautionary practice advocated by certain investigators as reviewed by Kosikowski (1963). Johnston (1960) states that such heating may decompose pharmaceutical antibiotics, leading to significant losses in sensitivity and to the production of false negatives. This interpretation is apparently in conflict with those evident in major scientific reviews, which conclude that penicillin is quite heat-stable and that other antibiotics, in general, have a high degree of heat resistance (Albright et al., 1961; Marth et al., 1959, and Overby, 1954).

During this study, penicillin was stable when heated at 82$^{o}$C for 1, 2, 3, 4, 5, and 6 minutes. Similarly, heating penicillin for 7, 8, 9 and 10 minutes, sizes of zones of inhibition were reduced little, if at all, between the heated and unheated penicillin (Table 12).

Results obtained in this survey showed that with punch hole technique, the prior heating of positive raw milks to 82$^{o}$C for 5 minutes had considerable merit. Most zones of nhibition that were detected during the assay of raw milk samples were lost after such heating.

### pH influence on zone size

High bacteria-count in milk leading to low pH arises from the following conditions: (a) inadequate refrigeration of milk milked in the evening awaiting delivery to the dairy plant the following day, (b) the interval between the time milk leaves the farm to the dairy plant. This depends also on the type of weather prevailing because warm weathers favor rapid growth of bacteria, and (c) inadequate refrigeration of milk transported en route to the laboratories for antibiotic testing.

Most of the milk samples were collected during the cold months and were kept cold with frozen freezer packs. On arrival to the laboratory, they were kept at +4$^{O}$C until analysed. However, on one occasion, milk samples were collected from Nakuru K.C.C. when the weather was quite hot. The pH of a number of raw milks was significantly lower than normal and this coincided with the ability of many of these milks to inhibit Micrococcus luteus on punch hole technique.

To investigate pH influence on zone size, 0.2M acetate buffer was used in this study. From the results shown in table 13, the inhibitory zones against M. luteus were apparent at pH values below 4.5.

The pH values of most milk samples tested were above 4.5 except only for a few cases which were below 4.5. The latter were neutralised by 0.5M phosphate buffer (pH 7.5) and retested. The effect of low pH on zoning was hereby eliminated.

Minimum inhibitory concentrations of antibiotics on starter cultures

Following treatment of mastitis or other infectious diseases with antibiotics, they may be found in the milk in sufficient concentrations to inhibit dairy starter microorganisms and cause economic losses to cheese and fermented milk industries.

The two cultures used for this study were Streptococcus lactis and Lactobacillus bulgaricus. Normally, Strept. lactis, and Strept. cremoris are used to produce right acidity in the following products: cultured butter milk, sour cream, cottage cheese and all types of cheese. Likewise, Lact. bulgaricus, Lact. lactis and Lact. helveticus are used for production of right acidity and flavour for the following: Bulgarian butter milk, yoghurt, Kefir, Koumiss, Swiss, Emmental, and Italian cheese.

On the basis of the results presented in this study (Table 14), it was apparent that Strept. lactis was more sensitive to both antibiotics than was Lact. bulgaricus. These observations also agree with reports by Mol (1975).

The inhibitory levels of penicillin and oxytetracycline on the two cultures during this study (Table 14) agree with those compiled by Kosikowski and Mocquot (1958) and Overby (1952).

Albright et al. (1961) reviewed that most starter cultures are retarded if concentration of penicillin is 0.05 units/ml. or greater and so milk from one treated quarter could inhibit bacterial action in 250 gallons assuming normal infusion dosage level is 100,000 units.

## Penicillin levels in milk of treated cows

The significance of penicillin in milk devolves chiefly around the question of its potentiality to cause allergic reactions, particularly in previously sensitized persons. For this reason, most research workers have developed the need for more explicit information regarding the time required for its elimination from the udder after administration by various routes.

The intramuscular and intrammamary routes were chosen for this study. The animals used for the experiment were all low-yielding cows and at their late stages of lactation. After the first day post-intramuscular injection the cows were milked twice a day. Likewise, the cows that received intramammary therapy were also milked at normal milking hours following treatment.

None of the pretreatment samples of milk from the cows treated intramuscularly and intramammarily were found to contain penicillin or other antibacterial substances at a concentration as high as 0.01 unit/ml. of milk.

After intramuscular injections of procaine penicillin G in aqueous suspension, there was not much variation in the concentrations found in different cows at each post-injection time (See Appendix 5). The quarter milk samples were also analysed. Slight variations in penicillin concentrations were observed in different cows and in different quarters of the same cow (see Appendix 6).

On the other hand, after intramammary infusion of penicillin-streptomycin suspension, as might be expected the highest concentrations of penicillin were found in the first milk samples collected following infusion. From this point, the levels progressively diminished at a rapid rate until the drug could no longer be detected.

There was much variation in the penicillin concentration in the milk from treated quarters of different cows as well as of the same cow, although they were treated with the same antibiotic preparation at the same dose (Appendix 7).

Similar variations have been pointed out by other investigators. For example, Funke (1961) demonstrated a uniform distribution in the mammary gland after parenteral application of $S^{35}$- penicillin in cows and goats; while he found an uneven distribution of the same drug after local administration.

Rasmussen (1964) demonstrated the same phenomenon in a similar work using intramammary application of sulphonamide preparations containing Food Green No. 4 and intravenous injection of the same sulphonamide. An uneven distribution after intramammary application and a uniform distribution after parenteral injection of the sulphonamide were shown.

The transfer of penicillin from treated quarters to non-treated quarters was in small quantities and was only observed in some of the animals for not more than 24 hours following treatment (see Appendix 8). Wide variations in the data reported in the literature (Albright, et al., 1961) may well be due to the lack of sensitivity of some assay procedures (Siddique et al., 1965).

It has been observed that antibiotics tend to persist in the milk of low producing cows for longer periods than in the milk of high producing cows. Some investigators, reviewed by Barnes (1956), have worked with various antibiotics and have presented the evidence of an inverse correlation relationship between the level of milk production and the levels of antibiotics in milk at given post-treatment intervals.

Furthermore, the length of time antibiotics remain in the udder is directly influenced by the type of antibiotic preparation used (Foley et al., 1949).

The results of this investigation showed that all milk from treated animals should be withheld for at least 2 days following intramuscular injection of Procaine penicillin in aqueous suspension (300,000 iu/ml.). Meanwhile, after intramammary application with penicillin-streptomycin suspension (Vetramycin® suspension), milk from treated quarters should be withheld for 4 days and milk from untreated quarters for 24 hours.

## CONCLUSION

The significance of antibiotic residues in milk centres chiefly around the question of:

(a) Some individuals developing sensitivity reactions to antibiotics (e.g. penicillin).

(b) Development of antibiotic resistant strains of micro-organisms and the problems of resistance transfer.

(c) Inhibition of growth of bacterial starter cultures which are involved in the production of fermented milk products.

A total of 1,725 milk samples were collected from K.C.C. centers for antibiotic testing. 89 samples (5.2%) were positive for heat-stable inhibitory substances and 29 of these were shown to contain penicillin, i.e. 1.7% of the total number of samples. The incidence of 5.2% is higher than in a number of other countries and could possibly be reduced.

Measures taken which might lead to prevention of or reduction in the incidence of antibiotic residues in milk are:

1. Restriction of the farmers' possibilities to purchase drugs including antibiotics.
2. Veterinarians do the treatment. Where certain cases (e.g. mastitis), require continued treatment, the veterinarian can leave sufficient amount of the drug to the farmer to follow up and he must instruct the farmer accordingly.
4. Proper instruction of the farmer given by the veterinarian as to the periods for which the milk should be withheld from the dairy. Milk from treated quarters

should be withheld for at least 4 days after intra-mammary treatment and 2 days after systemic treatment.

4. Control measures concerning the withholding of milk. Veterinarian informs the dairy plant manager of the treatments performed. The manager in turn, alerts the fellow who is in charge of recording the quantity of milk per supplier as the milk comes in. Milk from different areas is delivered to the dairy and milk samples could be taken at random at specific dates and sent to the laboratory for antibiotic testing. The exercise of sampling can be done a few times in a year.

Some or all of these measures have been adopted by a number of countries and have led to low percentages. For instance, in Denmark the incidence of antibiotic residues in milk has been reduced from 0.3% (1960) to 0.05% (1976).

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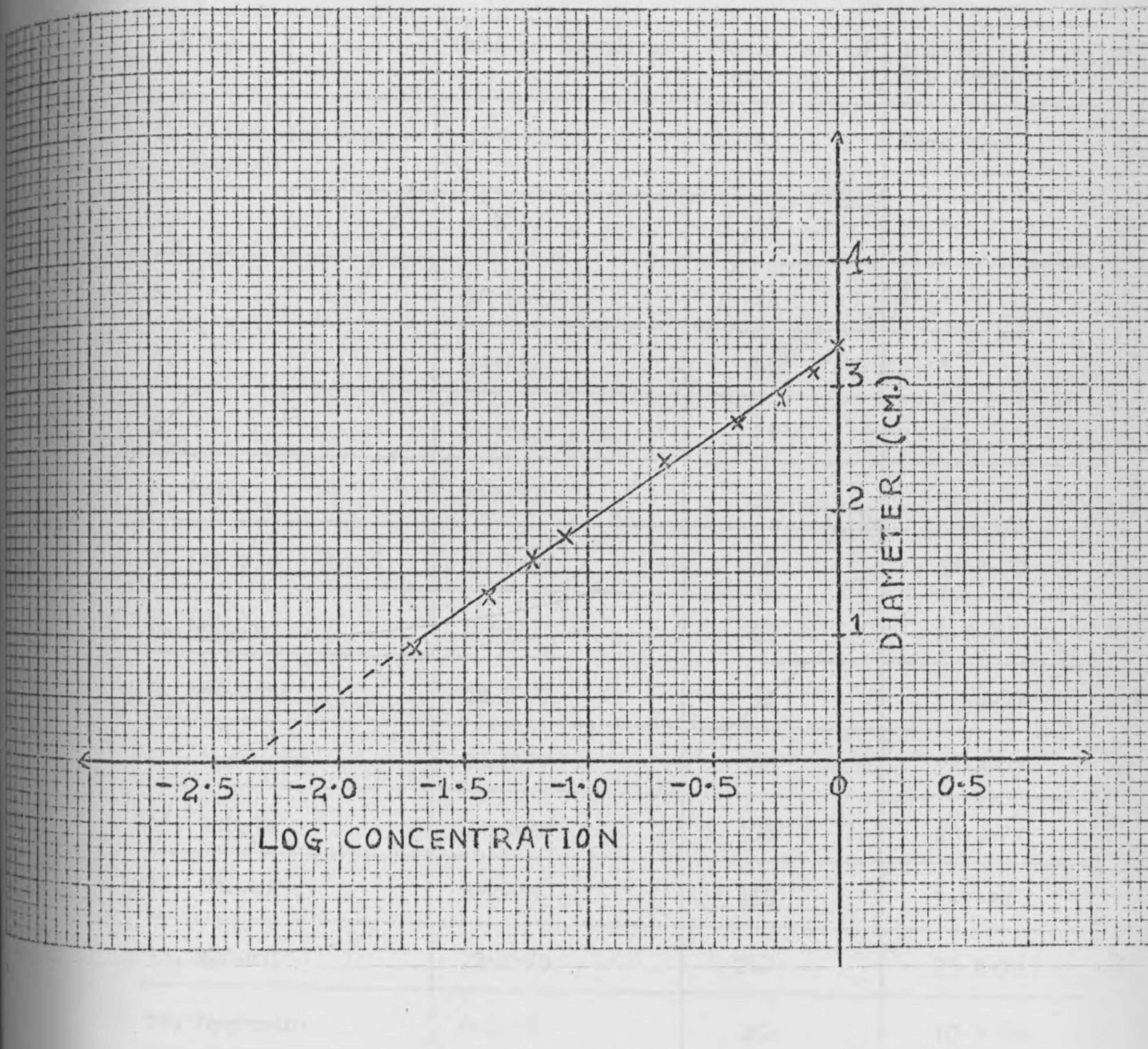
Appendix 1:

Standard curve for penicillin



Appendix 2:

A summary of milk sampling in the period 1977-1978

| K.C.C. | Date of Sampling | Total No. of samples | Initial testing of the samples |
|---|---|---|---|
| 1. Industrial Area - Nairobi | 12-4-77 | 67 | Same day |
| 2. " | 4-5-77 | 35 | " |
| 3. " | 30-5-77 | 48 | " |
| 4. " | 14-6-77 | 59 | " |
| 5. " | 20-6-77 | 74 | " |
| 6. Meru | 4-7-77 | 90 | 5-7-77 |
| 7. Kiganjo - Nyeri | 13-7-77 | 68 | 14-7-77 |
| 8. Nakuru | 5-8-77 | 90 | 6-8-77 |
| 9. Naivasha | 18-8-77 | 90 | 19-8-77 |
| 10. Nyahururu | 25-8-77 | 90 | 26-8-77 |
| 11. Kitale | 27-9-77 | 108 | 28-9-77 |
| 12. Naivasha | 8-2-78 | 150 | 9-2-78 |
| 13. Nakuru | 22-2-78 | 252 | 23-2-78 |
| 14. Nyahururu | 9-3-78 | 182 | 10-3-78 |
| 15. Kitale | 10-4-78 | 190 | 11-4-78 |
| 16. Eldoret | 28-7-78 | 132 | 29-7-78 |
| Total | | 1,725 | |

## APPENDIX 3:- TESTING FOR ANTIBIOTIC RESIDUES IN MILK COLLECTED FROM VARIOUS K.C.C. CENTERS

Nairobi K.C.C.

| Sample No. | Churn No. | Where From | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibitors |
|---|---|---|---|---|---|---|
| | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| 1 | 366 | Kirita-Kiambu | 1.0 | Neg. | | |
| 2 | " | " | 0.80 | " | | |
| 3 | " | " | Neg. | | | |
| 4 | " | " | " | | | |
| 5 | " | " | 1.20 | 1.20 | - | + |
| 6 | " | " | Neg. | | | |
| 7 | " | " | 1.65 | 1.50 | - | + |
| 8 | " | " | 1.0 | Neg. | | |
| 9 | " | " | Neg. | | | |
| 10 | " | " | " | | | |
| 11 | 334 | Nairobi | " | | | |
| 12 | 150 | Lower Kabete | " | | | |
| 13 | 229 | Nairobi | " | | | |
| 14 | 161 | Matakani-Machakos | " | | | |
| 15 | " | " | Neg. | | | |
| 16 | " | " | 0.90 | Neg. | | |
| 17 | " | " | Neg. | | | |
| 18 | " | " | 0.90 | Neg. | | |
| 19 | " | " | 0.80 | " | | |
| 20 | " | " | 1.20 | " | | |
| 21 | " | " | 1.05 | 1.0 | - | + |
| 22 | " | " | 0.80 | Neg. | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 23 | 161 | Matakani-Machakos | 0.90 | Neg. | | |
| 24 | 312 | Dondora-Athi River | Neg. | | | |
| 25 | 234 | Kiambu | 0.95 | 0.90 | - | + |
| 26 | 44 | " | 0.80 | Neg. | | |
| 27 | 306 | " | Neg. | | | |
| 28 | 231 | Limuru | 0.80 | Neg. | | |
| 29 | 101 | Nairobi | 1.20 | " | | |
| 30 | 329 | " | Neg. | | | |
| 31 | 99 | Limuru | " | | | |
| 32 | " | " | " | | | |
| 33 | " | " | " | | | |
| 34 | " | " | " | | | |
| 35 | " | " | " | | | |
| 36 | " | " | " | | | |
| 37 | " | " | " | | | |
| 38 | " | " | " | | | |
| 39 | " | " | " | | | |
| 40 | " | " | " | | | |
| 41 | 402 | Nairobi | " | | | |
| 42 | 434 | " | " | | | |
| 43 | 221 | " | " | | | |
| 44 | " | " | " | | | |
| 45 | " | " | " | | | |
| 46 | " | " | 0.90 | " | | |
| 47 | 35 | Kamuru | Neg | | | |
| 48 | " | Dondora-Athi River | " | | | |
| 49 | " | " " | " | | | |
| 50 | " | " " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 51 | 336 | Kamuru | Neg. | | | |
| 52 | " | " | " | | | |
| 53 | " | " | " | | | |
| 54 | " | " | 1.30 | 1.10 | - | + |
| 55 | " | " | Neg. | | | |
| 56 | " | " | " | | | |
| 57 | " | " | " | | | |
| 58 | " | " | " | | | |
| 59 | 188 | Kikuyu | " | | | |
| 60 | 189 | " | " | | | |
| 61 | 48 | " | " | | | |
| 62 | 440 | " | " | | | |
| 63 | " | " | " | | | |
| 64 | " | " | " | | | |
| 65 | " | " | 1.0 | Neg. | | |
| 66 | " | " | Neg. | | | |
| 67 | " | " | 1.15 | " | | |
| 68 | 165 | Nairobi | Neg. | | | |
| 69 | 296 | " | " | | | |
| 70 | 293 | Karen | " | | | |
| 71 | 250 | Lower Kabete | " | | | |
| 72 | 261 | Embakasi | " | | | |
| 73 | 414 | Karen | " | | | |
| 74 | 444 | Thika | " | | | |
| 75 | 38 | Karen | " | | | |
| 76 | 409 | Nairobi | " | | | |
| 77 | 193 | Kiambu | " | | | |
| 78 | 234 | Nairobi | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 79 | 22 | Kiambu | Neg. | | | |
| 80 | 405 | Thika | Neg. | | | |
| 81 | 441 | Nairobi | Neg. | | | |
| 82 | 156 | " | Neg. | | | |
| 83 | 329 | Kiambu | Neg. | | | |
| 84 | 447 | Nairobi | Neg. | | | |
| 85 | 119 | Ruiru-Kiambu | Neg. | | | |
| 86 | 391 | Limuru-Kiambu | Neg. | | | |
| 87 | 415 | Nairobi-Karen | Neg. | | | |
| 88 | 402 | Thika-Kiambu | Neg. | | | |
| 89 | 64 | Kiambu | Neg. | | | |
| 90 | 385 | Nairobi | Neg. | | | |
| 91 | 135 | Dondora-Athi River | Neg. | | | |
| 92 | 220 | " " | Neg. | | | |
| 93 | 198 | Machakos | Neg. | | | |
| 94 | 333 | " | Neg. | | | |
| 95 | 360 | " | Neg. | | | |
| 96 | 270 | " | Neg. | | | |
| 97 | 204 | Gatumani-Machakos | Neg. | | | |
| 98 | 263 | " " | Neg. | | | |
| 99 | 262 | " " | Neg. | | | |
| 100 | 151 | " " | Neg. | | | |
| 101 | 460 | Machakos Vet. Farm. | Neg. | | | |
| 102 | 207 | Machakos | Neg. | | | |
| 103 | 334 | Nairobi | Neg. | | | |
| 104 | 234 | " | 0.90 | Neg. | | |
| 105 | 431 | " | Neg. | | | |
| 106 | 366 | Kirita-Kiambu | Neg. | | | |

| (a) | (b) | (c) | | (d) | (e) | (f) | (g |
|---|---|---|---|---|---|---|---|
| 107 | 125 | Kabete | | Neg. | | | |
| 108 | 45 | " | | Neg. | | | |
| 109 | 226 | Dondora-Athi | River | Neg. | | | |
| 110 | 103 | " | " | Neg. | | | |
| 111 | 65 | " | " | Neg. | | | |
| 112 | 209 | " | " | " | | | |
| 113 | 379 | " | " | " | | | |
| 114 | 225 | " | " | " | | | |
| 115 | 89 | " | " | " | | | |
| 116 | 142 | " | " | " | | | |
| 117 | 214 | " | " | " | | | |
| 118 | 63 | " | " | " | | | |
| 119 | 2 | " | " | " | | | |
| 120 | 290 | " | " | " | | | |
| 121 | 140 | " | " | 1.20 | Neg. | | |
| 122 | 184 | Kiambu | | Neg. | | | |
| 123 | 348 | " | | " | | | |
| 124 | 410 | " | | " | | | |
| 125 | 231 | " | | " | | | |
| 126 | 99 | Limuru | | " | | | |
| 127 | 231 | " | | " | | | |
| 128 | 377 | Karen-Nairobi | | " | | | |
| 129 | 81 | Thika | | " | | | |
| 130 | 109 | " | | 1.10 | Neg. | | |
| 131 | 320 | " | | Neg. | | | |
| 132 | 330 | " | | " | | | |
| 133 | 362 | Makuyu-Muranga | | " | | | |
| 134 | 364 | Thika | | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 135 | 374 | Thika | Neg. | | | |
| 136 | 438 | " | " | | | |
| 137 | 514 | " | " | | | |
| 138 | 515 | " | " | | | |
| 139 | 517 | " | " | | | |
| 140 | 531 | Makuyu-Muranga | " | | | |
| 141 | 554 | Thika | " | | | |
| 142 | 437 | Mbooni-Machakos | " | | | |
| 143 | 161 | Lukenya-Machakos | " | | | |
| 144 | 101 | Kamiti-Nairobi | " | | | |
| 145 | 341 | Kithunguri | " | | | |
| 146 | 7 | Ndeiya | " | | | |
| 147 | 17 | " | " | | | |
| 148 | 133 | " | " | | | |
| 149 | 243 | " | " | | | |
| 150 | 392 | " | " | | | |
| 151 | 256 | Limuru-Kiambu | " | | | |
| 152 | 96 | " " | " | | | |
| 153 | 446 | Kiambu | " | | | |
| 154 | 427 | Kiambu-Limuru | " | | | |
| 155 | 115 | " " | " | | | |
| 156 | 384 | Kiambu | " | | | |
| 157 | 383 | " | " | | | |
| 158 | 217 | " | " | | | |
| 159 | 160 | " | " | | | |
| 160 | 100 | " | " | | | |
| 161 | 114 | " | " | | | |
| 162 | 398 | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 163 | 257 | Kiambu | Neg. | | | |
| 164 | 418 | " | " | | | |
| 165 | 321 | " | " | | | |
| 166 | 31 | " | " | | | |
| 167 | 187 | " | " | | | |
| 168 | 302 | " | " | | | |
| 169 | 146 | " | " | | | |
| 170 | 74 | " | " | | | |
| 171 | 167 | " | " | | | |
| 172 | 222 | " | " | | | |
| 173 | 210 | " | " | | | |
| 174 | 58 | Kiambu-Limuru | " | | | |
| 175 | 260 | " " | " | | | |
| 176 | 314 | " " | " | | | |
| 177 | 181 | " " | " | | | |
| 178 | 164 | Kiambu | " | | | |
| 179 | 124 | Kiambu-Limuru | " | | | |
| 180 | 62 | Kiambu | " | | | |
| 181 | 191 | " | " | | | |
| 182 | 123 | " | " | | | |
| 183 | 407 | Kiambu Kikuyu | " | | | |
| 184 | 337 | Kiambu | " | | | |
| 185 | 322 | " | " | | | |
| 186 | 286 | " | " | | | |
| 187 | 213 | " | " | | | |
| 188 | 78 | " | " | | | |
| 189 | 183 | " | " | | | |
| 190 | 159 | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 191 | 76 | Kiambu-Banana | Neg. | | | |
| 192 | 16 | Kiambu-Limuru | " | | | |
| 193 | 15 | Kiambu | " | | | |
| 194 | 110 | " | " | | | |
| 195 | 52 | Kiambu-Limuru | " | | | |
| 196 | 269 | Kiambu | " | | | |
| 197 | 381 | Dondora | " | | | |
| 198 | 51 | " | " | | | |
| 199 | 25 | " | " | | | |
| 200 | 230 | Kiambu-Ruiru | Neg. | | | |
| 201 | 130 | " " | " | | | |
| 202 | 117 | " " | " | | | |
| 203 | 168 | " " | " | | | |
| 204 | 199 | Nairobi | " | | | |
| 205 | 275 | Kiambu-Ruiru | " | | | |
| 206 | 238 | Kasaroni-Nairobi | " | | | |
| 207 | 313 | Lower Kabete | " | | | |
| 208 | 340 | Kiambu | " | | | |
| 209 | 350 | Kabete | " | | | |
| 210 | 446 | Nairobi | " | | | |
| 211 | 416 | " | " | | | |
| 212 | 378 | " | " | | | |
| 213 | 167 | Kiambu | 1.00 | 0.85 | 0.018 | |
| 214 | 15 | " | 1.10 | Neg. | | |
| 215 | 52 | Nairobi | Neg. | | | |
| 216 | 192 | Kiambu | " | | | |
| 217 | 83 | " | " | | | |
| 218 | 79 | " | " | | | |
| 219 | 72 | Limuru | " | | | |
| 220 | 13 | Limuru-Kiambu | 1.30 | Neg. | | |
| 221 | 132 | Kiambu | 1.20 | " | | |

| (a) | (b) | (c) | (d) | (e) | (f) | |
|---|---|---|---|---|---|---|
| 222 | 116 | Kiambu | 0.90 | Neg. | | |
| 223 | 406 | Nairobi | 0.90 | 0.85 | 0.018 | |
| 224 | 131 | Kiambu | 0.90 | 0.85 | 0.018 | |
| 225 | 248 | Limuru | 0.90 | 0.85 | 0.018 | |
| 226 | 395 | " | 1.15 | 0.85 | - | + |
| 227 | 448 | Githunguri | Neg. | | | |
| 228 | 443 | " | " | | | |
| 229 | 144 | " | " | | | |
| 230 | 442 | " | " | | | |
| 231 | 420 | Kiambu | 1.30 | Neg. | | |
| 232 | 247 | " | 1.05 | 0.85 | - | + |
| 233 | 258 | Limuru | Neg. | | | |
| 234 | 169 | Kiambu | 1.00 | 0.90 | 0.019 | |
| 235 | 60 | " | Neg. | | | |
| 236 | 29 | Thinguri-Kiambu | 0.85 | Neg. | | |
| 237 | 59 | Kiambu | 1.05 | " | | |
| 238 | 239 | " | 1.30 | 1.0 | 0.022 | |
| 239 | 208 | " | Neg. | | | |
| 240 | 119 | " | " | | | |
| 241 | 318 | " | 1.10 | Neg. | | |
| 242 | 176 | " | Neg. | | | |
| 243 | 42 | " | " | | | |
| 244 | 9 | " | " | | | |
| 245 | 244 | " | 1.0 | " | | |
| 246 | 429 | " | 1.15 | " | | |
| 247 | 26 | " | 1.60 | 0.95 | 0.021 | |
| 248 | 319 | " | 0.90 | 0.90 | 0.019 | |
| 249 | 126 | " | 1.40 | 1.0 | - | + |

| (a) | (b) | (c) | (d) | (e) | (f) | (g |
|---|---|---|---|---|---|---|
| 250 | 134 | Kiambu | Neg. | | | |
| 251 | 249 | " | " | | | |
| 252 | 397 | " | 1.08 | Neg. | | |
| 253 | 136 | " | Neg. | | | |
| 254 | 323 | " | " | | | |
| 255 | 20 | " | " | | | |
| 256 | 30 | " | " | | | |
| 257 | 14 | " | " | | | |
| 258 | 351 | Githunguri Society | " | | | |
| 259 | 376 | " " | " | | | |
| 260 | 349 | Komothai | " | | | |
| 261 | 308 | Gathiruiru | " | | | |
| 262 | 236 | Karen | " | | | |
| 263 | 282 | " | " | | | |
| 264 | 86 | " | " | | | |
| 265 | 432 | " | 1.10 | Neg. | | + |
| 266 | 121 | " | Neg. | | | |
| 267 | 411 | " | " | | | |
| 268 | 572 | " | " | | | |
| 269 | 428 | " | " | | | |
| 270 | 284 | " | " | | | |
| 271 | 339 | " | " | | | |
| 272 | 325 | " | " | | | |
| 273 | 363 | " | " | | | |
| 274 | 461 | " | 1.50 | 1.0 | - | + |
| 275 | 328 | " | Neg. | | | |
| 276 | 304 | " | " | | | |
| 277 | 291 | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 278 | 212 | Karen | Neg. | | | |
| 279 | 77 | " | " | | | |
| 280 | 389 | " | " | | | |
| 281 | 424 | " | 1.10 | Neg. | | |
| 282 | 305 | " | Neg. | | | |
| 283 | 297 | " | " | | | |

| No. | Churn No. | Where From | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibitors |
|---|---|---|---|---|---|---|
| | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| 1 | | Katheri I | Neg. | | | |
| 2 | | " | " | | | |
| 3 | | " | " | | | |
| 4 | | " | " | | | |
| 5 | | " | " | | | |
| 6 | | " | " | | | |
| 7 | | " | " | | | |
| 8 | | " | 1.10 | 0.90 | - | + |
| 9 | | " | Neg. | | | |
| 10 | | " | " | | | |
| 11 | | " | 0.90 | 0.90 | - | + |
| 12 | | Githongo | Neg. | | | |
| 13 | | " | " | | | |
| 14 | | " | " | | | |
| 15 | | " | " | | | |
| 16 | | " | " | | | |
| 17 | | " | " | | | |
| 18 | | " | " | | | |
| 19 | | " | 1.20 | Neg. | | |
| 20 | | " | 1.10 | 1.10 | 0.027 | |
| 21 | | " | Neg. | | | |
| 22 | | " | 1.10 | Neg. | | |
| 23 | | " | 1.50 | 0.90 | - | + |
| 24 | | " | Neg. | | | |
| 25 | | " | " | | | |
| 26 | | " | 1.15 | 1.0 | - | + |

| (a) | (b) | (c) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|
| 27 | | Githongo | Neg. | | | |
| 28 | | " | " | | | |
| 29 | | " | " | | | |
| 30 | | " | 1.10 | 0.90 | 0.019 | |
| 31 | | " | 1.30 | Neg. | | |
| 32 | | Mkando | 1.20 | " | | |
| 33 | | " | 1.20 | 1.0 | 0.022 | |
| 34 | | " | 0.90 | Neg. | | |
| 35 | | " | 0.90 | " | | |
| 36 | | Naari | 1.05 | " | | |
| 37 | | " | Neg. | - | | |
| 38 | | " | " | | | |
| 39 | | " | " | | | |
| 40 | | " | 0.90 | Neg. | | |
| 41 | | " | Neg. | | | |
| 42 | | " | " | | | |
| 43 | | " | 1.0 | 0.90 | - | + |
| 44 | | " | Neg. | | | |
| 45 | | " | " | | | |
| 46 | | Kithurune | 1.30 | Neg. | | |
| 47 | | " | 1.40 | " | | |
| 48 | | " | Neg. | - | | |
| 49 | | " | 1.15 | Neg. | | |
| 50 | | " | Neg. | | | |
| 51 | | " | 1.20 | Neg. | | |
| 52 | | " | 1.20 | " | | |
| 53 | | " | Neg. | - | | |
| 54 | | " | " | | | |
| 55 | | " | 0.90 | Neg | | |
| 56 | | " | 0.90 | " | | |
| 57 | | " | Neg. | - | | |
| 58 | | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|
| 59 | | Kithurune | Neg. | | | |
| 60 | | Katheri II | " | | | |
| 61 | | " | " | | | |
| 62 | | " | " | | | |
| 63 | | " | " | | | |
| 64 | | " | " | | | |
| 65 | | " | 1.40 | 1.0 | - | + |
| 66 | | " | Neg. | | | |
| 67 | | " | " | | | |
| 68 | | " | " | | | |
| 69 | | " | " | | | |
| 70 | | " | " | | | |
| 71 | | NKuene | " | | | |
| 72 | | " | " | | | |
| 73 | | " | " | | | |
| 74 | | " | " | | | |
| 75 | | " | " | | | |
| 76 | | " | " | | | |
| 78 | | " | " | | | |
| 79 | | " | " | | | |
| 80 | | " | " | | | |
| 81 | | " | " | | | |
| 82 | | " | " | | | |
| 83 | | " | 1.40 | 1.0 | - | + |
| 84 | | " | Neg. | | | |
| 85 | | " | 1.30 | 0.90 | - | + |
| 86 | | " | 1.20 | Neg. | | |
| 87 | | Abogeta | 0.90 | Neg. | | |
| 88 | | " | Neg. | | | |
| 89 | | " | " | | | |
| 90 | | " | " | | | |

Kiganjo K.C.C.

| Sample No. | Churn No. | Where From | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibitors |
|---|---|---|---|---|---|---|
| | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| 1 | 29 | Waraza F.C.S. | Neg. | | | |
| 2 | " | " | " | | | |
| 3 | " | " | " | | | |
| 4 | " | " | 0.90 | Neg. | | |
| 5 | " | " | Neg. | | | |
| 6 | 285 | Nyeri | " | | | |
| 7 | 56 | Ihururu F.C.S. | " | | | |
| 8 | " | " | " | | | |
| 9 | " | " | " | | | |
| 10 | " | " | " | | | |
| 11 | 60 | Gihaiga | " | | | |
| 12 | 14 A & B | Rware | 1.0 | Neg. | | |
| 13 | 38A | " | Neg. | | | |
| 14 | 59 | Mathenge | 0.90 | Neg. | | |
| 15 | 68 | Kimathi | Neg. | | | |
| 16 | 54 | Kirimara | 0.90 | Neg. | | |
| 17 | 233 | Gathi | Neg. | | | |
| 18 | 87 A,B& C | Tetu Dairy | 1.10 | Neg. | | |
| 19 | " | " | 1.10 | 0.85 | 0.018 | |
| 20 | " | " | 0.90 | 0.90 | - | + |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 21 | 87 A,B & C | Tetu Dairy | Neg. | | | |
| 22 | " | " | 0.90 | Neg. | | |
| 23 | " | " | 1.35 | 1.05 | 0.024 | |
| 24 | " | " | 0.90 | Neg. | | |
| 25 | 23 | Nyeri | Neg. | | | |
| 26 | 61 C | Mathira | " | | | |
| 27 | " | " | " | | | |
| 28 | " | " | 0.90 | Neg. | | |
| 29 | " | " | 1.0 | " | | |
| 30 | " | " | Neg. | | | |
| 31 | 188 | Island | " | | | |
| 32 | 57 A | Kirinyaga | " | | | |
| 33 | 64 | Uthaya | " | | | |
| 34 | " | " | 1.0 | 0.80 | - | + |
| 35 | 67 | Mweiga | Neg. | | | |
| 36 | 31 | Mweiga | 0.90 | Neg. | | |
| 37 | " | " | 0.85 | " | | |
| 38 | " | " | 1.0 | 0.85 | - | + |
| 39 | 17 | " | Neg. | | | |
| 40 | 15 A | " | " | | | |
| 41 | 83 | Ngukurani | " | | | |
| 42 | 93 | Gaiga | " | | | |
| 43 | 128 | Sweet water-Muranga | 0.90 | 0.90 | 0.019 | |
| 44 | 197 | Muranga Kiriti F.C.S. | 0.90 | 0.90 | 0.019 | |
| 45 | 73 | Gakindu | 0.90 | 0.85 | - | + |
| 46 | 205 | Ngobit | Neg. | | | |
| 47 | 209 | T. Falls Side | 0.80 | Neg. | | |
| 48 | 241 | " | 1.0 | " | | |
| 49 | 79 | " | Neg. | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 50 | 44 | T.Falls Side | Neg. | | | |
| 51 | 19 | " | 0.90 | Neg. | | |
| 52 | " | " | Neg. | | | |
| 53 | 71 | " | " | | | |
| 54 | 30 | " | " | | | |
| 55 | " | " | " | | | |
| 56 | 34 | " | " | | | |
| 57 | 193 | " | " | | | |
| 58 | " | " | " | | | |
| 59 | 180 | " | 1.0 | 0.85 | 0.018 | |
| 60 | " | " | Neg. | | | |
| 61 | " | " | " | | | |
| 62 | " | " | " | | | |
| 63 | 3 | " | " | | | |
| 64 | 16 | Gataragwa | " | | | |
| 65 | 164 | Mukurweini | " | | | |
| 66 | Route N | Timau | " | | | |
| 67 | " | " | " | | | |
| 68 | " | " | 1.0 | Neg. | | |

Nakuru K.C.C.

| Sample No. | Churn No. | Where From | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibitor |
|---|---|---|---|---|---|---|
| | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| 1 | 28 B | Sabatia | 1.10 | 0.85 | - | + |
| 2 | " | " | 0.95 | Neg. | | |
| 3 | " | " | Neg. | | | |
| 4 | " | " | 1.20 | Neg. | | |
| 5 | " | " | Neg. | | | |
| 6 | 189 | Pekera | " | | | |
| 7 | 772 | Chebonor Farm | 0.90 | Neg. | | |
| 8 | 226 | Rift Valley Dev. Tech. Farm | 1.0 | " | | |
| 9 | 18 | Hamilton Estate | Neg. | | | |
| 10 | " | " | " | | | |
| 11 | 532 | Monostry our Lady of Victory | 1.0 | Neg. | | |
| 12 | " | " | Neg. | | | |
| 13 | 408 | Lumbwa | " | | | |
| 14 | 525 | Suguna - Nakuru | " | | | |
| 15 | 256 | Nakuru | 0.90 | Neg. | | |
| 16 | 379 | " | Neg. | | | |
| 17 | 676 | " | " | | | |
| 18 | 853 | Bahati F.C.S. - Nakuru | 1.20 | 0.85 | - | + |
| 19 | 692 | Nakuru | Neg. | | | |
| 20 | 11299 | Rongai | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 21 | 239 | Elburgon | Neg. | | | |
| 22 | 1164 | Nakuru | " | | | |
| 23 | " | " | " | | | |
| 24 | 596 | Rongai | " | | | |
| 25 | " | " | " | | | |
| 26 | 983 | Solai - Akubi Farmers Ltd. | " | | | |
| 27 | 53 | Bahti-Githiga K.B.U. Farmers | " | | | |
| 28 | 76 | Nakuru | " | | | |
| 29 | 989A | " | 0.95 | Neg. | | |
| 30 | | Londiani | Neg. | | | |
| 31 | 27 | Nakuru | Neg. | | | |
| 32 | 166 | " | " | | | |
| 33 | 184 | Usalama Farmers-Nakuru | " | | | |
| 34 | 54 | Nakuru | " | | | |
| 35 | 37 | Cally estate | " | | | |
| 36 | " | " | " | | | |
| 37 | | Njoro - Kanziwa | " | | | |
| 38 | 238 | Njoro | " | | | |
| 39 | 510 | Nakuru | " | | | |
| 40 | " | " | 1.0 | Neg. | | |
| 41 | " | " | Neg. | | | |
| 42 | " | " | " | | | |
| 43 | 24 | " | " | | | |
| 44 | 369 | " | 0.90 | Neg. | | |
| 45 | 814 B | " | 0.90 | Neg. | | |
| 46 | | Rongai | 1.10 | " | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 47 | 272 B | Nakuru | Neg. | | | |
| 48 | 804 | Njoro | " | | | |
| 49 | 185 | Elburgon | 0.80 | Neg. | | |
| 50 | 120 | Rongai | Neg. | | | |
| 51 | " | " | " | | | |
| 52 | " | " | " | | | |
| 53 | " | " | " | | | |
| 54 | 196 | " | " | | | |
| 55 | 67 | Molo | " | | | |
| 56 | 1008 | Rongai | " | | | |
| 57 | 188 | " | 0.90 | Neg. | | |
| 58 | 52 | Menengai | 1.0 | " | | |
| 59 | 77 | Rongai | 0.90 | " | | |
| 60 | 236 | " | 0.90 | " | | |
| 61 | 646 | " | Neg. | | | |
| 62 | 624 | " | " | | | |
| 63 | 553 | " | " | | | |
| 64 | 1178 | " | " | | | |
| 65 | 141 B | " | 1.10 | 0.80 | - | + |
| 66 | 796 | " | 1.10 | Neg. | | |
| 67 | 288 | " | Neg. | | | |
| 68 | 906 | " | 1.10 | 0.85 | 0.018 | |
| 69 | 1208 | Nakuru | Neg. | | | |
| 70 | 559 | Rongai | " | | | |
| 71 | 831 | Nakuru | " | | | |
| 72 | 495 | " | " | | | |
| 73 | 787 | Rongai | " | | | |
| 74 | 709 | " | " | | | |
| 75 | 1141 | " | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 76 | 180 | Rongai | | Neg. | | | |
| 77 | 402 | " | | " | | | |
| 78 | 350 | " | | " | | | |
| 79 | 1344 | " | | " | | | |
| 80 | 169 | " | | " | | | |
| 81 | 99 | " | | " | | | |
| 82 | 1019 B | " | | " | | | |
| 83 | 992 | " | | " | | | |
| 84 | 524 | " | | " | | | |
| 85 | 651 | " | | " | | | |
| 86 | 866 | Nakuru | | " | | | |
| 87 | 842 | Elburgon- Septet Ltd. | | " | | | |
| 88 | 1 | " | | " | | | |
| 89 | 226 | Nakuru | | 0.95 | Neg. | | |
| 90 | " | " | | Neg. | | | |
| | | | PH | | | | |
| 91 | 247 | Njoro | 5.1 | Neg. | | | |
| 92 | 358 | " | 4.3 | " | | | |
| 93 | 863 | " | 4.7 | " | | | |
| 94 | 384 | " | 4.5 | 0.9 | Neg. | | |
| 95 | 239 | " | 4.7 | Neg. | | | |
| 96 | 704 | " | 4.5 | " | | | |
| 97 | 983 | Solai small society | 4.4 | " | | | |
| 98 | 27 | Mogotio | 4.4 | " | | | |
| 99 | 414 | Melangine co-op. | 4.6 | " | | | |
| 100 | " | " | 5.3 | " | | | |
| 101 | " | " | 4.5 | 0.9 | Neg. | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 102 | 19 | Njoro | 5.6 | Neg. | | | |
| 103 | 910/54/ 184 | Bahati | 4.5 | 1.0 | Neg. | | |
| 104 | " | " | 5.7 | Neg. | | | |
| 105 | " | " | 5.6 | " | | | |
| 106 | " | " | 4.7 | Neg. | | | |
| 107 | " | " | 5.4 | 1.0 | 0.9 | 0.019 | |
| 108 | 166, 508 | Nakuru | 5.5 | Neg. | | | |
| 109 | 256, 32 | Elbagon | 4.5 | 0.9 | Neg. | | |
| 110 | " | " | 4.4 | 1.0 | " | | |
| 111 | 1241 | Rongai | 4.3 | Neg. | | | |
| 112 | 559 | " | 4.5 | " | | | |
| 113 | " | " | 4.5 | 0.8 | Neg. | | |
| 114 | " | " | 4.4 | Neg. | | | |
| 115 | " | " | 4.3 | 1.0 | Neg. | | |
| 116 | " | " | 4.2 | 0.9 | " | | |
| 117 | " | " | 4.6 | 1.0 | " | | |
| 118 | " | " | 4.4 | Neg. | | | |
| 119 | " | " | 4.7 | 0.9 | Neg. | | |
| 120 | 866, 299 | Nakuru | 4.5 | 0.9 | " | | |
| 121 | 1485, 676 | Ndodori | 4.4 | 0.9 | " | | |
| 122 | " | " | 4.4 | 1.0 | " | | |
| 123 | | Menengai East | 4.4 | 1.0 | " | | |
| 124 | 787, 180 | Rongai-Njoro | 4.3 | Neg. | | | |
| 125 | | " | 4.4 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 126 | 787, 180 | Rongai-Njoro | 4.5 | 0.9 | Neg. | | |
| 127 | | " | 5.0 | Neg. | | | |
| 128 | | " | 5.1 | " | | | |
| 129 | | " | 4.5 | " | | | |
| 130 | | " | 4.5 | 1.0 | Neg. | | |
| 131 | | " | 4.4 | 1.0 | " | | |
| 132 | | " | 5.0 | Neg. | - | | |
| 133 | | " | 4.6 | 0.9 | Neg. | | |
| 134 | | " | 4.5 | 0.9 | " | | |
| 135 | | " | 4.9 | Neg. | - | | |
| 136 | | " | 4.5 | 1.0 | Neg. | | |
| 137 | | " | 4.5 | Neg. | | | |
| 138 | | " | 4.6 | 0.9 | Neg. | | |
| 139 | | " | 4.4 | 1.0 | Neg. | | |
| 140 | | " | 5.0 | 0.90 | 0.90 | - | + |
| 141 | | " | 4.6 | 0.8 | Neg. | | |
| 142 | | " | 4.5 | 1.0 | " | | |
| 143 | | " | 4.6 | 0.9 | " | | |
| 144 | | " | 5.0 | Neg. | - | | |
| 145 | 6 | Bahati farm | 4.9 | Neg. | | | |
| 146 | | " | 4.8 | " | | | |
| 147 | Mix-ture | Dondori/ Bahati | 4.6 | 0.9 | Neg. | | |
| 148 | 24, 272 | Dondori | 5.0 | 1.0 | Neg. | | |
| 149 | " | " | 5.2 | Neg. | | | |
| 150 | " | " | 4.9 | " | | | |
| 151 | 11118 | Rongai | 5.4 | " | | | |
| 152 | 624,646 | Njoro | 4.8 | " | | | |
| 153 | 553 | Ngata S.F.T. | 4.7 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 154 | 553 | Ngata S.F.T. | 4.8 | Neg. | | | |
| 155 | " | " | 4.5 | Neg. | | | |
| 156 | 1365, 1165 | Njoro | 4.4 | " | | | |
| 157 | 288, 796 | " | 5.3 | " | | | |
| 158 | " | " | 5.4 | " | | | |
| 159 | 141 | " | 4.9 | " | | | |
| 160 | 1446 | " | 4.6 | 0.90 | Neg. | | |
| 161 | 1334, 1356, 1103 | " | 4.7 | Neg. | | | |
| 162 | 72, 148 | Bahati | 4.7 | 0.80 | Neg. | | |
| 163 | 1092, 1491, 1178 | Njoro | 4.5 | Neg. | | | |
| 164 | 1208 | " | 4.7 | " | | | |
| 165 | 695 | Subukia | 4.2 | " | | | |
| 166 | 1113, 221 | Gicheha Farm-Rongai | 4.5 | " | | | |
| 167 | " | " | 4.9 | " | | | |
| 168 | " | " | 4.6 | " | | | |
| 169 | " | " | 5.0 | 1.0 | Neg. | | |
| 170 | " | " | 4.5 | 0.90 | " | | |
| 171 | " | " | 4.7 | 0.90 | " | | |
| 172 | " | " | 5.1 | Neg. | - | | |
| 173 | " | " | 5.0 | 0.90 | " | | |
| 174 | " | " | 4.5 | 1.0 | " | | |
| 175 | " | " | 5.4 | 0.90 | 0.90 | 0.019 | |
| 176 | 621 | Nakuru | 4.5 | 1.0 | Neg. | | |
| 177 | 33 | Subukia | 4.6 | 1.0 | " | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 178 | | Baruku | 4.5 | 1.0 | Neg. | | |
| 179 | | " | 4.5 | Neg. | | | |
| 180 | | " | 4.6 | " | | | |
| 181 | 417 | Nakuru | 4.5 | 1.0 | Neg.. | | |
| 182 | 1241 | Manduganda Farm | 4.3 | Neg. | | | |
| 183 | 583, 565 | Rongai | 4.3 | " | | | |
| 184 | 127 D | " | 4.4 | " | | | |
| 185 | " | " | 5.2 | " | | | |
| 186 | " | " | 5.0 | " | | | |
| 187 | | Menengai East-Soc. | 5.0 | " | | | |
| 188 | 1150, 1095 | Bahati | 4.7 | " | | | |
| 189 | 842 | Elbagon Soc. | 4.7 | " | | | |
| 190 | " | " | 4.8 | 0.90 | 0.90 | - | + |
| 191 | 999, 374 | Subukia | 4.9 | 0.90 | 0.90 | 0.019 | |
| 192 | 574 | " | 4.5 | Neg. | | | |
| 193 | " | " | 5.0 | " | | | |
| 194 | 609 | Rongai | 4.7 | " | | | |
| 195 | 235, 1011 | Njoro | 4.5 | 0.80 | Neg. | | |
| 196 | 59 | Ndodori | 4.6 | Neg. | | | |
| 197 | 871 | " | 4.3 | " | | | |
| 198 | | Bahati | 4.7 | 0.80 | Neg. | | |
| 199 | 12A 181B | " | 4.9 | 1.0 | " | | |
| 200 | | " | 4.5 | Neg. | | | |
| 201 | 245, 456 | Ndodori Soc. | 4.5 | 0.90 | Neg. | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 202 | 245, 456 | Ndodori Soc. (Ngorika co-op) | 4.5 | 0.90 | Neg. | | |
| 203 | " | " | 5.0 | Neg. | | | |
| 204 | " | " | 5.5 | " | | | |
| 205 | " | " | 4.5 | 1.0 | Neg. | | |
| 206 | " | " | 4.5 | 1.0 | " | | |
| 207 | " | " | 5.1 | Neg. | | | |
| 208 | 1255 | Nakuru | 4.9 | " | | | |
| 209 | 161 | Ndodori | 5.0 | " | | | |
| 210 | 1284, 34 | " | 5.4 | " | | | |
| 211 | | " | 4.6 | " | | | |
| 212 | | " | 4.7 | " | | | |
| 213 | 941 | Turi | 4.4 | " | | | |
| 214 | 1134 | Mogotio | 4.9 | " | | | |
| 215 | 865 | Bahati | 4.5 | 0.90 | Neg. | | |
| 216 | 908 | Elementeita | 4.6 | 0.90 | " | | |
| 217 | 226 | Njoro Technol Farm | 4.7 | 0.80 | " | | |
| 218 | " | " | 4.6 | 0.80 | " | | |
| 219 | " | " | 4.7 | Neg. | | | |
| 220 | " | " | 5.0 | " | | | |
| 221 | 1106 | Subukia S.F.T. | 5.1 | 0.90 | Neg. | | |
| 222 | 1344 | Njoro | 4.3 | Neg. | | | |
| 223 | 76 | " | 5.0 | " | | | |
| 224 | 1156 G | Njoro S.F.T. | 4.7 | " | | | |
| 225 | 1117 | Solai Soc. | 4.9 | " | | | |
| 226 | " | " | 5.1 | " | | | |
| 227 | " | " | 4.6 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 228 | 1327, 1041 | Elementeita | 5.0 | Neg. | | | |
| 229 | 369 | Ngawa Farm - Lanet | 4.9 | " | | | |
| 230 | 804 | Njoro | 4.5 | " | | | |
| 231 | " | " | 4.6 | " | | | |
| 232 | " | " | 5.2 | " | | | |
| 233 | 476, 273 | Bahati | 4.5 | " | | | |
| 234 | 909, 1304 | " | 4.5 | " | | | |
| 235 | | Ndodori | 4.4 | 1.0 | Neg. | | |
| 236 | | " | 4.3 | Neg. | | | |
| 237 | | " | 4.5 | 0.90 | Neg. | | |
| 238 | | " | 4.9 | Neg. | | | |
| 239 | | " | 4.7 | 0.80 | Neg. | | |
| 240 | | " | 4.5 | 0.90 | Neg. | | |
| 241 | 1315, 786, 280 | " | 5.0 | 0.80 | Neg. | | |
| 242 | | " | 4.5 | 0.90 | Neg. | | |
| 243 | 1379 | " | 4.7 | Neg. | | | |
| 244 | 1205 | Mau Narok | 4.9 | Neg. | | | |
| 245 | 414 | Melangine Soc. Ndodori | 4.6 | 0.90 | Neg. | | |
| 246 | " | Melangine Soc. | 5.0 | 0.80 | Neg. | | |
| 247 | 1059 | " | 4.9 | Neg. | | | |
| 248 | | " | 4.5 | " | | | |
| 249 | 106, 858 | Lanet | 4.5 | " | | | |
| 250 | 678, 11 | " | 4.7 | " | | | |
| 251 | | " | 4.9 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 252 | | Bahati | 5.0 | Neg. | | | |
| 253 | | " | 4.5 | 0.90 | Neg. | | |
| 254 | 151 | " | 4.6 | 1.0 | " | | |
| 255 | 159, 284, 1045 | " | 4.7 | 1.0 | " | | |
| 256 | 740, 111 | " | 4.8 | Neg. | | | |
| 257 | | " | 4.7 | " | | | |
| 258 | 420 | " | 4.7 | 1.0 | Neg. | | |
| 259 | 716, 115 | Rongai | 4.7 | Neg. | | | |
| 260 | | Njoro | 5.2 | " | | | |
| 261 | | " | 4.5 | " | | | |
| 262 | | " | 4.4 | " | | | |
| 263 | | " | 4.6 | 1.0 | Neg. | | |
| 264 | | " | 5.0 | Neg. | | | |
| 265 | 104 | Bahati | 4.4 | " | | | |
| 266 | 589 | Baruku | 5.1 | 1.0 | Neg. | | |
| 267 | " | " | 4.4 | 0.90 | " | | |
| 268 | 807 | Rongai-Njoro | 4.8 | 0.80 | " | | |
| 269 | 397 | " | 4.7 | Neg. | | | |
| 270 | | " | 4.5 | " | | | |
| 271 | 731 | " | 4.6 | " | | | |
| 272 | 231, 282 | Rongai | 4.4 | Neg. | | | |
| 273 | 815 | Nakuru | 4.6 | 1.0 | Neg. | | |
| 274 | | Mau Narok | 4.6 | 1.0 | " | | |
| 275 | | " | 4.4 | 1.0 | " | | |
| 276 | 1182 | " | 4.7 | Neg. | | | |
| 277 | 134 A | Londiani | 4.4 | 0.90 | Neg. | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) |
|---|---|---|---|---|---|---|
| 278 | 105 | Bongai | 4.5 | 1.0 | Neg. | |
| 279 | 429 | Ndodori-Matindiri-Soc. | 4.9 | Neg. | | |
| 280 | 641 | " | 4.9 | " | | |
| 281 | 1414, 1513 | " | 5.0 | 0.90 | Neg. | |
| 282 | 1471, 1036 | " | 4.9 | 1.0 | " | |
| 283 | | " | 4.5 | 1.0 | " | |
| 284 | 426 | " | 4.8 | Neg. | | |
| 285 | | Baruku | 4.7 | 1.0 | Neg. | |
| 286 | | Solai | 4.9 | Neg. | | |
| 287 | | Londiani Kipkerioni | 5.0 | " | | |
| 288 | | " | 4.5 | Neg. | | |
| 289 | 357, 1455 | " | 5.0 | " | | |
| 290 | 195, 1437 | " | 4.7 | " | | |
| 291 | 137, 700 B | " | 4.6 | " | | |
| 292 | | " | 4.6 | 0.85 | Neg. | |
| 293 | 656, 1177 | " | 4.6 | Neg. | | |
| 294 | 412, 444 | " | 4.6 | " | | |
| 295 | | " | 4.7 | " | | |
| 296 | | " | 4.4 | " | | |
| 297 | 745, 198 | " | 4.6 | 0.85 | Neg. | |
| 298 | | " | 4.5 | Neg. | | |
| 299 | | " | 4.5 | " | | |
| 300 | 1378 | " | 5.1 | Neg. | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 301 | 503 | Rongai | 4.6 | Neg. | | | |
| 302 | | Solai | 4.7 | " | | | |
| 303 | 552, 868 | " | 4.6 | " | | | |
| 304 | 1383, 1386 | " | 4.5 | | | | |
| 305 | | " | 4.4 | 0.80 | Neg. | | |
| 306 | 373, 200 | " | 5.0 | Neg. | | | |
| 307 | 1105, 404 | " | 4.9 | " | | | |
| 308 | 73 | " | 4.9 | " | | | |
| 309 | | " | 4.9 | " | | | |
| 310 | 1280, 788 | Londiani Kipkerioni | 4.3 | " | | | |
| 311 | 290, 174 | " | 4.4 | " | | | |
| 312 | | " | 4.5 | " | | | |
| 313 | | " | 4.9 | " | | | |
| 314 | | " | 4.8 | " | | | |
| 315 | | " | 4.6 | " | | | |
| 316 | 135, 1118 | " | 4.5 | 0.90 | Neg. | | |
| 317 | | " | 4.4 | 0.90 | " | | |
| 318 | | " | 4.6 | 0.90 | " | | |
| 319 | | " | 5.1 | Neg. | | | |
| 320 | | " | 4.5 | " | | | |
| 321 | 1400 | Ole Nguoni | 5.2 | " | | | |
| 322 | 1405, 1227 | " | 4.3 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 323 | 1209, 1233 | Ole Nguoni | 4.9 | Neg. | | | |
| 324 | | Solai | 4.7 | " | | | |
| 325 | 192, 780 | " | 4.7 | " | | | |
| 326 | " | " | 4.8 | " | | | |
| 327 | " | " | 4.9 | " | | | |
| 328 | 89c, 283 | Mogotio | 5.0 | " | | | |
| 329 | 886 B | " | 5.1 | " | | | |
| 330 | 89A, 98 | " | 4.8 | " | | | |
| 331 | " | " | 4.7 | " | | | |
| 332 | " | " | 4.9 | " | | | |
| 333 | " | " | 4.9 | " | | | |
| 334 | 101 | Elemtaita | 4.5 | " | | | |
| 335 | 1117 | Solai society | 4.6 | " | | | |
| 336 | | Elbagon & Molo | 4.3 | " | | | |
| 337 | | " | 4.9 | " | | | |
| 338 | | " | 4.8 | " | | | |
| 339 | 4A, 1081 | " | 5.2 | " | | | |
| 340 | | Molo | 4.9 | " | | | |
| 341 | | " | 4.9 | " | | | |
| 342 | | " | 5.0 | " | | | |

| Sample No. | Churn No. | Where From | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibi tors |
|---|---|---|---|---|---|---|
| | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| 1 | | Gilgil | Neg. | | | |
| 2 | | " | " | | | |
| 3 | | " | " | | | |
| 4 | | " | " | | | |
| 5 | | " | " | | | |
| 6 | | Manera-Nsa | " | | | |
| 7 | | Govt. Farm - Nsa. | 1.0 | Neg. | | |
| 8 | | " | 1.0 | 0.90 | 0.019 | |
| 9 | | " | Neg. | | | |
| 10 | | " | 1.0 | Neg. | | |
| 11 | | " | 1.0 | 0.90 | - | + |
| 12 | | Marula Farm - Nsa. | 1.0 | Neg. | | |
| 13 | | " | 0.90 | " | | |
| 14 | | Kairu Farm - N. Kinangop | Neg. | | | |
| 15 | | N. Kinangop | 0.85 | Neg. | | |
| 16 | | 62 Members Co-op. | 0.80 | " | | |
| 17 | 428 | Tulaga F.C.S. - N. Kinangop | Neg. | | | |
| 18 | " | " | " | | | |
| 19 | " | " | " | | | |
| 20 | " | " | " | | | |
| 21 | " | " | " | | | |
| 22 | " | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 23 | 428 | Tulaga F.C.S. - N. Kinangop | 0.90 | Neg. | | |
| 24 | " | " | Neg. | | | |
| 25 | " | " | " | | | |
| 26 | " | " | " | | | |
| 27 | | S. Kinangop | " | | | |
| 28 | | " | " | | | |
| 29 | | " | " | | | |
| 30 | | " | " | | | |
| 31 | | " | " | | | |
| 32 | | " | " | | | |
| 33 | | " | " | | | |
| 34 | | " | " | | | |
| 35 | | " | " | | | |
| 36 | | " | " | | | |
| 37 | | " | " | | | |
| 38 | | " | " | | | |
| 39 | | " | " | | | |
| 40 | | " | " | | | |
| 41 | 425 | Muruaki F.C.S. - N. Kinangop | " | | | |
| 42 | " | " | " | | | |
| 43 | " | " | " | | | |
| 44 | " | " | " | | | |
| 45 | " | " | " | | | |
| 46 | " | " | " | | | |
| 47 | | N. Kinangop | " | | | |
| 48 | | " | " | | | |
| 49 | | Gilgil - Chokoreria F.C.S. | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 50 | | Gilgil - Chokoreria F.C.S. | Neg. | | | |
| 51 | | " | " | | | |
| 52 | | " | 0.90 | Neg. | | |
| 53 | | " | Neg. | | | |
| 54 | | " | " | | | |
| 55 | | Nyakairo Rugongo - Naivasha | 1.0 | 0.85 | 0.018 | |
| 56 | | Maraguchu F.C.S. - Naivasha | Neg. | | | |
| 57 | | " | " | | | |
| 58 | | " | " | | | |
| 59 | | " | " | | | |
| 60 | | Eburru F.C.S | " | | | |
| 61 | | " | " | | | |
| 62 | | " | 0.90 | Neg. | | |
| 63 | | " | 1.0 | " | | |
| 64 | | " | 0.90 | 0.90 | - | + |
| 65 | 427 | Kahuru - N.Kinangop | 0.90 | Neg. | | |
| 66 | " | " | Neg. | | | |
| 68 | " | " | " | | | |
| 69 | " | " | " | | | |
| 70 | " | " | " | | | |
| 71 | " | " | " | | | |
| 72 | | N. Kinangop | " | | | |
| 73 | 720 | New Karati Farm | 1.0 | Neg. | | |
| 74 | " | " | 1.10 | 0.90 | - | + |
| 75 | | N. Kinangop | Neg. | | | |
| 76 | | Olaragwai F.C.S.8 | " | | | |
| 77 | | " | " | | | |
| 78 | | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 79 | | Olaragwai F.C.S. | Neg. | | | |
| 80 | | " | " | | | |
| 81 | | " | " | | | |
| 82 | | " | " | | | |
| 83 | | " | 0.90 | Neg. | | |
| 84 | 413 | Mawingo F.C.S. N. Kinangop | 1.0 | " | | |
| 85 | " | " | 1.0 | 1.0 | - | + |
| 86 | " | " | Neg. | | | |
| 87 | " | " | 1.0 | 0.90 | - | + |
| 88 | " | " | Neg. | | | |
| 89 | " | " | " | | | |
| 90 | " | " | " | | | |
| 91 | 412 | Olaragwai F.C.S. N. Kinangop | Neg. | | | |
| 92 | " | " | " | | | |
| 93 | 274 | Olaragwai Farm - Naivasha | " | | | |
| 94 | 412 | " | 0.80 | 0.85 | - | + |
| 95 | " | " | Neg. | | | |
| 96 | " | " | " | | | |
| 97 | " | " | " | | | |
| 98 | " | " | 1.0 | 0.90 | - | + |
| 99 | 739 | Naivasha | Neg. | | | |
| 100 | 720 | New Karati Farm - N. Kinangop | 1.0 | 0.90 | 0.019 | |
| 101 | " | " | Neg. | | | |
| 102 | 705 | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 103 | 414 | Turasha F.C.S. - N. Kinangop | Neg. | | | |
| 104 | " | " | 1.0 | 0.90 | - | + |
| 105 | " | " | Neg. | | | |
| 106 | 655 | Gilgil | " | | | |
| 107 | 630 | S. Lake | " | | | |
| 108 | 743 | " | " | | | |
| 109 | 694, 696 | " | 1.0 | 1.0 | - | + |
| 110 | 305 | Loldia Ltd. - S. Lake | Neg. | | | |
| 111 | " | " | " | | | |
| 112 | " | " | " | | | |
| 113 | " | " | " | | | |
| 114 | 737 | Ndereti Eastate - S.Lake | " | | | |
| 115 | " | " | " | | | |
| 116 | " | " | " | | | |
| 117 | | N. Kinangop | " | | | |
| 118 | | " | " | | | |
| 119 | 427 | Kahuru F.C.S. - N. Kinangop | 0.90 | 0.90 | - | + |
| 120 | " | " | Neg. | | | |
| 121 | " | " | " | | | |
| 122 | " | " | " | | | |
| 123 | " | " | " | | | |
| 124 | " | " | 1.0 | 0.90 | - | + |
| 125 | " | " | Neg. | | | |
| 126 | 714, 749 | Naivasha | " | | | |
| 127 | 714, 716 | " | 1.0 | 0.95 | - | + |
| 128 | 716, 281 | " | Neg. | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 129 | 685 | Naivasha | Neg. | | | |
| 130 | 740, 714 B | N. Kinangop | " | | | |
| 131 | 425 | Muruaki F.C.S. - N. Kinangop | " | | | |
| 132 | " | " | 0.90 | Neg. | | |
| 133 | " | " | 0.90 | " | | |
| 134 | " | " | Neg. | - | | |
| 135 | " | " | " | | | |
| 136 | " | " | 0.90 | Neg. | | |
| 137 | " | " | Neg. | | | |
| 138 | " | " | " | | | |
| 139 | " | " | " | | | |
| 140 | " | " | " | | | |
| 141 | " | " | " | | | |
| 142 | | " | " | | | |
| 143 | 701 | N. Kinangop | 0.90 | 0.90 | - | + |
| 144 | 428 | Tulaga F.C.S. - N. Kinangop | 1.0 | 1.0 | - | + |
| 145 | " | " | Neg. | | | |
| 146 | " | " | " | | | |
| 147 | " | " | " | | | |
| 148 | " | " | " | | | |
| 149 | " | " | " | | | |
| 150 | " | " | " | | | |
| 151 | " | " | " | | | |
| 152 | " | " | " | | | |
| 153 | | Gilgil | " | | | |
| 154 | | " | " | | | |
| 155 | | " | " | | | |
| 156 | | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 157 | | Gilgil | Neg. | | | |
| 158 | 627, 734 | " | " | | | |
| 159 | 734 | Gachuiro Co-op. Society | " | | | |
| 160 | 734 | Gilgil | " | | | |
| 161 | 734, 717 | " | " | | | |
| 162 | 429 | Karati F.C.S. - S. Kinangop | 0.90 | Neg. | | |
| 163 | " | " | Neg. | | | |
| 164 | " | " | " | | | |
| 165 | " | " | 0.85 | Neg. | | |
| 166 | " | " | Neg. | | | |
| 167 | " | " | " | | | |
| 168 | " | " | " | | | |
| 169 | " | " | " | | | |
| 170 | " | " | " | | | |
| 171 | " | " | " | | | |
| 172 | " | " | " | | | |
| 173 | " | " | " | | | |
| 174 | " | " | " | | | |
| 175 | 419 | Bomboo Forest F.C.S. | " | | | |
| 176 | 689 | S.S. Bomboo Forest F.C.S. | " | | | |
| 177 | 432,4501 | " | " | | | |
| 178 | 408 | S.Kinangop F.C.S. | " | | | |
| 179 | " | " | " | | | |
| 180 | " | " | " | | | |
| 181 | " | " | " | | | |
| 182 | " | " | " | | | |
| 183 | " | " | " | | | |
| 184 | " | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|
| 185 | 408 | S. Kinangop F.C.S. | 0.80 | Neg. | | |
| 186 | " | " | Neg. | | | |
| 187 | " | " | " | | | |
| 188 | " | " | " | | | |
| 189 | " | " | " | | | |
| 190 | 747 | S. Kinangop | " | | | |
| 191 | 457, 757 | " | " | | | |
| 192 | 426 | Kitiri F.C.S. S. Kinangop | " | | | |
| 193 | " | " | " | | | |
| 194 | " | " | " | | | |
| 195 | " | " | 0.80 | 0.80 | 0.016 | |
| 196 | " | " | Neg. | | | |
| 197 | " | " | " | | | |
| 198 | " | " | " | | | |
| 199 | " | " | " | | | |
| 200 | " | " | " | | | |
| 201 | " | " | 0.90 | 0.90 | 0.019 | |
| 202 | 452 | Njabini F.C.S. - S. Kinangop | Neg. | | | |
| 203 | " | " | " | | | |
| 204 | " | " | " | | | |
| 205 | " | " | " | | | |
| 206 | " | " | " | | | |
| 207 | " | " | " | | | |
| 208 | 674 | Githioro member - S. Kinangop | " | | | |
| 209 | " | " | 0.90 | Neg. | | |
| 210 | 654 | Githioro F.C.S. - S. Kinangop | 1.0 | 0.90 | - | + |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 211 | 406 | Kipipiri F.C.S. - N. Kinangop | Neg. | | | |
| 212 | " | " | " | | | |
| 213 | " | " | " | | | |
| 214 | " | " | " | | | |
| 215 | " | " | " | | | |
| 216 | | Gilgil | " | | | |
| 217 | | S. Lake | " | | | |
| 218 | 282 | Kongoni Farm | " | | | |
| 219 | " | " | " | | | |
| 220 | 405 | N. Kinangop - Malewa F.C.S. | 0.80 | Neg. | | |
| 221 | " | " | Neg. | | | |
| 222 | " | " | " | | | |
| 223 | " | " | " | | | |
| 224 | " | " | " | | | |
| 225 | 415 A | Mumui F.C.S. N. Kinangop | " | | | |
| 226 | 415 B | " | " | | | |
| 227 | 431 | Nandarashi F.C.S. - N. Kinangop | " | | | |
| 228 | " | " | " | | | |
| 229 | " | " | " | | | |
| 230 | " | " | " | | | |
| 231 | 413 | Mawingo F.C.S. - N. Kinangop | " | | | |
| 232 | " | " | " | | | |
| 233 | " | " | " | | | |
| 234 | 430 | Mukungi F.C.S. - N. Kinangop | " | | | |
| 235 | " | " | " | | | |
| 236 | " | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 237 | 730 | Geta F.C.S. - N. Kinangop | Neg. | | | |
| 238 | " | " | " | | | |
| 239 | " | " | " | | | |
| 240 | " | " | 0.90 | Neg. | | |

Nyahururu K.C.C.

| Sample No. | Churn No. | Where From | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibitor |
|---|---|---|---|---|---|---|
| | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| 1 | | Silibwet | 0.90 | 0.90 | - | + |
| 2 | | Losoogwa | Neg. | | | |
| 3 | | " | " | | | |
| 4 | | " | " | | | |
| 5 | | " | 0.90 | Neg. | | |
| 6 | | Silibwet | Neg. | | | |
| 7 | | Leshau | 0.90 | Neg. | | |
| 8 | | " | Neg. | | | |
| 9 | | " | " | | | |
| 10 | | " | " | | | |
| 11 | | " | " | | | |
| 12 | | Lesirko | " | | | |
| 13 | | " | 1.0 | 0.80 | - | + |
| 14 | | Mungetho | 1.0 | 0.95 | - | + |
| 15 | | Silibwet | Neg. | | | |
| 16 | | Marmanet | " | | | |
| 17 | | Githunguchu | " | | | |
| 18 | | Kanyagia | " | | | |
| 19 | | Raiciri | 0.90 | Neg. | | |
| 20 | | Kanyagia | Neg. | | | |
| 21 | | Marmanet | 0.90 | Neg. | | |
| 22 | | Ol-Joro-Orok | Neg. | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 23 | | Leshau | 0.90 | Neg. | | |
| 24 | | " | Neg. | | | |
| 25 | | "8 | " | | | |
| 26 | | " | " | | | |
| 27 | | Oraimutia | " | | | |
| 28 | | Ol-Joro-Orok | " | | | |
| 29 | | Mungeho | " | | | |
| 30 | | Marmanet | 1.0 | Neg. | | |
| 31 | | " | Neg. | | | |
| 32 | | Njumu Ltd. | " | | | |
| 33 | | Wiumiriri Estate | " | | | |
| 34 | | Mukuruei-ini | " | | | |
| 35 | | Nyahururu F.C.S. | " | | | |
| 36 | | Marmanet | " | | | |
| 37 | | " | " | | | |
| 38 | | " | " | | | |
| 39 | | " | " | | | |
| 40 | | Kanyagia | " | | | |
| 41 | | Ndaragwa | " | | | |
| 42 | | Pesi F.C.S. | " | | | |
| 43 | | " | " | | | |
| 44 | | " | " | | | |
| 45 | | " | " | | | |
| 46 | | Nyairoko | " | | | |
| 47 | | " | 0.90 | Neg. | | |
| 48 | | " | 0.85 | 0.85 | - | + |
| 49 | | " | 1.0 | 0.95 | - | + |
| 50 | | " | 0.85 | Neg. | | |
| 51 | | " | Neg. | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (F) |
|---|---|---|---|---|---|---|
| 52 | | Olkalou-Salient | Neg. | | | |
| 53 | | " | " | | | |
| 54 | | " | " | | | |
| 55 | | " | " | | | |
| 56 | | " | " | | | |
| 57 | | " | 1.0 | Neg. | | |
| 58 | | Karagoini | Neg. | | | |
| 59 | | " | 0.90 | " | | |
| 60 | | " | Neg. | | | |
| 61 | | Olkalou-Salient | " | | | |
| 62 | | " | " | | | |
| 63 | | " | " | | | |
| 64 | | " | 1.0 | Neg. | | |
| 65 | | " | 1.0 | 0.90 | - | + |
| 66 | | " | 0.90 | 0.90 | - | + |
| 67 | | " | Neg. | | | |
| 68 | | " | " | | | |
| 69 | | " | " | | | |
| 70 | | " | " | | | |
| 71 | | " | 1.0 | 0.90 | - | + |
| 72 | | Tetu Farm | 0.90 | Neg. | | |
| 73 | | " | Neg. | | | |
| 74 | | " | " | | | |
| 75 | | " | " | | | |
| 76 | | " | " | | | |
| 77 | | Kianjoro F.C.S. | 1.0 | 0.85 | - | + |
| 78 | | " | 1.0 | Neg. | | |
| 79 | | " | Neg. | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 80 | | Mwais Ltd. | | 0.90 | Neg. | | |
| 81 | | " | | Neg. | | | |
| 82 | | " | | " | | | |
| 83 | | " | | " | | | |
| 84 | | " | | 0.90 | Neg. | | |
| 85 | | " | | Neg. | | | |
| 86 | | Muricho | | " | | | |
| 87 | | Lesirko | | " | | | |
| 88 | | " | | " | | | |
| 89 | | " | | " | | | |
| 90 | | " | | " | | | |
| 91 | 143, 628 | Siribwet F.C.S. | PH 5.6 | Neg. | | | |
| 92 | 640, 143 | " | 5.7 | " | | | |
| 93 | 686 | Richau | 5.3 | " | | | |
| 94 | 613 | Rimutia | 5.9 | " | | | |
| 95 | | " | 5.7 | " | | | |
| 96 | 186 | Muruai Farm | 5.5 | " | | | |
| 97 | 923 | Samburu Farm - Ol Jarok | 5.9 | " | | | |
| 98 | 316 | Richau Pondo [illegible] | 5.8 | " | | | |
| 99 | 826 | Nyahururu Farm | 5.1 | " | | | |
| 100 | | " | 5.8 | " | | | |
| 101 | 339 | Siribwet | 5.6 | " | | | |
| 102 | 132 | Ol-Jorok West Soc. | 5.4 | " | | | |
| 103 | 166 | Ngai Ndeithi Co. | 5.6 | " | | | |
| 104 | " | " | 5.6 | " | | | |
| 105 | 586 | Reshau Pondo Farm Co. | 5.6 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 106 | | Siribwet | 6.0 | Neg. | | | |
| 107 | 138 | Shamata F.C.S. | 5.5 | " | | | |
| 108 | " | " | 5.6 | " | | | |
| 109 | 48 | Karago-in FCS | 5.7 | " | | | |
| 110 | 227 | Nyahururu | 6.0 | " | | | |
| 111 | 870 | Simbara | 5.9 | " | | | |
| 112 | | " | 5.8 | " | | | |
| 113 | 965 | Resaku | 5.4 | " | | | |
| 114 | " | " | 5.5 | " | | | |
| 115 | 300 | " | 6.2 | " | | | |
| 116 | 343 | Rimutia | 5.7 | " | | | |
| 117 | " | " | 5.6 | " | | | |
| 118 | 209 | Rechau | 5.8 | " | | | |
| 119 | 13 | Jamuhuri estate | 5.5 | " | | | |
| 120 | " | " | 5.2 | " | | | |
| 121 | " | " | 5.4 | " | | | |
| 122 | 136 | Large Scale Farm Co. | 6.0 | " | | | |
| 123 | 154 | Jamuhuri estate | 5.2 | " | | | |
| 124 | " | " | 5.7 | " | | | |
| 125 | " | " | 5.8 | " | | | |
| 126 | " | " | 5.8 | " | | | |
| 127 | 167 | Kanembe FCS | 5.4 | " | | | |
| 128 | " | " | 6.0 | 1.0 | 0.90 | 0.019 | |
| 129 | 440 | Karai FCS | 5.5 | Neg. | | | |
| 130 | " | " | 5.3 | " | | | |
| 131 | 432 | Resirko-Ol Jorok | 5.3 | " | | | |
| 132 | 355 | Ngano Farm | 4.9 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 133 | 616 | Ol-Jorok | 5.4 | Neg. | | | |
| 134 | 143 | Mukeu FCS | 5.9 | " | | | |
| 135 | " | " | 5.8 | " | | | |
| 136 | 70 | Ol-Jorok | 4.8 | " | | | |
| 137 | 303 | Ol-Jorok-West FCS | 5.6 | " | | | |
| 138 | " | " | 5.4 | " | | | |
| 139 | " | " | 5.3 | " | | | |
| 140 | " | " | 6.3 | " | | | |
| 141 | 880 | Ol-Jorok West | 5.8 | " | | | |
| 142 | 399 | Ol-Jorok - Kagera Farm | 5.8 | " | | | |
| 143 | 15 | Rosogwa F.C.S. | 5.8 | " | | | |
| 144 | " | " | 5.0 | " | | | |
| 145 | " | " | 5.7 | " | | | |
| 146 | " | " | 5.1 | " | | | |
| 147 | 82 | Mung'etho F.Co-op. | 5.5 | " | | | |
| 148 | " | " | 6.0 | " | | | |
| 150 | 266 | Ol-Jorok-Kangui | 6.3 | " | | | |
| 151 | 415 | " | 5.5 | " | | | |
| 152 | | " | 5.5 | 1.0 | Neg. | | |
| 153 | 473 E | Sielent Ol-Jorok F.C.S. | 6.2 | Neg. | | | |
| 154 | 473 Z | " | 5.7 | " | | | |
| 155 | 120 | Pesi FCS | 4.9 | " | | | |
| 156 | " | " | 5.4 | " | | | |
| 157 | " | " | 6.1 | " | | | |
| 158 | 142 | Simbara FCS | 6.2 | " | | | |
| 159 | " | " | 5.9 | " | | | |
| 160 | " | " | 4.9 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g?) |
|---|---|---|---|---|---|---|---|
| 161 | 621 | Raichiri | 6.0 | Neg. | | | |
| 162 | 682 | " | 5.7 | " | | | |
| 163 | 347 | Resirko FCS | 5.5 | " | | | |
| 164 | " | " | 5.8 | " | | | |
| 165 | " | " | 5.9 | " | | | |
| 166 | 84 | " | 6.1 | " | | | |
| 167 | 67 B | Muhohetu F. Co. | 5.5 | " | | | |
| 168 | " | " | 5.6 | 1.0 | 0.95 | - | + |
| 169 | 62 A | " | 5.8 | Neg. | | | |
| 170 | " | " | 6.0 | " | | | |
| 171 | 775 | Subukia Co. | 5.7 | " | | | |
| 172 | 22 | Wiumiririe F.C.S. | 5.8 | " | | | |
| 173 | 777 | Sumbukia Co. | 5.8 | " | | | |
| 174 | 842 | Laikipia West Maru Monet Co. | 5.6 | 1.0 | 0.95 | - | + |
| 175 | " | " | 6.2 | Neg. | | | |
| 176 | | Laikipia West Maru Monet Co. | 5.6 | " | | | |
| 177 | 216 | Riruruti Farm | 5.9 | " | | | |
| 178 | 245 | Maru-Monet | 5.7 | " | | | |
| 179 | 409 | Simbara | 5.8 | " | | | |
| 180 | " | " | 5.1 | " | | | |
| 181 | 124 | Richau Elijah Farm | 5.4 | " | | | |
| 182 | 114 | Marumanet FCS | 5.9 | " | | | |
| 183 | " | " | 6.1 | " | | | |
| 184 | " | " | 6.0 | " | | | |
| 185 | " | " | 5.4 | " | | | |
| 186 | 174 | Magutu Farm | 5.8 | " | | | |
| 187 | | " | 5.5 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 188 | 617 | Muruthi Cattle Co. | 5.7 | Neg. | | | |
| 189 | 862 | Ndururi Cattle Co. | 5.9 | " | | | |
| 190 | 685 | Ndimi Co. | 6.3 | " | | | |
| 191 | 759 | A.R.Swift-<br>Subukia | 6.0 | " | | | |
| 192 | 792 | A.R.SWift -<br>Gituamba FCS | 5.6 | " | | | |
| 193 | 903 | Subukia - wei farm | 5.9 | " | | | |
| 194 | 791 | Subukia -<br>Ngamini farm | 5.8 | " | | | |
| 195 | 776 | Kaptarakwa farm -<br>(Subukia) | 5.4 | " | | | |
| 196 | " | " | 5.5 | " | | | |
| 197 | " | " | 5.0 | " | | | |
| 198 | 787 | Keanwe Farm -<br>Subukia | 5.0 | " | | | |
| 199 | " | " | 4.9 | " | | | |
| 200 | 748 | Munanda Farm | 5.7 | " | | | |
| 201 | 751 | Mundanda | 5.8 | " | | | |
| 202 | " | " | 5.6 | " | | | |
| 203 | 750 | Tetu -<br>Subukia Co. | 5.9 | " | | | |
| 204 | 346 | Olaimutia FCS | 6.0 | " | | | |
| 205 | " | " | 5.4 | " | | | |
| 206 | " | " | 5.6 | 1.0 | Neg. | | |
| 207 | " | " | 5.8 | Neg. | | | |
| 208 | " | " | 6.4 | " | | | |
| 209 | " | " | 5.3 | " | | | |
| 210 | 471 | Ol-Jorok | 5.8 | " | | | |
| 211 | | Ol-Jorok | 5.5 | " | | | |
| 212 | 143 | Mukfu FCS | 5.9 | " | | | |
| 213 | " | " | 5.8 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 214 | 452 | Kanyagia | 5.7 | Neg. | | | |
| 215 | " | " | 5.7 | " | | | |
| 216 | 473 S | Salient Ol-Kalou | 5.4 | " | | | |
| 217 | " | " | 6.0 | " | | | |
| 218 | " | " | 5.7 | 0.9 | Neg. | | |
| 219 | 473 R | Salient Ol-Jarok | 5.2 | Neg. | | | |
| 220 | " | " | 5.1 | Neg. | | | |
| 221 | 422 | " | 5.4 | " | | | |
| 222 | 246 | Salient | 5.7 | " | | | |
| 223 | 86 | Mukurue-Ini FCS | 8.2 | " | | | |
| 224 | " | " | 5.9 | " | | | |
| 225 | " | " | 4.9 | " | | | |
| 226 | 473 W | Salient-Kiriwa | 6.0 | " | | | |
| 227 | " D | " | 4.9 | " | | | |
| 228 | " D | " | 5.0 | " | | | |
| 229 | " D | " | 5.5 | 1.0 | Neg. | | |
| 230 | " E | " | 5.5 | Neg. | | | |
| 231 | " E | " | 5.6 | " | | | |
| 232 | 473 K | " | 5.5 | " | | | |
| 233 | " Y | Ol-Kalou Salient | 4.8 | " | | | |
| 234 | " J | " | 6.2 | " | | | |
| 235 | " J | " | 5.7 | " | | | |
| 236 | " J | " | 5.4 | " | | | |
| 237 | 985 H | Salient Ndami FCS | 5.5 | " | | | |
| 238 | 945 | Ol-Kalau Kaimboga FCS | 5.8 | " | | | |
| 239 | " | " | 6.2 | " | | | |
| 240 | 985 G | Ol-Kalou - Ndami FCS | 5.9 | " | | | |
| 241 | " | | 4.9 | " | | | |

| (a) | (b) | (c) | (ci) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 242 | 473 X | Ol-Kalou-Ndami FCS | 6.1 | Neg. | | | |
| 243 | 570 | Wanjoki FCS | 6.3 | " | | | |
| 244 | " | " | 5.9 | " | | | |
| 245 | " | " | 5.7 | " | | | |
| 246 | 570 | " | 5.7 | " | | | |
| 247 | " | " | 5.5 | " | | | |
| 248 | " | " | 5.6 | " | | | |
| 249 | 560 | West Ol-Kalou FCS | 5.1 | 1.0 | Neg. | | |
| 250 | " | " | 4.9 | 1.0 | " | | |
| 251 | " | " | 5.1 | 1.0. | " | | |
| 252 | " | " | 5.4 | Neg. | | | |
| 253 | 563 | Ol-Kalou Farmers Co-op. | 5.7 | " | | | |
| 254 | " | " | 5.6 | " | | | |
| 255 | | " | 5.4 | " | | | |
| 256 | 598 | " | 5.8 | " | | | |
| 257 | " | " | 6.3 | " | | | |
| 258 | 596 | New Riricua Milk Producing Company | 5.2 | " | | | |
| 259 | " | " | 5.7 | " | | | |
| 260 | " | " | 5.6 | " | | | |
| 261 | 568 | Ol-Kalou South FCS | 5.4 | 1.0 | Neg. | | |
| 262 | " | " | 5.8 | Neg. | | | |
| 263 | " | " | 5.0 | " | | | |
| 264 | 65 | Kanyagia FCS | 5.5 | 0.90 | Neg. | | |
| 265 | " | " | 5.9 | Neg. | | | |
| 266 | 377 | Ndaragwa FCS | 5.9 | " | | | |
| 267 | 486 | " | 6.0 | " | | | |
| 268 | " | " | 5.8 | " | | | |
| 269 | " | " | 5.7 | " | | | |
| 270 | " | " | 5.9 | " | | | |
| 271 | " | " | 5.8 | " | | | |
| 272 | 116 | " | 5.8 | " | | | |

| Sample No. | Churn No. | Where From | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibitor. |
|---|---|---|---|---|---|---|
| | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| 1 | | Cherangani | 0.90 | Neg. | | |
| 2 | | " | Neg. | | | |
| 3 | | " | " | | | |
| 4 | | " | " | | | |
| 5 | | " | " | | | |
| 6 | | " | " | | | |
| 7 | | " | " | | | |
| 8 | | " | 0.90 | 0.85 | 0.018 | |
| 9 | | " | Neg. | | | |
| 10 | | Kapomboi | " | | | |
| 11 | | " | 0.90 | 0.90 | - | + |
| 12 | | " | Neg. | | | |
| 13 | | " | " | | | |
| 14 | | " | 1.0 | 0.85 | - | + |
| 15 | | " | Neg. | | | |
| 16 | | " | " | | | |
| 17 | | " | " | | | |
| 18 | | " | 1.0 | Neg. | | |
| 19 | | Gatwe Farm | Neg. | | | |
| 20 | | Cherangani | " | | | |
| 21 | | " | " | | | |
| 22 | | " | 1.0 | 0.85 | - | + |

| (a) | (b) | (c) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|
| 23 | | Cherangani | Neg. | | | |
| 24 | | " | " | | | |
| 25 | | " | " | | | |
| 26 | | Soy | " | | | |
| 27 | | " | " | | | |
| 28 | | " | " | | | |
| 29 | | " | " | | | |
| 30 | | " | " | | | |
| 31 | | Lulu Farm | " | | | |
| 32 | | Siuna Farm | " | | | |
| 33 | | Cherangani | " | | | |
| 34 | | " | " | | | |
| 35 | | " | 0.90 | Neg. | | |
| 36 | | " | Neg. | | | |
| 37 | | " | " | | | |
| 38 | | " | " | | | |
| 39 | | " | " | | | |
| 40 | | " | " | | | |
| 41 | | " | " | | | |
| 42 | | " | " | | | |
| 43 | | " | " | | | |
| 44 | | " | 1.0 | Neg. | | |
| 45 | | " | Neg. | | | |
| 46 | | " | " | | | |
| 47 | | " | " | | | |
| 48 | | " | " | | | |
| 49 | | Nzoia | " | | | |
| 50 | | Nzoia Farmers | " | | | |
| 51 | | " | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 52 | | Saboti | 1.0 | Neg. | | |
| 53 | | " | Neg. | | | |
| 54 | | " | " | | | |
| 55 | | " | " | | | |
| 56 | | " | " | | | |
| 57 | | " | " | | | |
| 58 | | " | " | | | |
| 59 | | " | " | | | |
| 60 | | " | " | | | |
| 61 | | Kachibora - Cherangani | Neg. | | | |
| 62 | | " | " | | | |
| 63 | | " | " | | | |
| 64 | | " | " | | | |
| 65 | | " | " | | | |
| 66 | | " | " | | | |
| 67 | | Kibomet | " | | | |
| 68 | | " | " | | | |
| 69 | | " | " | | | |
| 70 | | " | " | | | |
| 71 | | " | " | | | |
| 72 | | Hume | " | | | |
| 73 | | Moi's bridge | " | | | |
| 74 | | " | " | | | |
| 75 | | Pembeni Farm | " | | | |
| 76 | | " | " | | | |
| 77 | | Endebess | 0.90 | 0.85 | 0.018 | |
| 78 | | Kiborimos Farm | Neg. | | | |
| 79 | | " | " | | | |
| 80 | | Nzoia Section | " | | | |

| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|
| 81 | | Nzoia Section | Neg. | | | |
| 82 | | " | " | | | |
| 83 | | " | 0.90 | 0.85 | - | + |
| 84 | | " | Neg. | | | |
| 85 | | " | " | | | |
| 86 | | Ziwa | " | | | |
| 87 | | " | " | | | |
| 88 | | " | " | | | |
| 89 | | " | " | | | |
| 90 | | " | " | | | |
| 91 | | " | " | | | |
| 92 | | Ndalu | " | | | |
| 93 | | " | " | | | |
| 94 | | " | " | | | |
| 95 | | " | " | | | |
| 96 | | " | 0.85 | Neg. | | |
| 97 | | " | 0.90 | 0.85 | - | + |
| 98 | | Segero | Neg. | | | |
| 99 | | " | 0.90 | 0.85 | 0.018 | |
| 100 | | " | Neg. | | | |
| 101 | | " | " | | | |
| 102 | | " | " | | | |
| 103 | | " | 0.90 | Neg. | | |
| 104 | | " | Neg. | | | |
| 105 | | " | " | | | |
| 106 | | " | " | | | |
| 107 | | Tenai | " | | | |
| 108 | | Kamukuyua | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 109 | 315 | A.D.C. Farm - Kwanza Center | PH<br>5.9 | Neg. | | | |
| 110 | " | " | 5.7 | " | | | |
| 111 | " | " | 5.9 | " | | | |
| 112 | " | " | 6.0 | " | | | |
| 113 | " | " | 5.7 | " | | | |
| 115 | " | " | 6.0 | " | | | |
| 116 | 247 | " | 5.4 | " | | | |
| 117 | " | " | 5.5 | " | | | |
| 118 | " | " | 5.4 | " | | | |
| 119 | " | " | 6.0 | " | | | |
| 120 | 726 | Kitale area | 5.3 | " | | | |
| 121 | | Kitale area | 5.6 | " | | | |
| 122 | 351 | Saboti center | 5.6 | " | | | |
| 123 | " | " | 5.5 | " | | | |
| 124 | 595 | Cherangani | 5.7 | " | | | |
| 125 | " | " | 5.8 | " | | | |
| 126 | 512 | Kwanza | 5.8 | " | | | |
| 127 | " | " | 5.9 | " | | | |
| 128 | " | " | 6.0 | " | | | |
| 129 | " | " | 6.18 | " | | | |
| 130 | 431 | " | 4.9 | " | | | |
| 131 | " | " | 5.3 | " | | | |
| 132 | | Kitale area | 5.8 | " | | | |
| 133 | | " | 5.9 | " | | | |
| 134 | 129 | Ndalu area | 5.9 | " | | | |
| 135 | | " | 5.9 | " | | | |
| 136 | 552 | " | 6.0 | " | | | |
| 137 | | " | 5.3 | " | | | |
| 138 | | " | 5.4 | | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 139 | | Ndalu area | 5.5 | Neg. | | | |
| 140 | | Kitale | 5.6 | " | | | |
| 141 | | " | 5.9 | " | | | |
| 142 | | " | 5.9 | " | | | |
| 143 | | Saboti | 5.8 | " | | | |
| 144 | 1013, 1893 | Endebess | 5.1 | " | | | |
| 145 | 140 | Saboti | 5.0 | " | | | |
| 146 | " | " | 4.9 | " | | | |
| 147 | " | " | 6.0 | " | | | |
| 148 | | " | 5.8 | " | | | |
| 149 | | " | 5.6 | " | | | |
| 150 | 577, 825 | " | 5.9 | 0.90 | Neg. | | |
| 151 | 350 | " | 5.8 | 0.85 | " | | |
| 152 | | " | 6.0 | Neg. | | | |
| 153 | 996, 79 | " | 5.3 | " | | | |
| 154 | 79 | " | 5.2 | " | | | |
| 155 | | " | 5.4 | " | | | |
| 156 | 601, 1675 | Ndalu center | 5.6 | " | | | |
| 157 | | " | 5.9 | " | | | |
| 158 | 1078, 240 | " | 5.8 | " | | | |
| 159 | | " | 5.8 | " | | | |
| 160 | | " | 6.0 | " | | | |
| 161 | | Cherangani | 6.1 | " | | | |
| 162 | | " | 5.9 | 0.90 | Neg. | | |
| 163 | | " | 5.8 | 0.90 | 0.90 | - | + |
| 164 | | " | 5.7 | Neg. | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g |
|---|---|---|---|---|---|---|---|
| 165 | | Cherangani | 5.7 | Neg. | | | |
| 166 | | " | 5.2 | " | | | |
| 167 | | " | 5.0 | " | | | |
| 168 | | " | 6.1 | " | | | |
| 169 | | " | 6.0 | " | | | |
| 170 | | " | 5.3 | 0.90 | Neg. | | |
| 171 | | " | 5.4 | Neg. | | | |
| 172 | | " | 5.3 | 1.0 | Neg. | | |
| 173 | 415, 534 | " | 6.0 | Neg. | | | |
| 174 | | " | 5.1 | " | | | |
| 175 | | " | 5.6 | " | | | |
| 176 | | Soy | 5.9 | " | | | |
| 177 | | " | 5.1 | " | | | |
| 178 | | " | 5.5 | " | | | |
| 179 | | " | 5.2 | " | | | |
| 180 | | " | 5.8 | " | | | |
| 181 | 978, 122 | " | 5.3 | 0.90 | Neg. | | |
| 182 | | " | 5.3 | Neg. | | | |
| 183 | | " | 5.0 | " | | | |
| 184 | 193, 985 | Cherangani | 4.9 | " | | | |
| 185 | | Kiminini | 4.8 | " | | | |
| 186 | | " | 6.1 | " | | | |
| 187 | | " | 6.0 | " | | | |
| 188 | | " | 5.1 | " | | | |
| 189 | | Cherangani | 5.9 | " | | | |
| 190 | | " | 5.8 | " | | | |
| 191 | | " | 5.8 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 192 | | Cherangani | 5.5 | Neg. | | | |
| 193 | | " | 5.4 | " | | | |
| 194 | | " | 5.5 | " | | | |
| 195 | | " | 6.0 | " | | | |
| 196 | | " | 5.8 | " | | | |
| 197 | | " | 5.8 | " | | | |
| 198 | | " | 5.2 | " | | | |
| 199 | | " | 5.0 | " | | | |
| 200 | | " | 5.5 | " | | | |
| 201 | | " | 5.9 | " | | | |
| 202 | | Kiminini | 5.1 | " | | | |
| 203 | | " | 5.0 | " | | | |
| 204 | | Cherangani | 5.6 | " | | | |
| 205 | | " | 5.6 | " | | | |
| 206 | | " | 6.0 | " | | | |
| 207 | 969, 790 | " | 6.1 | " | | | |
| 208 | | " | 6.0 | " | | | |
| 209 | | " | 5.2 | Neg. | | | |
| 210 | | " | 5.6 | 0.90 | Neg. | | |
| 211 | | " | 5.7 | Neg. | | | |
| 212 | | " | 5.7 | " | | | |
| 213 | | " | 4.9 | " | | | |
| 214 | 971 | " | 4.8 | " | | | |
| 215 | | " | 5.6 | " | | | |
| 216 | | Kisawai | 5.5 | " | | | |
| 217 | | " | 5.8 | " | | | |
| 218 | | " | 5.7 | " | | | |
| 219 | | " | 5.7 | " | | | |
| 220 | | " | 5.6 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 221 | | Endebess | 5.4 | Neg. | | | |
| 222 | | Kitale area | 5.3 | Neg. | | | |
| 223 | | " | 5.3 | " | | | |
| 224 | 378, 66 | Saboti | 5.2 | " | | | |
| 225 | 66, 1013 | " | 5.0 | " | | | |
| 226 | 514, 1941 | Moi's bridge | 4.9 | " | | | |
| 227 | 103 | Saboti | 4.9 | " | | | |
| 228 | 103, 1910 | " | 6.0 | " | | | |
| 229 | 1840, 1397 | Kapomboi | 5.6 | " | | | |
| 230 | 1379, 743 | " | 5.5 | " | | | |
| 231 | 743, 787 | " | 5.6 | " | | | |
| 232 | 787 | " | 5.7 | " | | | |
| 233 | | " | 5.7 | " | | | |
| 234 | | " | 5.9 | " | | | |
| 235 | | " | 5.9 | " | | | |
| 236 | | " | 5.9 | " | | | |
| 237 | | Sikhendu | 6.1 | " | | | |
| 238 | | " | 5.8 | " | | | |
| 239 | | " | 5.8 | " | | | |
| 240 | | " | 5.0 | " | | | |
| 241 | | " | 5.1 | " | | | |
| 242 | | " | 5.0 | " | | | |
| 243 | | Kwanza center | 5.6 | " | | | |
| 244 | 113 | " | 5.7 | " | | | |
| 245 | 1896 | " | 5.6 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 246 | 1896, 1360 | Kwanza center | 4.9 | Neg. | | | |
| 247 | | " | 6.0 | " | | | |
| 248 | | " | 5.9 | " | | | |
| 249 | | " | 5.4 | " | | | |
| 250 | | " | 5.3 | " | | | |
| 251 | | " | 5.5 | 1.0 | Neg. | | |
| 252 | | " | 5.8 | Neg. | | | |
| 253 | | " | 5.8 | " | | | |
| 254 | | " | 5.0 | " | | | |
| 255 | | " | 5.4 | " | | | |
| 256 | | Endebess | 5.6 | 1.0 | Neg. | | |
| 257 | | Ndalu | 5.6 | Neg. | | | |
| 258 | | " | 5.6 | " | | | |
| 259 | 638 | " | 4.9 | " | | | |
| 260 | | " | 4.8 | " | | | |
| 261 | 35 | " | 5.6 | " | | | |
| 262 | 35 | " | 5.7 | " | | | |
| 263 | | " | 5.9 | " | | | |
| 264 | | " | 5.6 | " | | | |
| 265 | | " | 5.4 | " | | | |
| 266 | | " | 5.3 | " | | | |
| 267 | | " | 5.3 | " | | | |
| 268 | | " | 5.0 | " | | | |
| 269 | | " | 5.2 | " | | | |
| 270 | | " | 5.9 | " | | | |
| 271 | | Mois bridge | 5.8 | " | | | |
| 272 | | " | 5.8 | 0.85 | Neg. | | |
| 273 | | " | 4.9 | Neg. | | | |
| 274 | | " | 5.0 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
|---|---|---|---|---|---|---|---|
| 275 | 732 | Moi's bridge | 4.7 | Neg. | | | |
| 276 | 732 | " | 5.0 | " | | | |
| 277 | 62 | " | 5.9 | " | | | |
| 278 | | Ziwa | 5.8 | " | | | |
| 279 | | " | 5.7 | " | | | |
| 280 | | " | 5.7 | " | | | |
| 281 | | " | 5.6 | " | | | |
| 282 | | " | 5.7 | " | | | |
| 283 | | " | 5.8 | " | | | |
| 284 | | " | 5.5 | " | | | |
| 285 | | " | 5.5 | " | | | |
| 286 | | " | 5.6 | " | | | |
| 287 | | " | 5.9 | " | | | |
| 288 | | " | 6.0 | " | | | |
| 289 | | " | 6.1 | " | | | |
| 290 | 343 | " | 5.4 | " | | | |
| 291 | | Saboti area | 5.6 | " | | | |
| 292 | | " | 5.7 | 0.85 | Neg. | | |
| 293 | | " | 5.7 | Neg. | | | |
| 294 | 351 | " | 5.6 | " | | | |
| 295 | | " | 5.5 | " | | | |
| 296 | | " | 5.0 | " | | | |
| 297 | | " | 5.8 | " | | | |
| 298 | | " | 5.7 | " | | | |

| Sample No. | Churn No. | Where From | PH | Zone Presence & Size (cm) | | Positive Penicillin (Units/ml) | Other Inhibitor |
|---|---|---|---|---|---|---|---|
| | | | | Raw Milk | Heated Milk | | |
| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | (g) |
| 1 | 2930 | Kimumu S.F.T. (Eldoret) | 6.0 | Neg. | | | |
| 2 | 2513 | Sugutek S.F.T. (Eldoret) | 6.0 | " | | | |
| 3 | 826 | Kabkong - Eldoret | 5.5 | " | | | |
| 4 | " | " | 5.9 | " | | | |
| 5 | 146 | Kaptagat - Eldoret | 5.6 | " | | | |
| 6 | | Eldoret | 5.2 | " | | | |
| 7 | | " | 6.1 | " | | | |
| 8 | 725, 3414, 234 | Kipsinende-Eld. | 6.0 | " | | | |
| 9 | 2879, 3042 | Vasin Ngishu Farmers-Eldoret | 6.0 | " | | | |
| 10 | | Sirika Farm-Eld. | 5.9 | " | | | |
| 11 | | " | 5.9 | " | | | |
| 12 | | Kambi-Ndege Farmers (Eldoret) | 5.8 | " | | | |
| 13 | 380 | Plateau- Eld. | 5.0 | " | | | |
| 14 | 201 | Mayo Farm - Eldoret | 5.8 | " | | | |
| 15 | 223, 783 | Kambi - Kaku (Eldoret) | 6.0 | " | | | |
| 16 | 3577, 2661 | Kamugunji Farm - (Eldoret) | 5.7 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 17 | | Mutwot Farm - Eldoret | 5.7 | Neg. | | | |
| 18 | | " | 5.3 | " | | | |
| 19 | | " | 5.4 | " | | | |
| 20 | | " | 6.1 | " | | | |
| 21 | | Siani Farm - Eldoret | 5.6 | " | | | |
| 22 | | Tuiyo Farm - Eldoret | 5.5 | " | | | |
| 23 | | " | 5.5 | " | | | |
| 24 | | " | 5.4 | " | | | |
| 25 | | " | 5.9 | " | | | |
| 26 | | " | 5.4 | " | | | |
| 27 | | " | 6.2 | " | | | |
| 28 | | " | 5.7 | " | | | |
| 29 | 409 | Kaptagat - Eldoret | 5.7 | " | | | |
| 30 | 164 | Kamani Farm | 5.7 | " | | | |
| 31 | | Sosiani Area | 5.8 | " | | | |
| 32 | | " | 5.3 | " | | | |
| 33 | | " | 5.7 | " | | | |
| 34 | | " | 5.8 | " | | | |
| 35 | 829 | " | 5.4 | " | | | |
| 36 | 829 | Ngenyilel Farms - settlement | 4.9 | " | | | |
| 37 | " | " | 5.8 | " | | | |
| 38 | " | " | 5.7 | " | | | |
| 39 | | Moiben | 5.3 | " | | | |
| 40 | | " | 5.5 | " | | | |
| 41 | | " | 5.9 | " | | | |

| (a) | (b) | (c) | c(i | (d) | (e) | (f) | (- |
|---|---|---|---|---|---|---|---|
| 42 | 3463 | Nandi Hills | 5.8 | Neg. | | | |
| 43 | | Racecourse | 5.9 | " | | | |
| 44 | | Turbo | 6.0 | " | | | |
| 45 | 765 | Sosiani settlement | 5.8 | " | | | |
| 46 | | " | 5.3 | " | | | |
| 47 | | " | 5.2 | " | | | |
| 48 | | " | 6.1 | " | | | |
| 49 | | " | 5.5 | " | | | |
| 50 | | " | 5.5 | " | | | |
| 51 | 166 | Elgeyo border | 5.4 | " | | | |
| 52 | | Sergoit | 5.9 | " | | | |
| 53 | | " | 5.8 | " | | | |
| 54 | | " | 5.9 | " | | | |
| 55 | | Mutwot Farm | 5.7 | " | | | |
| 56 | | " | 5.7 | " | | | |
| 57 | | " | 5.0 | " | | | |
| 58 | | " | 5.2 | " | | | |
| 59 | | Sokchok Farm - Eldoret | 4.9 | " | | | |
| 60 | | " | 5.9 | " | | | |
| 61 | | " | 5.8 | " | | | |
| 62 | | Plateau | 5.3 | " | | | |
| 63 | | " | 5.4 | " | | | |
| 64 | | Kipsombe Farm - Eldoret | 5.5 | " | | | |
| 65 | | " | 5.5 | " | | | |
| 66 | | " | 6.1 | " | | | |
| 67 | | " | 5.3 | " | | | |
| 68 | | " | 5.4 | " | | | |
| 69 | | " | 5.4 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (d) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 70 | 411 | Sergoit Farmers Settlement- Eld. | 5.9 | Neg. | | | |
| 71 | " | " | 5.9 | " | | | |
| 72 | " | " | 5.7 | " | | | |
| 73 | " | " | 5.7 | " | | | |
| 74 | " | " | 5.0 | " | | | |
| 75 | | Tulwet | 5.3 | " | | | |
| 76 | | " | 5.3 | " | | | |
| 77 | | Kapwele - Eld. | 6.1 | " | | | |
| 78 | | " | 5.4 | " | | | |
| 79 | | " | 5.8 | " | | | |
| 80 | | Kesendany Famers-Eldoret | 5.2 | " | | | |
| 81 | | " | 5.2 | " | | | |
| 82 | | " | 6.0 | " | | | |
| 83 | | Kipsamo Farmers - Eldoret | 5.4 | " | | | |
| 84 | | " | 5.9 | " | | | |
| 85 | | " | 5.3 | " | | | |
| 86 | | " | 5.1 | " | | | |
| 87 | | " | 5.1 | " | | | |
| 88 | | Lutiet Farm - Eldoret | 5.8 | " | | | |
| 89 | | " | 5.7 | " | | | |
| 90 | | Yamumbi Farm - Eldoret | 5.7 | " | | | |
| 91 | | " | 5.9 | " | | | |
| 92 | | " | 5.6 | " | | | |
| 93 | | " | 6.1 | " | | | |
| 94 | | Sangalo Farm - Nandi | 5.5 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 95 | | Sangalo Farm - Nandi | 5.6 | Neg. | | | |
| 96 | | " | 5.8 | " | | | |
| 97 | | " | 5.9 | " | | | |
| 98 | | " | 5.5 | " | | | |
| 99 | | " | 5.3 | " | | | |
| 100 | | " | 5.4 | " | | | |
| 101 | 221 | Ndalat Farmers Nandi- Settlement | 5.3 | " | | | |
| 102 | " | " | 5.3 | " | | | |
| 103 | " | " | 5.0 | " | | | |
| 104 | " | " | 5.6 | 1.10 | 1.10 | - | + |
| 105 | " | " | 4.9 | Neg. | | | |
| 106 | | " | 5.3 | " | | | |
| 107 | | " | 5.9 | " | | | |
| 108 | | Ngechek Farmers- Nandi | 5.1 | " | | | |
| 109 | | " | 5.7 | " | | | |
| 110 | | " | 5.8 | " | | | |
| 111 | | " | 5.9 | " | | | |
| 112 | | " | 5.5 | " | | | |
| 113 | | Kipkaren Salien- Nandi | 5.3 | " | | | |
| 114 | | " | 5.7 | " | | | |
| 115 | | " | 5.9 | " | | | |
| 116 | | " | 5.8 | " | | | |
| 117 | | " | 5.5 | " | | | |
| 118 | | Mutwot - Nandi | 5.3 | " | | | |
| 119 | | " | 5.9 | " | | | |
| 120 | 555, 1968 | Kipkabus - Eld. | 5.6 | " | | | |
| 121 | | Sambul Farmers - Eldoret | 5.3 | " | | | |

| (a) | (b) | (c) | c(i) | (d) | (e) | (f) | ( |
|---|---|---|---|---|---|---|---|
| 122 | | Sambul Farmers - Eldoret | 5.5 | Neg. | | | |
| 123 | | " | 5.7 | " | | | |
| 124 | 12 | Kabongo Farm - Eldoret | 5.8 | " | | | |
| 125 | | Kasses Farmers - Eldoret | 4.9 | " | | | |
| 126 | | " | 6.1 | " | | | |
| 127 | | " | 5.9 | " | | | |
| 128 | | " | 5.8 | 1.0 | 1.0 | - | + |
| 129 | | " | 5.8 | Neg. | | | |
| 130 | | " | 5.9 | " | | | |
| 131 | | " | 5.7 | " | | | |
| 132 | | Kimnyimis Farmers-Nandi | 5.6 | " | | | |

Appendix 4:

Determination of minimum inhibitory concentrations of penicillin and oxytetracycline on *Lact. bulgaricus* and *Strept. lactis*.

I. Starter culture: *Lactobacillus bulgaricus*

A. Plate agar diffusion test

| Antibiotic | Dilution | Concentration | Size of zone of Inhibition (cm) |
|---|---|---|---|
| Oxytetracycline (Terramycin Q-50) (50 mg./ml) | 1:10 | 5,000 μg/ml | 2.8 |
| | 1:100 | 500 μg/ml | 2.3 |
| | 1:1,000 | 50 μg/ml | 2.0 |
| | 1:10,000 | 5 " | 1.4 |
| | 1:100,000 | 0.5 " | Neg. |
| Procaine penicillin G (300,000 units/ ml) | 1:10,000 | 30 unit/ml | 2.25 |
| | 1:100,000 | 3 " | 1.8 |
| | 1:1,000,000 | 0.3 " | 1.0 |
| | 1:10,000,000 | 0.03 " | Neg. |
| | 1:100,000,000 | 0.003 " | Neg. |

Neg. = no zone appearing

B. Broth Test:

| Antibiotic | Amount taken from 50 µg/ml into 10 ml. M.R.S. broth | Concentrations | Growth (turbidity) |
|---|---|---|---|
| Oxytetra-cycline (Terramycin Q-50) | 1.0 ml. | 5 µg/ml | - |
| | 0.80 ml. | 4 " | - |
| | 0.60 ml. | 3 " | - |
| | 0.40 " | 2 " | - |
| | 0.20 " | 1 " | - |
| | 0.19 " | 0.95" | - |
| | 0.18 " | 0.90" | - |
| | 0.17 " | 0.85" | - |
| | 0.16 " | 0.80" | - |
| | 0.15 " | 0.75" | - |
| | 0.14 " | 0.70 µg/ml* | - |
| | 0.13 " | 0.65 " | + |
| | 0.12 " | 0.60 " | + |
| | 0.11 | 0.55 " | + |
| | 0.10 | 0.50 " | + |
| | 0.09 " | 0.45 | |
| | Amount taken from 10 unit/ml into 10 ml broth | | |
| Procaine penicillin G | 0.90 ml. | 0.90 unit/ml | - |
| | 0.80 ml. | 0.80 " | - |
| | 0.70 " | 0.70 " | - |
| | 0.60 " | 0.60 " | - |
| | 0.50 " | 0.50 " | - |

B. Broth test cont'd:

| Antibiotic | Amount taken from 10 unit/ml into 10 ml. M.R.S. broth | Concentration | Growth (turbidity) |
|---|---|---|---|
| Procaine penicillin | 0.40 ml. | 0.40 unit/ml | - |
| | 0.30 " | 0.30 " | + |
| | 0.20 " | 0.20 " | + |
| | 0.10 " | 0.10 " | + |
| | 0.50 " | 0.50 " | - |
| | 0.40 " | 0.40 " | - |
| | 0.39 " | 0.39 unit/ml* | - |
| | 0.38 " | 0.38 " | + |
| | 0.37 " | 0.37 " | + |
| | 0.36 " | 0.36 " | + |
| | 0.35 " | 0.35 " | + |
| | 0.34 " | 0.34 " | + |
| | 0.33 " | 0.33 " | + |
| | 0.32 " | 0.32 " | + |
| | 0.31 " | 0.31 " | + |
| | 0.30 " | 0.30 " | + |
| Benzyl penicillin sodium salt 1,000,000 units | 0.60 ml | 0.60 unit/ml | - |
| | 0.55 " | 0.55 " | - |
| | 0.51 " | 0.51 " | - |
| | 0.50 " | 0.50 " | - |
| | 0.45 " | 0.45 " | - |
| | 0.44 " | 0.44 " | - |
| | 0.43 " | 0.43 " | - |

B. Broth test cont'd:

| Antibiotic | Amount taken from 10 units/ ml. into 10 ml M.R.S. broth | Concentration | Growth (Turbidity) |
|---|---|---|---|
| Benzyl peni-cillin sodium salt 1,000,000 units | 0.42 ml. | 0.42 units/ml | - |
| | 0.41 " | 0.41 " | - |
| | 0.40 " | 0.40 " | - |
| | 0.39 " | 0.39 unit/ml* | - |
| | 0.38 " | 0.38 " | + |
| | 0.37 " | 0.37 " | + |
| | 0.36 " | 0.36 " | + |
| | 0.35 " | 0.35 " | + |
| | 0.34 " | 0.34 " | + |
| | 0.33 " | 0.33 " | + |
| | 0.32 " | 0.32 " | + |
| | 0.31 " | 0.31 " | + |
| | 0.30 " | 0.30 " | + |

- = No visible growth (broth is clear)

+ = Visible growth characterised by turbidity of broth

* = Minimum inhibitory concentration (MIC)

2. Starter culture: Streptococcus lactis

A. Plate agar diffusion test

| Antibiotic | Dilution | Concentration | Size of zone of inhibition (cm) |
|---|---|---|---|
| Oxytetracycline (Terramycin Q-50) (50 mg/ml) | 1:10 | 5,000 μg/ml | 3.45 |
| | 1:100 | 500 " | 2.7 |
| | 1:1,000 | 50 " | 1.7 |
| | 1:10,000 | 5 " | 1.1 |
| | 1:100,000 | 0.5 " | Neg. |
| Procaine peni- cillin G (300,000 units/ ml) | 1:10,000 | 30 units/ml | 3.3 |
| | 1:100,000 | 3 " | 2.9 |
| | 1:1,000,000 | 0.3 " | 1.0 |
| | 1:10,000,000 | 0.03 " | Neg. |
| | 1:100,000,000 | 0.003 " | Neg. |

Neg. = No zone appearing

B. <u>Broth test</u>

| Antibiotic | Amount taken from 50 µg/ml into 10 ml. Dextrose broth | Concentration | Growth (turbidity) |
|---|---|---|---|
| Oxytetracycline (Terramycin Q-50) | 1.0 ml. | 5 µg/ml | - |
| | 0.80 " | 4 " | - |
| | 0.60 " | 3 " | - |
| | 0.40 " | 2 " | - |
| | 0.20 " | 1 " | - |
| | 0.19 " | 0.95 " | - |
| | 0.18 " | 0.90 " | - |
| | 0.17 " | 0.85 " | - |
| | 0.16 " | 0.80 " | - |
| | 0.15 " | 0.75 " | - |
| | 0.14 " | 0.70 " | - |
| | 0.13 " | 0.65 " | - |
| | 0.12 " | 0.60 µg/ml* | - |
| | 0.11 " | 0.55 " | + |
| | 0.10 " | 0.50 " | ± |
| | 0.09 " | 0.45 " | ± |
| | 0.08 " | 0.40 " | + |
| | 0.07 " | 0.35 " | + |
| | 0.06 " | 0.30 " | + |

B. <u>Broth test cont'd</u>:

| Antibiotic | Amount taken from 10 iu/ml into 10 ml. Dextrose broth | Concentration | Growth (turbidity) |
|---|---|---|---|
| Procaine penicillin G | 0.50 ml. | 0.50 unit/ml | - |
| | 0.40 " | 0.40 " | - |
| | 0.30 " | 0.30 " | - |
| | 0.20 " | 0.20 " | + |
| | 0.10 " | 0.10 " | + |
| | 0.01 " | 0.01 " | + |
| | 0.02 " | 0.02 " | + |
| | 0.03 " | 0.03 " | + |
| | 0.30 " | 0.30 " | - |
| | 0.29 " | 0.29 " | - |
| | 0.28 " | 0.28 " | - |
| | 0.27 " | 0.27 " | - |
| | 0.26 " | 0.26 unit/ml* | + |
| | 0.25 " | 0.25 " | + |
| | 0.24 " | 0.23 " | + |
| | 0.23 " | 0.23 " | + |
| | 0.22 " | 0.22 " | + |
| | 0.21 " | 0.21 " | + |
| | 0.20 " | 0.20 " | + |
| Benzyl penicillin sodium salt (1,000,000 units) | 0.30 " | 0.30 " | - |
| | 0.29 " | 0.29 " | - |
| | 0.28 " | 0.28 " | - |
| | 0.27 " | 0.27 " | - |
| | 0.26 " | 0.26 unit/ml* | - |
| | 0.25 " | 0.25 " | + |

B. Broth test cont'd:

| Antibiotic | Amount taken from 10 units/ml into 10 ml. Dextrose broth | Concentration | Growth (turbidity) |
|---|---|---|---|
| Benzyl Pen- | 0.24 ml. | 0.24 unit/ml | + |
| icillin sodi- | 0.23 " | 0.23 " | ± |
| um salt | 0.22 " | 0.22 " | + |
| | 0.21 " | 0.21 " | + |
| | 0.20 " | 0.20 " | + |

- = No visible growth (broth is clear)

+ = Visible growth characterised by turbidity of the broth

* = Minimum inhibitory concentration (MIC)

Appendix 5:

Concentration and duration of detectable levels of penicillin in milk following intramuscular injection of procaine penicillin G (300,000 units per ml) in aqueous suspension

| Post injection time (hrs) | Penicillin(units per ml) in milk* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | cow 1 | cow 2 | cow 3 | cow 4 | cow 5 | cow 6 | Average** | S.D. |
| 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 2 | 0.038 | 0.019 | 0.000 | 0.022 | 0.021 | 0.022 | 0.020 | 0.012 |
| 4 | 0.067 | 0.030 | 0.021 | 0.042 | 0.042 | 0.042 | 0.041 | 0.015 |
| 6 | 0.094 | 0.050 | 0.038 | 0.084 | 0.106 | 0.084 | 0.096 | 0.026 |
| 8 | 0.159 | 0.119 | 0.100 | 0.142 | 0.178 | 0.119 | 0.136 | 0.029 |
| 22 | 0.084 | 0.060 | 0.060 | 0.067 | 0.119 | 0.084 | 0.079 | 0.023 |
| 32 | 0.022 | 0.027 | 0.019 | 0.032 | 0.032 | 0.022 | 0.026 | 0.005 |
| 46 | 0.000 | 0.019 | 0.000 | 0.018 | 0.000 | 0.000 | 0.006 | 0.010 |
| 56 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 70 | - | - | - | - | - | - | - | - |
| 80 | - | - | - | - | - | - | - | - |
| 95 | - | - | - | - | - | - | - | - |
| 105 | - | - | - | - | - | - | - | - |

* = Pooled quarter milk samples of each cow

** = Average readings for 6 cows. Control pooled quarter milk samples, before injection, were negative

S.D. = standard deviation

\- = negative

Appendix 6:

Concentration and duration of detectable levels of penicillin in quarter milk samples following intramuscular injection of procaine penicillin G (300,000 units/ml) in aqueous suspension

| Cow | Milk yield per day (gall.) | Quarter milk samples | Penicillin (units per ml) in milk | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (Hrs.) 1 | 2 | 4 | 6 | 8 | 22 | 32 | 46 | 56 | 70 | 80 | 95 | 105 |
| 1 | 2 | RFQ | 0.000 | 0.042 | 0.119 | 0.142 | 0.237 | 0.159 | 0.030 | 0.019 | 0.00 | - | - | - | - |
| | | RHQ | 0.000 | 0.019 | 0.024 | 0.067 | 0.084 | 0.060 | 0.022 | 0.000 | 0.00 | - | - | - | - |
| | | LFQ | 0.000 | 0.050 | 0.100 | 0.119 | 0.237 | 0.150 | 0.024 | 0.000 | 0.00 | - | - | - | - |
| | | LHQ | 0.000 | 0.022 | 0.030 | 0.060 | 0.100 | 0.060 | 0.019 | 0.000 | 0.00 | - | - | - | - |
| 2 | 2 | RFQ | 0.000 | 0.022 | 0.056 | 0.178 | 0.212 | 0.071 | 0.034 | 0.022 | 0.00 | - | - | - | - |
| | | RHQ | 0.000 | 0.000 | 0.024 | 0.067 | 0.075 | 0.048 | 0.000 | 0.000 | 0.00 | - | - | - | - |
| | | LFQ | 0.000 | 0.000 | 0.014 | 0.071 | 0.067 | 0.050 | 0.014 | 0.021 | 0.00 | - | - | - | - |
| | | LHQ | 0.000 | 0.019 | 0.030 | 0.067 | 0.047 | 0.024 | 0.000 | 0.000 | 0.00 | - | - | - | - |
| 3 | 2 | RFQ | 0.000 | 0.000 | 0.000 | 0.024 | 0.032 | 0.022 | 0.000 | 0.000 | 0.00 | - | - | - | - |
| | | RHQ | 0.000 | 0.022 | 0.071 | 0.100 | 0.056 | 0.030 | 0.000 | 0.000 | 0.00 | - | - | - | - |
| | | LFQ | 0.000 | 0.000 | 0.047 | 0.030 | 0.022 | 0.000 | 0.000 | 0.000 | 0.00 | - | - | - | - |
| | | LHQ | 0.000 | 0.027 | 0.032 | 0.047 | 0.034 | 0.000 | 0.000 | 0.000 | 0.00 | - | - | - | - |
| 4 | 1½ | RFQ | 0.000 | 0.019 | 0.032 | 0.056 | 0.119 | 0.060 | 0.032 | 0.019 | 0.00 | - | - | - | - |
| | | RHQ | 0.000 | 0.022 | 0.030 | 0.060 | 0.067 | 0.047 | 0.027 | 0.000 | 0.00 | | | | |
| | | LFQ | 0.000 | 0.019 | 0.027 | 0.119 | 0.100 | 0.071 | 0.034 | 0.016 | 0.00 | - | - | - | - |
| | | LHQ | 0.000 | 0.000 | 0.022 | 0.032 | 0.038 | 0.022 | 0.019 | 0.000 | 0.00 | - | - | - | - |
| 5 | 1½ | RFQ | 0.000 | 0.019 | 0.034 | 0.056 | 0.150 | 0.178 | 0.119 | 0.022 | 0.00 | - | - | - | - |
| | | RHQ | 0.000 | 0.000 | 0.027 | 0.084 | 0.119 | 0.142 | 0.100 | 0.034 | 0.00 | - | - | - | - |
| | | LFQ | 0.000 | 0.022 | 0.047 | 0.084 | 0.142 | 0.159 | 0.119 | 0.024 | 0.00 | - | - | - | - |
| | | LHQ | 0.000 | 0.019 | 0.042 | 0.084 | 0.119 | 0.188 | 0.119 | 0.022 | 0.00 | - | - | - | - |
| 6 | 1½ | RFQ | 0.000 | 0.022 | 0.047 | 0.067 | 0.100 | 0.084 | 0.019 | 0.000 | 0.00 | - | - | - | - |
| | | RHQ | 0.000 | 0.022 | 0.047 | 0.100 | 0.119 | 0.100 | 0.022 | 0.000 | 0.00 | - | - | - | - |
| | | LFQ | 0.000 | 0.022 | 0.038 | 0.100 | 0.119 | 0.100 | 0.022 | 0.000 | 0.00 | - | - | - | - |
| | | LHQ | 0.000 | 0.019 | 0.034 | 0.071 | 0.119 | 0.067 | 0.019 | 0.000 | 0.00 | - | - | - | - |

RFQ = Right Fore quarter; RHQ = Right hind quarter; LFQ = Left Fore quarter; LHQ = Left hind quarter
Control quarter milk samples, taken before injections, were negative

\- = negative

Appendix 7:

Concentration and duration of detectable levels of penicillin in quarter milk samples following intramammary infusion of Vetramycin® suspension at the rate of one tube (4.2 ml.) per quarter (i.e. Left fore quarter and Right hind quarter).

| | | | Penicillin (units per ml) in milk | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cow | Milk yield per day (gallons) | Treated Quarters | DAY 1 EVEN | DAY 1 MORN | DAY 2 EVEN | DAY 2 MORN | DAY 3 EVEN | DAY 3 MORN | DAY 4 EVEN | DAY 4 MORN | DAY 5 EVEN | DAY 5 MORN | DAY 6 EVEN | DAY 7 MOR | DAY 7 EVEN |
| | | | 10 hr | 24 hr | 34 hr | 48 hr | 58 hr | 72 hr | 82 hr | 96 hr | 106 hr | 120 hr | 130 hr | 144 | 154 |
| 1 | 1½ | LFQ | >1 | >1 | >1 | 0.447 | 0.266 | 0.119 | 0.022 | 0.000 | 0.000 | - | - | - | - |
| | | RHQ | >1 | >1 | >1 | 1.890* | 0.708 | 0.119 | 0.078 | 0.022 | 0.000 | - | - | - | - |
| 2 | 1½ | LFQ | >1 | >1 | >1 | 0.119 | 0.032 | 0.022 | 0.018 | 0.000 | 0.000 | - | - | - | - |
| | | RHQ | >1 | >1 | >1 | 1.890* | 0.708 | 0.119 | 0.022 | 0.000 | 0.000 | - | - | - | - |
| 3 | 2 | LFQ | >1 | >1 | >1 | 0.596 | 0.060 | 0.042 | 0.000 | 0.000 | 0.000 | - | - | - | - |
| | | RHQ | >1 | >1 | >1 | 0.795 | 0.159 | 0.022 | 0.000 | 0.000 | 0.000 | - | - | - | - |
| 4 | 2 | LFQ | >1 | >1 | >1 | 0.317 | 0.119 | 0.027 | 0.000 | 0.000 | 0.000 | - | - | - | - |
| | | RHQ | >1 | >1 | >1 | 0.100 | 0.266 | 0.084 | 0.032 | 0.000 | 0.000 | - | - | - | - |
| 5 | 2 | LFQ | >1 | >1 | >1 | 0.188 | 0.038 | 0.000 | 0.000 | 0.000 | 0.000 | - | - | - | - |
| | | RHQ | >1 | >1 | >1 | 1.680* | 0.842 | 0.159 | 0.027 | 0.000 | 0.000 | - | - | - | - |
| 6 | 2 | LFQ | >1 | >1 | >1 | 0.266 | 0.100 | 0.019 | 0.000 | 0.000 | 0.000 | - | - | - | - |
| | | RHQ | >1 | >1 | >1 | 0.159 | 0.027 | 0.000 | 0.000 | 0.000 | 0.000 | - | - | - | - |
| Average | | | >1 | >1 | >1 | 0.779 | 0.277 | 0.061 | 0.17 | 0.002 | 0.000 | - | - | - | - |
| S.D. | | | | | | 0.682 | 0.300 | 0.056 | 0.023 | 0.007 | 0.000 | | | | |

Hrs. = hours

>1 = greater than 1 unit/ml penicillin

* = approximated figures obtained by extrapolation of the standard curve for penicillin  - = negative

LFQ = Left fore quarter; RHQ = Right hind quarter

Control quarter milk samples, taken before infusions, were negative  S.D. = standard deviation

Appendix 8:

Concentration of penicillin in milk from non-infused quarters following infusion of two quarters.

| Post-infusion time (hrs) | Penicillin concentration (units/ml) in milk | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C1 | | C2 | | C3 | | C4 | | C5 | | C6 |
| - | RF | RF | LH | RF | LH | RF | LH | RF | LH | RF | LH |
| 10 | - | 0.224 | 0.100 | 0.022 | 0.022 | - | - | - | 0.032 | - | 0.084 |
| 24 | - | 0.022 | 0.019 | - | - | - | - | - | - | - | 0.042 |
| 34-154 | - | - | - | - | - | - | - | - | - | - | - |

C1, ----, C6 = Cow 1, ---, Cow 6

RF = Right fore quarter

LH = Left hind quarter

\- = Negative

Appendix 9:

Percentage of milk samples positive for antibiotics

| Country | Year | No. of samples | Positive (%) | | Year | No. of samples | Positive (%) | Assay sensitivity (unit/ml) |
|---|---|---|---|---|---|---|---|---|
| U.S.A. | 1954 | 94 | 3.2* | --------- | 1960-67 | ? | 0.50* | 0.05 |
| England & Wales | 1961 | 975,000 | 6.1* | --------- | 1969 | 975,000 | 0.90* | 0.005-0.02 |
| Scotland | 1956 | ? | 5.9* | -------- | 1966 | 90,833 | 1.60* | 0.005-0.02 |
| Northern Ireland | 1965 | 17,000 | 1.7 | -------- | 1966 | 17,000 | 1.30 | 0.01 |
| Netherlands (i) | 1958 | 155 | 45.2 | -------- | 1971 | 215,241 | 1.10 | 0.01-0025 |
| (ii) | 1960 | 14,078 | 11.1 | -------- | 1971 | 1,577,922 | 1.40 | 0.01-0.0025 |
| Denmark | 1960 | 9,175 | 0.23* | -------- | 1976 | 189,416 | 0.05* | 0.02 |
| Australia | 1961-62 | 1,523 | 3.6 | -------- | 1963-64 | 2,127 | 2.0 | 0.03 |
| Kenya | - | - | - | -------- | 1977-78 | 1,725 | 5.2* | 0.01 |

* Penicillin and other "unnatural" inhibitors included