(updated)
REGULATIONS RELATING TO MILK AND DAIRY PRODUCTS

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The Minister of Health has, in terms of section 15(1) of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972), made the regulations in the Schedule.

SCHEDULE

Definitions

1. In these regulations any expression to which a meaning has been assigned in the Act shall bear such meaning and, unless inconsistent with the context -

"butter" means the product the fat of which consists exclusively of butter fat and the composition of which complies with the fat-content requirements prescribed in the Regulations relating to Dairy Products and Imitation Dairy Products (Government Notice No. R. 2581 of 20 November 1987, as amended) made under the Agricultural Products Standards Act, 1990 (Act No. 119 of 1990) (hereinafter referred to as "the Dairy and Imitation Dairy Products Regulations");

"buttermilk" means the milky by-product of the butter-making process;

"cheese" means the product that is obtained from a coagulum of –

(a) milk or milk constituents;
(b) cream;
(c) partly or wholly skimmed milk;
(d) reconstituted (prepared) milk;
(e) buttermilk;
(f) concentrated milk; or
(g) a combination of the above products,

by the removal of the whey, and that has undergone ripening to a greater or lesser extent and that may in addition have been further processed;

"closed container" means a clean container that is impervious to liquid, leak proof and will protect the product therein from contamination under normal conditions of storage, handling and transport;

"coliform bacteria" means rod-shaped, gram negative aerobic and facultatively anaerobic non-spore forming bacteria that ferment lactose, producing gas and acid in the process, by using the mediums and methods prescribed in paragraph 4, 5 or 11 of Annex A;

"composite dairy products" means a product as defined in the Dairy and Imitation Dairy Products Regulations;

"cream" means the fluid dairy product with a fat content as prescribed by the Dairy and Imitation Dairy Products Regulations;

"culture" means a liquid or powder containing one or more acceptable selected micro-organisms used in the manufacturing of cultured buttermilk, sour cream, sour milk, yoghurt or any other type of fermented milk product;

"cultured buttermilk" means buttermilk or pasteurized or reconstituted (prepared) milk which has been inoculated with a culture;

"dairy product" means a product as defined in the Dairy and Imitation Dairy Products Regulations;

"Escherichia coli" means the organism that produces gas at 44^0C +/- $0,25^0$C in brilliant green 2% (m/v) bile broth and produces indole in tryptone water at the same temperature when incubated for 24 hours, when using the method described in paragraph 2 of Annex A or, alternatively, when the violet red bile MUG agar method is used, the colonies that fluoresce blue in the surrounding mediums under an ultraviolet light after incubation for 24 +/- 1 hour at 30^0C;

"extraneous" means of external origin;

"food additive" means a substance as defined in the Regulations governing the Labelling and Advertising of Foodstuffs (Government Notice No. R. 2034 of 29 October 1993, as amended) (hereinafter referred to as "the Labelling and Advertising of Foodstuffs Regulations");

"hermetically sealed container" means an unopened container which cannot be opened without breaking or damaging such container or a seal, adhesive label or other part of or attachment to such container and which is intended to protect its contents against the entry of micro-organisms;

"imitation dairy product" means a product as defined in the Dairy and Imitation and Dairy Product Regulations;

"milk" means the normal mammary gland secretion obtained from lactating cows of the bovine species, goats or sheep;

"milk powder" means the product obtained by the removal of water only from milk, partly skimmed milk or wholly skimmed milk, with or without food additives permitted by the Act;

"modified dairy product" means a product as defined in the Dairy and Imitation Dairy Products Regulations;

"pasteurisation" means the heat treatment, as described in Annex B, of a dairy product or an imitation dairy product so that -

(a) all vegetative pathogens are destroyed; and
(b) in the case of milk, the result of the phosphatase test described in paragraph 3 of Annex A is negative

and, if the product concerned does not undergo further processing, the cooling thereof to below 5^0C immediately after having been thus heat treated;

"presumptive test" means a test the positive result of which invites the presumption that a substance is present after which the presumption must be proven to be true by using more sophisticated and accurate test methods;

"primary dairy product" means a product as defined in the Dairy and Imitation Dairy Products Regulations;

"raw cream" means cream that has undergone pasteurisation, sterilization or ultra high temperature treatment;

"raw milk" means milk that has not undergone pasteurization, sterilization or ultra high temperature treatment;

"reconstituted (prepared) milk" means the product obtained by re-constituting milk powder with water so that it complies with all the requirements for milk as prescribed in the Dairy and Imitation Dairy Products Regulations;

"skimmed milk" means milk the fat of which has been removed to comply with the fat-content requirements prescribed in the Dairy and Imitation Dairy Product Regulation;

"skimmed milk powder" means the product obtained by the drying of skimmed milk;

"sour cream or cultured cream" means the product obtained from pasteurized cream that has been inoculated with a culture in order for it to develop certain microbial flora under controlled conditions;

"sour milk or cultured milk" means the product obtained from pasteurized milk that has been inoculated with a culture in order for it to develop certain microbial flora under controlled conditions;

"sterilization" means the heat treatment above 100°C, after packaging, of a dairy product or an imitation dairy product so that the product concerned will be resistant to microbiological deterioration for a period of at least 14 days if kept at a temperature of 30°C +/- 1°C;

"the Act" shall means the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972);

"UHT" or **"ultra high temperature treatment"** means the process whereby milk or a dairy product is subjected to heat treatment above 100°C and aseptically packaged so that the end product, after incubation for not less than 14 days at a temperature of 30°C +/- 1°C, is free from spoilage by micro-organisms; and

"yoghurt" means the product obtained from pasteurized milk or reconstituted milk which has been inoculated with a yoghurt culture and which is allowed to ferment under controlled conditions;

Restrictions

2. No person shall use or sell raw milk intended for further processing which -
   (a) contains the following:
      (i) Antibiotics or other antimicrobial substances in amounts that exceed the maximum residue levels stipulated in the Regulations governing Maximum Limits for Veterinary Medicine and Stock Remedy Residues that my be present in Foodstuffs (Government Notice No. R. 1809 of 3 July 1992, as amended) (hereinafter referred to as the Maximum Limits for Veterinary Medicines and Stock Remedy Residues Regulations) or which virtue of a presumptive test is suspected to contain antibiotics or other antimicrobial substances in amounts that exceed such maximum residue levels;

(ii) pathogenic organisms, extraneous matter or any inflammatory product or other substances which for any reason whatsoever may render the milk unfit for human consumption;

(b) gives a positive result when subjected to the clot-on-boiling test described in paragraph 6 of Annex A

(c) gives a standard plate count of more than 200 000 colony forming units per 1,0ml when subjected to the standard plate count test described in paragraph 7 of Annex A or the dry rehydrated film method for standard colony count described in paragraph 10 of Annex A;

(d) (i) on application of the test described in paragraph 4(4) of Annex A, exceeds the most probable number (MPN) of 10,0 coliform bacteria per 1,0 ml milk or, if the test for coliforms described in paragraph 5 or 11 of Annex A is used, the number of colony forming units exceeds 20 per milliliter of milk; or

(ii) on application of the modified Eijkmann test, the VRB MUG agar method, or the dry rehydrated film method described in paragraphs 2, 5 and 11, respectively, of Annex A, is found to contain any *Escherichia coli* in 0,01ml or raw milk. When the Eijkmann test is used, or any *Escherichia coli* in 1,0ml of raw milk if the methods described in paragraph 5 or 11 of Annex A are used;

(e) when subjected to the Standard Methods for Counting Somatic Cells in Bovine Milk*, is found to contain an average of 500 000 or more somatic cells per 1,0ml of bovine milk or an average of 750 000 or more cells per 1,0ml goat's or sheep's milk after three successive readings at intervals of at least seven days during the test period, of which shows any other signs of abnormal secretary activity of the mammary gland(s);

* The Standard Method for Counting Somatic Cells in Bovine Milk is set forth in International Dairy Federation (IDF) Bulletin No. 114 of 1979.

(f) fails the ethanol stability test described in paragraph 9 of Annex A; and

(g) is not packed in a closed container.

3. (1) No person shall after two years from the date of publication of these regulations sell any raw milk, raw cream, raw skimmed milk, raw reconstituted (prepared) milk, raw reconstituted (prepared) skimmed milk or raw milk that has become sour, except in the areas of jurisdiction of the local authorities listed in Annex C.

(2) Any local authority that is of the opinion that it can exercise sufficient control over the selling of the raw dairy products referred to in subparagraph (1) may request the Minister, in writing through the relevant provincial health department, to be listed in Annex C.

(3) Any local authority that is listed in Annex C may request the Minister in writing to delete its name from the list.

4. (1) No person shall sell for consumption raw milk, raw cream, raw skimmed milk, raw reconstituted (prepared) milk or raw reconstituted (prepared) skimmed milk which -

(a) contains the following:

(i) Antibiotics or other antimicrobial substances in amounts that exceed the maximum residue levels stipulated in the Maximum Limits for Veterinary Medicine and Stock Remedy Residues Regulations or which by virtue of a presumptive test is suspected to contain antibiotics or other antimicrobial substances in amounts that exceed such maximum residue levels;

(ii) pathogenic organisms, extraneous matter or any inflammatory product or other substance which for any reason whatsoever may render the product unfit for human consumption;

(b) gives a standard plate count of more than 50 000 colony forming units (CFUs) per 1,0ml of the product when subjected to the standard plate count test described in paragraph 7 of Annex A or the dry rehydrated film method for standard colony count described in paragraph 10 of Annex A.

(c) gives a positive result when subjected to the clot-on-boiling test desribed in paragraph 6 of Annex A;

(d) fails the ethanol stability test described in paragraph 9 of Annex A;

(e) on execution of the modified Eijkmann test, the VRB MUG agar method or the dry rehydrated film method described in paragraphs 2, 5 and 11, respectively, of Annex A, is found to contain any *Escherichia coli* in 1,0ml of fluid or 1,0g of cream;

(f) (i) on subjection to the Standard Routine Method for the Counting of Coliform Bacteria in Raw Milk of the International Dairy Federation's International IDF 73:1985, or any revised version thereof, or on application of the VRB MUG agar method described in paragraph 5 of Annex A or on using the dry rehydrated film method described in paragraph 11 of Annex A, is found to contain more than 20 coliform bacteria in 1,0ml of fluid; or

(ii) on subjection to the coliform bacteria test described in paragraph 4(4) of Annex A, exceeds the most probable number (MPN) of 10,0 coliform bacteria per 1,0ml of fluid or 1,0g of semi-solid product;

(g) in the case of raw milk, on subjection to the Standard Method for Counting Somatic Cells in Bovine Milk, is found to contain an average of 500 000 or more somatic cells per 1,0ml of bovine milk or an average of 750 000 or more cells per 1,0ml of goat's or sheep's milk after three successive readings at intervals of at least seven days during the test period, or which shows any other signs of abnormal secretory activity of the mammary gland(s);

(h) is not packed in a closed cntainer;

(i) does not bear clearly on the label the words: "Unpasteurised" / "Ongepasteuriserd" or "Raw milk" / "Rou melk";

(j) when the milk is sold in the consumer's own container, is tapped from a container which does not bear a label clearly indicating the words: "Unpasteurised" / "Ongepasteuriseerd" or "Raw milk" / "Rou melk";

(k) (i) is not derived from a herd enrolled in the Bovine Tuberculosis Scheme and the Bovine Brucellosis Scheme which have been established in terms of the Animal Diseases Act, 1984 (Act No. 35 of 1984); or

(ii) is not derived fro a herd which annually tests negative for tuberculosis and brucellosis.

5. No person shall sell for consumption raw milk that has become sour which -

(a) contains the following:

(i) Antibiotics or other antimicrobial substances in amounts that exceed the maximum residue levels stipulated in the Maximum Limits for Veterinary Medicine and Stock Remedy Residues Regulations or which by virtue of a presumptive test is suspected to contain antibiotics or other antimicrobial substances in amounts that exceed such maximum residue levels;

(ii) pathogenic organisms, extraneous matter or any inflammatory product or other substance which for any reason whatsoever may render the raw milk unfit for human consumption;

(b) on application of the modified Eijkmann test or the VRB MUG agar method described in paragraphs 2 and 5 respectively, of Annex A, is found to contain any *Escherichia coli* in 1,0ml of the product;

(c) on subjection to the VRB MUG agar method or the dry rehydrated film method described in paragraphs 5 and 11 of Annex A, respectively, contains more than 50 coliform bacteria per 1,0ml of the product;

(d) is not packed in a closed container; and

(e) does not bear clearly on the label the words: "Unpasteurised sour milk" / "ongepasteuriseerde suur melk" or "Raw sour milk" / "Rou suur melk";

(f) when the milk is sold in the consumer's own container, is tapped from a container which does not bear a label clearly indicating the words: "Unpasteurised sour milk"/ "Ongepasteuriseerde suur melk" or "Raw sour milk" / "Rou suur melk".

6. No person shall sell -

(a) pasteurised milk, pasteurised reconstituted (prepared) milk, pasteurized skimmed milk, pasteurized reconstituted (prepared) skimmed milk or pasteurized cream which -

(i) contains the following:

(aa) Antibiotics or other antimicrobial substances in amounts that exceed the maximum residue levels stipulated in the Maximum Limits for Veterinary Medicine and Stock Remedy Residues Regulations or which by virtue of a presumptive test (for example the Kundrat test) is suspected to contain antibiotics or other antimicrobial substances in amounts that exceed such maximum residue levels;

(bb) pathogenic organisms, extraneous matter or any inflammatory product or other substance which for any reason whatsoever may render the product unfit for human consumption;

(ii) has been shown by the Aschaffenburg and Mullen phosphate test described in paragraph 3 of Annex A or any other test, provided its accuracy equals that of the aforementioned test, to yield the equivalent of 10 micrograms or more of p-nitrophenol per 1,0ml;

(iii) (aa) on execution of the VRB MUG agar method or the dry rehydrated film method is found to contain more than 10,0 coliform bacteria in 1,0ml or exceeds the most probable number (MPN) of 10,0 coliform bacteria per 1,0ml of milk or 1,0g of semi-solid product; or

(bb) on execution of the modified Eijkmann test, the VRB MUG agar method or the dry rehydrated film method described in paragraphs 2, 5 and 11, espectively, of Annex A, is found to contain any *Escherichia coli* in 1,0ml of milk or 1,0g of semi-solid product;

(iv) gives a standard plate count of more than 50 000 colony forming units (CFUs) per 1,0ml of fluid or per 1,0g of semi-solid product when subjected to the tests described in paragraph 7 or 10 of Annex A;

(v) is not packed in a hermetically sealed container when sold to the ultimate consumer: Provided that in cases where the consumer supplies his or her own empty container to be filled from a bulk tank or container, the filled container need to be hermetically sealed;

(b) sterilised cream, sterilised milk, sterilised reconstituted (prepared) milk or UHT cream or UHT milk which -

(i) contains the following:

(aa) Antibiotics or other antimicrobial substances in amounts that exceed the maximum residue levels stipulated in the Maximum Limits for Veterinary Medicine and Stock Remedy Residues Regulations or which by virtue of a presumptive test is suspected to contain antibiotics or other antimicrobial substances in amounts that exceed such maximum residue levels;

(bb) pathogenic organisms, extraneous matter or any inflammatory product or other substances which for any reason whatsoever may render any such product unfit for human consumption;

(ii) (aa) shows an increase in titratable acidity greater than 0,02, expressed as grams of lactic acid per 100ml of milk, on application of the test described in paragraph 8 of Annex A after incubation at 30°C +/- 1°C for 14 days;

(bb) shows any signs of coagulation or blown containers after incubation;

(iii) is not packed in a hermetically sealed container when sold to the ultimate consumer.

7. Subject to the provisions of the Act, no person shall sell any dairy product or composite product which -

(a) contains the following:

(i) Antibiotics or other antimicrobial substances in amounts that exceed the maximum residue levels stipulated in the Maximum Limits for Veterinary Medicine and Stock Remedy Residues Regulations or which by virtue of a presumptive test is suspected to contain antibiotics or other antimicrobial substances in amounts that exceed such maximum residue levels;

(ii) pathogenic organisms, extraneous matter or any inflammatory product or other substance which for any reason whatsoever may render any such product unfit for human consumption;

(b) in the case of milk powder or skimmed milk powder, contains more than 50 000 colony forming units per gram on application of the standard plate count test described in paragraph 7 of Annex A;

(c) with the exception of ripened cheese -

(i) on execution of the test described in paragraph 4 of Annex A or the test described in International Standard IDF 73A:1985, contains more than 50 coliform bacteria per 1,0ml of fluid or 1,0g of solid or semi-solid product;

(ii) on execution of the modified Eijkmann test or the VRB MUG agar method described in paragraphs 2 and 5, respectively, of Annex A, is found to contain any *Escherichia coli* in 1,0ml of fluid or 1,0g of solid or semi-solid product;

(d) in the case of ripened cheese -

(i) on execution of the test described in paragraph 4 of Annex A or the test described in International Standard IDF 73A: 1985 contains more than 1 000 coliform bacteria per 1,0g of the product;

(ii) on execution of the modified Eijkmann test or the VRB MUG agar method described in paragraphs 2 and 5, respectively, of Annex A, is found to contain any *Escherichia coli* per 1,0 ml of fluid or 1,0g of solid or semi-solid product;

(e) is not packed in a hermetically sealed package or in a closed package.

8. No person shall sell any dairy product or composite dairy product which contains any food additive not permitted by regulation.

9. No person shall sell milk, cream or any dairy product that is not derived from the mammary gland(s) of lactating cows of the bovine species or of goats or sheep unless it is labeled in accordance with the requirements of the labeling and Advertising of Foodstuffs Regulations promulgated under the Act.

10. No pasteurised milk, pasteurised cream or pasteurised reconstituted (prepared) milk which is returned to the milk processing plant shall be resold or processed for resale.

11. In determining whether milk, dairy products and composite dairy products meet the requirements laid down in regulations 2, 4, 5, 6 or 7, the tests prescribed therein shall be conducted and these tests shall be conclusive for the said purpose

Repeal of regulations

12. The regulations published by Government Notice No. R. 258 of 8 February 1985, as amended by Government Notice No. R. 2706 of 15 November 1991, are hereby repealed.

ANNEX A

METHODS FOR THE TESTING OF MILK, CREAM AND DAIRY PRODUCTS

1. (1) (a) The tests set forth in Annex A shall be conducted in appropriate cases in order to ascertain the suitability of milk, cream and dairy products for human consumption. Samples shall not be frozen but shall be kept at a temperature below 5°C and shall be tested within 48 hours of collection: Provided that these requirements shall not apply to dried dairy products, sterilized milk, UHT milk and condensed dairy products in their unopened containers

(c) For the purpose of Annex A "milk" shall include milk that has undergone pasteurization or sterilization or ultra high temperature treatment, and cream.

MICROBIOLOGICAL TESTS

(2) (a) All distilled water used in the preparation of mediums shall be glass distilled water or water of similar purity.

(b) All glassware used in the tests prescribed by this Annex shall be sterile.

(c) The sterility of all glassware, media and diluents shall be checked by -

(i) testing representative control tubes, control dishes and growth media used in each batch of tests;

(ii) using the growth medium referred to in this Annex.

(d) All pipettes of the blow-out type shall be suitably plugged with non-absorbent cotton wool.

(e) All glassware used for volumetric measurement shall have an accuracy level at least equal to National Physical Research Laboratory Grade B.

(f) All chemicals used in the preparation of the solutions and media referred to in this Annex shall, except where otherwise prescribed, be of an analytical reagent grade or a grade suitable for the preparation of bacteriological media.

(g) Appropriate dehydrated culture media, where such preparations are available, may be used instead of the media prescribed: Provided that such dehydrated media shall conform to the description given and yield equivalent results: Provided further that any peptone, bile salts, tryptone, yeast extract and ox bile used shall be of a standard equivalent to the reference standard kept by the South African Bureau of Standards.

Modified Eijkmann test for *Escherichia coli*

2. (1) The modified Eijkmann test shall be carried out in the manner set out below.

(2) Thoroughly mix the sample of milk or cream and, if the cream is too thick for easy handling, heat it to a temperature not higher than 37°C.

(3) After taking all necessary precautions to prevent contamination of the sample, inoculate three tubes containing 10ml (m/v) of brilliant green bile broth and fitted with an inverted Durham fermentation tube for the detection of gas using a 1 ml pipette with the equivalent of 0,01ml in the case of raw milk intended for pasteurization and 1ml in the case of pasteurised milk, reconstituted (prepared) milk, pasteurized cream and cultured dairy products. In the case of solid or semi-solid dairy products, inoculate tubes containing double-strength brilliant green bile broth with 10ml of a 1:10 dilution of the dairy product.

(4) For the measurement of the 0,01ml quantities to be tested in the case of milk, prepare decimal dilutions in accordance with the standard plate count method described in

paragraph 7(1)(a) and (b), substituting 11,0ml of milk for 11,0g of milk powder or skimmed milk powder.

(5) Incubate the inoculated brilliant green bile broth for 48 hours in a water bath keeping the temperature of the water bath at 44°C +/- 0,15°C.

(6) If the incubation prescribed in subparagraph (5) leads to the formation of gas as seen in the Durham tube, transfer and inoculum of 0,2ml from each brilliant green bile broth tube in which gas has formed to a separate tube of tryptone water.

(7) Incubate the tryptone water tubes referred to in subparagraph (6) in the water bath referred to in subparagraph (5) at 44°C +/- 0,25°C for 24 +/- 2 hours.

(8) After the said 24 +/- 2 hours, test the tryptone water in the tubes for indole production by adding 0,5ml of Kovac's reagent.

(9) The formation of a rose-coloured ring at the interface of the two liquids indicates the presence of indole.

(10) A positive result of gas and indole in any of the three tubes inoculated with the prescribed volume of the same milk shall be taken to indicate the presence of *Escherichia coli*.

(11) Prepare the (m/v) brilliant green bile broth, the tryptone water and the Kovac's reagent as follows:

(a) (i) The composition of the brilliant green bile broth shall be as follows:
Ox bile……20g
Peptone……10g
Lactose……10g
1 per cent (m/v) aqueous solution of brilliant green……1,3ml
Distilled water……1l

(ii) Dissolve the constituents in the distilled water.

(iii) Adjust the pH to a value of 7,2 to 7,4.

(iv) Distribute the medium in 10ml quantities among test tubes containing an inverted Durham fermentation tube and then sterilize them in an autoclave at 121°C for at least 15 minutes.

(v) In order to prepare double-strength brilliant green bile broth, use half the quantity of distilled water.

(b) (i) The composition of the tryptone water shall be as follows:
Tryptone……10g
Sodium chloride……5g
Distilled water……up to 1l

(ii) Dissolve the constituents in the distilled water by warming the mixture slightly.

(iii) Cool to 20-25°C and adjust the pH with sodium hydroxide solution or hydrochloric acid solution to between 7,4 and 7,5.

(iv) Dispense the medium in 5ml aliquots in test tubes. Autoclave the dispensed medium at 121°C for at least 15 minutes.

(c) (i) The composition of the Kovac's reagent shall be as follows:
Paradimethylaminobenzaldehyde……5g
Concentrated hydrochloric acid……25ml
Amyl alcohol (pyridine free)……75ml

(ii) Dissolve paradimethylaminobenzaldehyde in the amyl alcohol and add the hydrochloric acid.

(iii) After preparation, the reagent should be yellow in colour.

(iv) Place the reagent in an amber-coloured glass stoppered vessel and store in a cool, dark place.

(v) Do not use the reagent within 24 hours after preparation.

Aschaffenburg and Mullen phosphatase test

3. (1) The phosphatase test shall be carried out in the manner set out below.
(2) Test each sample as soon as possible after its arrival at the laboratory.
(3) If the sample is not tested immediately on its arrival at the laboratory, keep it at a temperature below 5°C, but not frozen, until it is tested.
(4) Raise the temperature of the sample to 20-25°C immediately before it is tested.
(5) Take the following precautions during or in connection with he testing of a sample;
(a) Except in the case of cultured dairy products, do not test a sample that shows signs of spoiling or souring.
(b) Use a clean pipette for each sample of milk or cream and ensure that no pipette is contaminated with saliva.
(c) Do not perform the test in direct sunlight.
(d) Use only distilled water throughout the test.
(6) Whenever practicable, use reagents of analytical quality for this test. Prepare the buffer substrate solution as follows:
(a) Buffer solution: Dissolve 3,5g of anhydrous sodium carbonate and 1,5g of sodium bicarbontate in distilled water and fill up with water to 1l solution in a volumetric flask.
(b) Keep the solid substrate, disodium p-nitrophenyl phosphate, in a refrigerator.
(c) Buffer substrate solution:
(i) Place 150mg of the substrate in a standard 100ml volumetric measuring flask and fill to the 100ml mark with the buffer solution.
(ii) Store the solution in a refrigerator and protect from light.
(iii) When distilled water is used for purposes of comparison, the solution must give a reading of less than the standard 10 on the comparator disc APTW 5 or APTW 7 when viewed in transmitted light through a 5mm cell in the all-purpose comparator.
(iv) Do not use the solution for longer than one week.
(7) Use the following apparatus for the test:
(a) A lovibond all-purpose comparator with a stand for work in reflected light.
(b) A Lovibond comparator disc APTW 5 or APTW 7.
(c) Two fused-glass cells, 25mm deep, or test tubes of colourless lass, 13,5mm internal diameter, conforming to B.S. 625, fitted with non-p-nitrophenol-containing stoppers, for use in the Lovibond all purpose 1000 comparator.
(d) A waterbath capable of being maintained at 39°C +/- 0,5°C.
(e) A pipette to deliver 5,0ml.
(f) A supply of 1,0ml straight-sided pipettes.
(g) A 1l volumetric flask.
(h) A 100ml standard volumetric flask.
(8) (a) After use, empty each tube, rinse it in water, wash well in hot water containing soda, rinse in hot water and then in distilled water and dry, or clean by some other equally effective method.
(b) If, after treatment in accordance with (a) of this subparagraph, a test tube does not appear to be clean, repeat the treatment but, in addition, after rising it in hot water, place it in hydrochloric acid and then rinse it again in hot water and then in distilled water and dry it, or clean it by some other equally effective method.
(c) Clean new glassware by dipping it in a solution of chromic acid consisting of five volumes of 8% (m/v) potassium dichromate and four volumes of concentrated sulphuric acid added slowly and carefully to the mixture of dichromate and water.
(d) Keep the solution referred to in (c) of this subparagraph covered and discard it when it turns green.

(e) After cleaning new glassware in the manner described above, rinse it in hot water, then rinse it in distilled water and dry.

(f) Pipettes should be rinsed in cold water and then cleaned by soaking for 24 hours in a solution of chromic acid in a 250ml glass cylinder or other suitable container, and thereafter well rinsed in hot water and then in distilled water and dried, or cleaned by some other equally effective method.

(g) Glassware used for the test shall not be used for any other purpose and shall be kept separate from all other apparatus in the laboratory.

(9) The test shall be carried out in the manner as set out below:

(a) Transfer 5ml of the buffer substrate solution to a test tube using a pipette, stopper the test tube and bring the contents to a temperature of 37°C +/- 0,5°C.

(b) Add 1 ml of the milk or cream to be tested, replace the stopper of the test tube and mix the contents well by shaking.

(c) Incubate the test tube for 2 hours +/- 1 minute at 37°C +/- 0,5°C.

(d) With each series of samples, incubate one control sample prepared from 5ml of buffer substrate solution and 1ml of boiled milk or cream of the same type as that undergoing the test.

(e) After incubation, remove the test tube from the water bath and mix the contents well.

(f) Place the control sample on the left-hand ramp of the stand and the test sample on the right.

(g) Take the readings in reflected light by looking down onto the two apertures with the comparator facing a good source of daylight.

(h) If artificial light is needed for matching, use a daylight type of illumination.

(i) Revolve the disc until the colour of the test sample matches that of the control sample.

(j) Record readings falling between two standards by affixing a plus or minus sign to the figure for the nearest standard.

Coliform Bacteria Test

4. (1) The coliform bacteria test for milk, reconstituted (prepared) milk, pasteurised milk, pasteurised cream and dairy products shall be carried out in the manner set out below or by using VRB MUG agar method described in paragraph 5 of Annexure A.

(2) Mix the milk, ream or dairy products thoroughly before sampling from bulk.

(3) (a) Thoroughly mix samples of milk, skimmed milk, buttermilk or cream. If it is too thick for easy handling, cream may be heated to a temperature not exceeding 37°C. Prepare the 1:10 dilution (m/m) by adding 1ml of the product to 9ml of the sterile diluent (phosphate buffer or peptone saline solution) or 11ml of the product to 99ml of the diluent (paragraph 7).

(b) Thoroughlt mix viscous or semi-solid cultured dairy products and place 11g of the mixed product in a sterile wide-mouthed container. Add 99ml of heated (40°C) sterile 2% (m/v) sodium citrate solution and shake the mixture until homogeneous dispersion is obtained. This constitutes the 1:10 dilution (m/m) of the product. Further tenfold dilutions are prepared in the sterile diluent (paragraph 7).

(4) The most probable number (MPN) of coliform bacteria shall be determined as follows:

(a) Inoculate three test tubes each containing 10ml of double-strength brilliant green bile broth as described in paragraph 2(11)(a)(i) to (v) and a Durham tube with 10ml of the 1:10 dilution of the product. This inoculation corresponds to 1g or 1ml of the product sample in each tube.

(b) Inoculate three tubes containing 10ml single-strength brilliant green bile broth and a Durham tube with 1 ml of the 1:10 dilution of the product. This inoculation corresponds to 0,1g or 0,1ml of sample in each tube.

(c) Inoculate three tubes each containing 10ml of single-strength brilliant green bile broth and a Durham tube with 1ml of the 1:100 dilution or 0,1 dilution of the

product. This inoculation corresponds to 0,01g or 0,01ml of the sample in each tube.

(d) Mix carefully, making sure that no air bubbles are shaken into the Durham tubes.

(e) After preparing the initial dilutions, proceed without delay with the preparation of further dilutions and inoculations.

(f) Incubate the inoculated tubes for 48 +/- 2 hours at 30°C +/- 1°C.

(g) A tube containing sufficient gas to fill the concavity of the Durham tube shall be recorded as positive. A positive result shall also be recorded if the Durham tube contains less than the said amount of gas but effervescence occurs when the side of the test tube is tapped. Record the number of positive results.

(h) In the case of fruit yoghurt and other products containing a fermentable substance other than lactose, confirm the presence of lactose fermenters by transferring one loop full of the contents of each tube showing gas production to fresh tubes of single-strength billiant green bile broth, incubating these tubes for 48 +/- 2 hours at 30°C +/- 1°C and examining them for gas production.

(i) The number of positive tubes (after confirmation, in the case of products containing fermentable substances other than lactose) for each dilution is used for determining the MPN of coliform bacteria per 1,0g or 1,0ml of the product in accordance with the following table:

Number of positive			MPN of coliform in	Number of positive tubes			MPN of coliforms in
1,0g or 1,0ml	0,1g or 0,1ml	0,01g or 0,01ml	1,0g or 1,0ml	1,0g or 1,0ml	0,1g or 0,1ml	0,01g or 0,01ml	1,0g or 1,0ml
0	0	0	0,0	2	2	2	3,5
0	0	1	0,3	2	2	3	4,0
0	1	0	0,3	2	3	0	3,0
0	1	1	0,6	2	3	1	3,5
0	2	0	0,6	2	3	2	4,0
1	0	0	0,4	3	0	0	2,5
1	0	1	0,7	3	0	1	4,0
1	0	2	1,1	3	0	2	6,5
1	1	0	0,7	3	1	0	4,5
1	1	1	1,1	3	1	1	7,5
1	2	0	1,1	3	1	2	11,4
1	2	1	1,5	3	1	3	16,0
1	3	0	1,6	3	2	0	9,5
2	0	0	0,9	3	2	1	15,0
2	0	1	1,4	3	2	2	20,0
2	0	2	2,0	3	2	3	30,0
2	1	0	1,5	3	3	0	25,0
2	1	1	2,0	3	3	1	45,0
2	1	2	3,0	3	3	2	110,0
2	2	0	2,0	3	3	3	110,0
2	2	1	3,0	3	3	3	100,0

(5) Cultured products with developed acidity shall be tested within 48 hours of their manufacture.

Violet red bile (MUG) agar method for coliforms and *Escherichia coli*

5. (1) The coliform organism test and the test for *Escherichia coli* in milk, reconstituted (prepared) milk, pasteurised milk, pasteurised cream and dairy products shall be carried out in the manner set out below.

(2) Prepare the samples as follows:

(a) Thoroughly mix samples of milk, skimmed milk, buttermilk or cream. If it is too thick for easy handling, cream may be heated to a temperature not exceeding 37°C. Prepare the 1:10 dilution (m/m) by adding 1ml of the product to 9ml of sterile diluent or 11ml of the product to 99ml of diluent.

(b) Thoroughly mix viscous or semi-solid cultured dairy products and place 11g of the product in a sterile wide-mouthed container. Then add 99ml of heated (40°C) sterile 2% (m/v) sodium citrate solution and shake the mixture until homogeneous dispersion has been obtained. This constitutes the 1:10 dilution of the product. Prepare further tenfold dilution in the sterile diluent.

(3) The violet red bile agar is prepared as follows:

	g/l
Brain heart infusion	7,0
Peptone	4,0
Lactose	9,0
Bile salts No. 3	1,5
Neutral red	0,03
Crystal violet	0,002
MUG (4-methylumbelliferyl B-D-glucuronide)	0,1
Sodium chloride	4,5
Disodium phosphate	1,0
Agar	13,0*

* When testing for *Escherichia coli*, add the MUG reagent, if not already included in the media, according to the manufacturer's instructions

Note: (i) The preparation of the samples should not be carried out in direct sunlight; and

(ii) normal aseptic precautions should be taken when necessary.

(4) The test shall be conducted as follows:

(a) Prepare dilutions so as to obtain plates with colony counts of more than 10, if possible, and fewer than 150. In the case of milk and liquid dairy products, make sure that the micro-organisms in the test sample are distributed as evenly as possible by inverting the sample container 25 times. If foam is formed, it should be allowed to disperse. The interval between mixing and removing the test portion should not be longer than three minutes. Remove 1ml of the test sample with a sterile pipette and add to 9ml of the diluent (or 10ml of the test sample to 90ml of the diluent or 11ml of the test sample to 99ml of the diluent). Shake this primary dilution thoroughly. In this way, a 10^{-1} dilution is obtained.

(b) Now prepare further dilutions by transferring, using a sterile pipette, 1ml of the primary dilution to another test tube containing 9ml of sterile diluent, avoiding contact between the pipette and the diluent. A fresh pipette should be used for each dilution.

Alternatively, transfer 10ml of the primary dilution to a bottle containing 90ml of the sterile diluent, or 11ml of the primary dilution to 99ml of the sterile diluent.

Mix thoroughly either by aspirating 10 times with a fresh pipette or by mixing mechanically for 5 to 10 seconds to obtain the 10^{-2} dilution. The frequency of rotation in the case of mechanical mixing shall be such that the liquid moves two or three centimeters up the side of the vessel while being mixed. If necessary, repeat this procedure, using the 10^{-2} and further dilutions to obtain 10^{-3}, 10^{-4}, etcetera, dilutions until the appropriate number of micro-organisms has been obtained.

Note: The time lapse between the initial measurement of the test portion, the preparation of the primary dilution and the mixing of the dilutions and mediums shall not be longer than 15 minutes.

(c) Use a pipette to transfer 1ml of the liquid product or the appropriate dilutions to the center of two petri dishes. Touch a dry area in the petri dish with the tip of the pipette. Use a fresh pipette to inoculate each dilution.

(d) Pour about 15ml of the VRB MUG agar at 45°C +/- 1°C into each petri dish. Mix immediately after pouring by rotating the petri dish sufficiently to obtain evenly dispersed colonies after incubation. Allow to solidify on a cool horizontal surface.

After complete solidification, pour about 4ml of the VRB agar at 45°C +/- 1°C onto the surface of the inoculated medium and allow to solidify. Prepare a control dish with 15ml of the medium to check its sterility.

Note: In order to ensure that the temperature of the medium is 45°C +/- 1°C before pouring, place a thermometer into a 1,5% agar solution portion in a separate container identical to that used for the medium. This control portion should be exposed to the same heating and cooling as the medium.

(e) Incubate the plates in an adverted position. Do not stack them more than six high. Stack of plates should be separated from one another and from the sides and top of the incubator. Incubate 30°C +/- 1°C into for 24°C +/- 2 hours.

(f) Examine the plates under a 366 nm ultra violet light. All colonies showing a blue fluorescence in the surrounding medium are counted. Then examine the plates under normal light and count the coliform organisms. Select the plates with more than 10 and fewer than 150 colonies. Count the dark red-coloured colonies with a diameter of at least 0,5mm, characteristic of coliform organisms. These dark pink to red colonies are usually surrounded by a red zone in the medium. Confirm the count by following the procedure described in subparagraph (g). Calculate the number of coliform organisms per gram or per milliliter, taking into account the result of the confirmatory test. Five or more fluorescent colonies are regarded as positive for *Escherichia coli*.

(g) The confirmatory test is done by inoculating five colonies of each type, if available, into tubes of brilliant green lactose bile broth containing a Durham tube and incubating at 30°C +/- 1°C for 24°C +/- 2 hours. Consider colonies that show gas formation in the Durham tube to be coliform organisms.

The clot-on-boiling test

6. (1) Thoroughly mix the milk before sampling.
(2) Pour 5ml of milk into a test tube.
(3) Place the tube in boiling water.
(4) Ensure that the level of the boiling water is higher than the milk level.
(5) Stand the test tube of milk in the boiling water for five minutes.
(6) Remove the test tube from the water and tilt the tube almost horizontally without shaking the milk inside.
(7) Wait until a thin film is formed on the milk.
(8) The result is positive if all the milk clots or if floccules are seen to be adhering to the sides of the tube when it is returned to the vertical position.

Note: Colostrum in milk will result in a positive clot-on-boiling test result. The heat stability of the milk is also affected by other factors.

Standard plate count

7. (1) Mix raw milk or pasteurized milk thoroughly immediately before sampling from bulk:
(a) The 1:10 dilution (m/m) of raw or pateurised shall be prepared in the manner set forth in paragraphs 4(3)(a) and (b) of this Annex.
(b) In the case of milk powder and skimmed milk powder the 1:10 dilution (m/m) shall be prepared as follows:

Place 99ml of sterile diluent* into a sterile wide-mouthed container equipped with a rubber stopper or a screw top and heat it to 47°C +/- 2°C by placing it in a water bath at this temperature. Weigh 11g of the powder into a sterile aluminium weighing boat or glass container equipped with a rubber stopper or a screw top and heat it to 47°C +/- 2°C by placing it in a water bath at this temperature.

Quickly add the powder to the warm diluent and turn the diluent bottle slowly in order to wet the powder. Then shake the bottle 25 times using up and down movements of 300mm. Replace the bottle in the water bath for an additional five minutes and shake it at intervals. In order to facilitate the reconstitution of the powder, a few grams of sterile glass beads may be added to the diluent. Prepare additional tenfold dilutions in sterile diluent (at room temperature) as required.

(2) Using a fresh pipette, transfer 1ml of each of the dilutions at least in duplicate to sterile petri dishes, beginning with the highest concentration and ending with the lowest.

(3) To each dish add 10ml of the standard plate count agar** which has been melted beforehand and cooled to 45°C +/- 1°C.

(4) Mix the contents of each dish thoroughly using horizontal rotational movement while the medium is still fluid.

(5) Once the medium has set, invert the dishes and incubate at 30°C +/- 1°C for 72+/-2 hours.

(6) At the end of the incubation period remove the dishes from the incubator and count the colony-forming units (CFU) with the aid of magnification under uniform artificial illumination.

(7) To count the CFUs of each dish, spreader-free dishes containing 30 – 300 CFUs are selected; count all the CFUs and calculate the number of CFUs per ml or per gram.

(8) If the number of CFUs of each dish exceeds 300, count the CFUs in portions of the dish representative of the CFU distribution and use this count to determine the total number per dish. Proceed as in (7) above, but record as an "estimated" plate count.

* **Diluents:**

Phosphate buffer solution:

Potassium dihydrogen orthophosphate……5,08g
Disodium hydrogen orthophosphate……13,63g
In 2l distilled water

OR

Peptone saline solution

Peptone……1,0g
Sodium chloride……8,5g
In 1l distilled water

Dissolve the components in the water, heating if necessary. Adjust the pH so that, after sterilization, it is 7,0+/-0,1 at 25°C.

****** **Plate count agar**

Tryptone (pancreatic digestive product of casein)……5g
Yeast extract……2,5g
Glucose……1g
Agar (bacterial grade)……15g
Distilled water……1l
Final pH of sterilized medium……7,0+/-0,1

Sterilise for at least 15 minutes at 121°C.

Titratable acidity

8. (1) Pipette 9ml of milk into a white dish.

(2) Add either 10 drops or 0,5 ml of a 1,6% phenolphthalein indicator solution in 50% ethanol to the milk.

(3) Titrate with 0,1N NaOH solution until the first tinge of pink appears, that persists for 30 seconds.

(4) To express the titratable acidity of the milk as the percentage of lactic acid, divide by 10 the number of milliliters of 0,1 NaOH used in the test.

Stability test with ethanol

9. Mix one volume of 68% (v/v) aqueous ethanol with one volume of milk or cream. If there are no signs of coagulation, the milk or cream shall be deemed to have passed the ethanol stability test.

Dry rehydrated film method for standard colony count

10. (1) Mix milk or cream thoroughly before sampling from bulk.

(2) Prepare a 1:10 dilution by adding 1ml to 9ml of sterile phosphate buffer. Mix well. Prepare a 1:100 dilution by adding 1ml of the 1:10 dilution to 9ml of sterile phosphate buffer. Mix well. Prepare a 1:1 000 dilution by adding 1ml of the 1:100 dilution to 9ml of sterile phosphate buffer. The final pH should be between 6,6 and 7,4.

(3) Place the films for aerobic bacterial counting on a flat surface and label them. Lift the top film and carefully transfer 1ml of the 1:1 000 dilution to the center of the bottom film by holding the pipette perpendicular to the film. Release the top film to drop onto the sample. Repeat the process with the 1:100 dilution of the sample.

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

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

(6) Remove the films from the incubator at the end of the incubation period and count the colony forming units (CFUs) with the aid of magnification under uniform artificial illumination as follows:

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

(b) An estimated count can be made on films with the CFUs exceeding 250 by counting at least four squares or 20 per cent of the growth area. Calculate the number of viable bacteria per milliliter of milk and record as an "estimated" amount.

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

Phosphate buffer

Potassium dihydrogen orthophosphate……5,08g
Disodium hydrogen orthophosphate in 2l distilled water……13,63g
Sterilize for 15 minutes at 121°C

Dry rehydrated film for standard colony count

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

Dry rehydrated film method for coliform and *Escherichia coli* count

11. (1) Mix milk thoroughly before sampling from bulk. The pH should be between 6,6 and 7,4.

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

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

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

(5) Stack the films in piles of no more than 20 and incubate the films, with the clear sides up, at 32°C +/- 1°C for 24+/-2 hours.

(6) At the end of the incubation period remove the films from the incubator and count the colonies with the aid of magnification under uniform artificial illumination as follows (Re-incubate films for a further 24+/-2 hours to detect any additional *Escherichia coli* growth):

(a) Blue colonies associated with gas are *Escherichia coli* and red colonies associated with gas are coliform colonies. Colonies that are not associated with gas are not counted as coliform colonies. All the red and blue colonies with gas represent the coliform colony count.

(b) Films with 15 - 150 colonies should be counted. An estimated count can be made on films where the colonies exceed 150 by counting at least 4 squares or 20 per cent of the growth area. Calculate the number of viable coliform colonies per milliliter of milk and report it as an "estimated" coliform colony count.

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

Dry rehydrated film for coliform and *Escherichia coli* counts

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

ANNEXURE B

PASTEURISATION

1. The pasteurization of milk shall be performed -

(a) by heating every particle of the milk to as temperature of at least 63°C (not exceeding 65,5°C) and keeping it at that temperature for at least 30 minutes, which heating shall be followed by cooling within 30 minutes to a temperature lower than 5°C (this process is referred to as the "holder method" or the "batch method"); or

(b) by heating every particle of the milk to a temperature of at least 72°C and keeping it at that temperature for at least 15 seconds, which heating shall be followed immediately by cooling to a temperature lower than 5°C (this process is hereinafter referred to as the "high-temperature short-time method"); or

(c) by any other method prescribed by regulation:

Provided that milk shall in no instance be deemed to have been pasteurized if it fails to pass the Aschaffenburg and Mullen phosphate test descried in paragraph 3 of Annexure A or any other test, provided the accuracy thereof equals that of the Aschaffenburg and Mullen phosphatase test.

2. Cream and milk or dairy products containing added sweeteners shall be pasteurized as follows:

(a) by heating every particle of the product to a temperature of at least 66°C and keeping it at this temperature for at least 30 minutes; or

(b) by eating every particle of the product to a temperature of at least 74°C and keeping it at this temperature for at least 15 seconds; or

(c) by any other method prescribed by regulation:

Provided that such product shall in no instance be deemed to have been pasteurized if it fails to pass the Aschaffenburg and Mullen phosphatase test described in paragraph 3 of Annexure A or any other test, provided the accuracy thereof equals that of the Ascaffenburg and Mullen phosphatase test.

3. The process of pasteurization, if carried out according to the high-temperature short-time method, shall be mechanically controlled with regard to the temperature range of the milk and the period for which milk is kept at the prescribed temperature, and the apparatus concerned shall be calibrated monthly to ensure the correctness of the pasteurization process.

4. Thermographic recording of temperatures of pasteurization by any method shall be made and retained for at least four weeks.

ANNEXURE C

LOCAL AUTHORITIES IN WHOSE AREAS OF JURISDICTION RAW DAIRY PRODUCTS LISTED IN REGULATIONS (1) MAY BE SOLD

Albertinia
Aliwal North
Belfast
Benoni
Bethal
Boksburg
Carolina
Centurion
Clocolan
Colesberg
De Aar
Ellisras/Marapong
Ermelo
Excelsior
Ficksburg
Great Brak River
Greater Warmbaths
Hanover
Harrismith
Jamestown
Koster
Ladismith
Ladybrand
Ladysmith
Lady Grey
Machadodorp
Malmesbury
Marquard
Mashae-Fourie
Middelburg
Nylstroom
Paul Roux
Parys
Petrusville
Petrus Steyn/Mamafubedu

Phalaborwa
Phillipstown
Pietermaritzburg-Msunduzi
Pietersburg/Polokwane
Piet Retief
Prince Albert
Reddersburg
Richmond
Senekal
Steynsrus/Matlwagtlwang
Strydenburg
Swartruggens
Volksrust
Wakkerstroom
Waterval-Boven
Wepener
West Coast
Winelands
Tzaneen
Zeerust