Subsidiary Legislation made under ss. 6, 23 and 58.

Cream (Heat Treatment) Regulations, 1987

LN.1987/157

Commencement **1.3.1988**

ARRANGEMENT OF REGULATIONS

Title and commencement.

1.(1) These regulations may be cited as the Cream (Heat Treatment) Regulations, 1987.

(2) There regulations shall come into operation on the 1st day of March, 1988

Interpretation.

2.(1) In these regulations, unless the context otherwise require–

"catering establishment" means a restaurant, canteen, club, public house, school, hospital or other establishment (including a vehicle or a fixed or mobile stall) where, in the course of a business, food is prepared for delivery to the ultimate consumer for immediate consumption;

"clotted cream" means cream which has been produced and separated by the scalding, cooling and skimming of milk or cream;

"container" includes a bottle;

"cream" means cream from cows' milk and includes cream to which permitted ingredients have been added;

"cream processor" means a person who subjects cream to heat treatment;

"permitted ingredient" means an ingredient permitted to be added to cream by regulation 5(1) or 5(2) of the Cream Regulations 1987;

"sale" includes possession for sale, offer for sale, and exposure for sale and 'sell' shall be construed accordingly;

(2) Any reference in these regulations to a numbered regulation or schedule shall, unless the reference is to a regulation of, or schedule to, specified regulations, to be construed as a reference to the regulation or schedule so numbered in these regulations

(3) Any reference in a regulation of or a schedule or a part of a schedule to these regulations to a numbered paragraph shall be construed as a reference to the paragraph so numbered in that regulation, schedule or part of a schedule (unless the reference specifies a different regulation, schedule or part of a schedule).

Exemptions.

3. These regulations shall not apply to any cream which is intended to be exported to any place outside Gibraltar.

Heat treatment of cream.

4.(1) (a) No person shall sell cream intended for human consumption unless the general requirements of Schedule 1 in connection with the heat treatment of that cream and the special requirements of-

(i) Schedule 2, Part I, in connection with such heat treatment by pasteurisation,

(ii) Schedule 2, Part II, in connection with such heat treatment by sterilisation, or

(iii) Schedule 2, Part III, in connection with such heat treatment by the ultra high temperature method are satisfied.

(b) The provisions as to sampling set out in Schedule 3, Part I, shall apply for the purposes of Schedule 2, the tests set out in Schedule 3, Parts II and III, shall apply for the purposes of Schedule 2, Part I, and the test set out in Schedule 3, Part IV, shall apply for the purposes of Schedule 2, Parts II and III.

(2) Paragraph (1) shall not apply in respect of the sale of cream for use in the preparation for human consumption of food other than cream.

Records.

5. Every cream processor shall keep accurate records of-

(a) the quantities of cream purchased by him, of milk purchased or produced by him for cream production and of cream sold and delivered by him, and

(b) the names and addresses of the person–

(i) from whom cream was purchased by him,

(ii) from whom milk was purchased by him for cream production, and

(iii) to whom cream was sold and delivered by him otherwise than by retail-and retain each such record for a period of 12 months from the date of the transaction to which it relates.

Penalties.

6. If any person contravenes or fails to comply with any provision of these regulations, he shall be guilty of an offence and shall be liable on summary conviction to a fine not exceeding £1,000.

Application of various sections of the Act

7. Sections 46(2) and (3) (which relate to prosecutions), 47(1) and (2) (which relate to evidence of analysis), 49 (which relates to the power of a court to require analysis by the Government Chemist in the United Kingdom), 50 (which relates to a contravention due to some person other than the person charged), 51(2) (which relates to the conditions under which a warranty may be pleaded as a defence) and 52 (which relates to offences in relation to warranties, and certificates of analysis) of the Act shall apply for the purposes of these regulations as if references therein to proceedings, or a prosecution, under or taken or brought under the Act included references to proceedings, or a prosecution as the case may be, taken or brought for an offence under these regulations and as if the reference in the said Section 49 to subsection (3) of Section 46 included a reference to that subsection as applied by these regulations.

SCHEDULE 1.

Regulation 4.

General requirements in connection with the heat treatment of cream.

Every cream processor shall take such measures as are adequate to ensure that any cream heat treated in accordance with these regulations shall, until such cream has been put into the containers in which it is to be supplied to consumers or to a catering establishment (and those containers have been closed so as to prevent contamination), be kept apart at all times from other cream, milk or food containing cream or milk.

SCHEDULE 2.

Regulation 4.

Special requirements in connection with the heat treatment of cream.

PART I-

PASTEURISATION.

1. The cream shall be pasteurised, that is to say it shall be treated in accordance with paragraph 2.

2.(1) Where the cream is to be pasteurised separately from the remainder of the milk of which it forms part, the cream shall be separated from the milk and shall be heated–

(a) to a temperature of not less than 63°C and retained at that temperature for not less than 30 minutes,

(b) to a temperature of not less than 72°C and retained at that temperature for not less than 15 seconds, or

(c) to such other temperature for such other period as has equivalent effect to paragraph (a) or (b) of his subparagraph in relation to the elimination of vegetative pathogenic organisms in the cream.

(2) Where the cream is to be pasteurised together with the remainder of the milk of which it forms part, the milk shall be heated in accordance with subparagraph (1)(a), (1)(b) or (l)(c) of this paragraph, following which the cream shall be separated in a hygienic manner.

3. The cream shall be cooled as soon as practicable after pasteurisation (allowance being made for such delay as is reasonable in respect of the addition of permitted ingredients, the production of clotted cream and cream of high viscosity and the whipping of cream to be sold as whipped cream) to achieve a final temperature of not more than 10°C, following which the temperature of the cream shall not rise above 10°C so long as the cream is on appropriate premises (as described in paragraph 7).

4. The whole of any apparatus in which milk or cream is pasteurised, cooled, packaged, transported or handled (or subjected to any process allowance for which is made in paragraph 3) shall be so constructed as to protect the milk or cream adequately from risk of atmospheric contamination by dust or otherwise (there being taken into account the nature of any process allowance for which is made in paragraph 3).

5. The apparatus in which the milk or cream is to be heated shall (except in respect of the production of clotted cream or pasteurisation otherwise than by a continuous-flow method) be provided with a device which shall automatically divert the flow or prevent the onward flow of any milk or cream which is not raised to the appropriate temperature required by paragraph 2.

6. Except in respect of the production of clotted cream:-

(a) indicating and recording thermometers shall be installed in suitable places in the pasteurising apparatus in order to indicate and record the temperature at which the milk or cream has been retained and to which the milk or cream is colled; and

(b) the record of those recording thermometers shall give clear readings of the minimum temperatures achieved and they shall be dated and preserved for a period of not less than 3 months.

7.(l)

(a) Cream which is pasteurised in bottles shall be supplied to the consumer or to a catering establishment in those bottles.

(b) Those bottles shall be securely closed before or during pasteurisation.

(2)

(a) Where cream is pasteurised otherwise than in bottles, the following things shall, subject to paragraph (b) of this subparagraph, be done as soon as practicable after pasteurisation (allowance being made for such delay as is reasonable in respect of the addition of permitted ingredients, the production of clotted cream and cream of high viscosity and the whipping of cream to be sold as whipped cream) at appropriate premises (that is to say the premises where pasteurisation has taken place or other premises to which the cream has been transported, after pasteurisation, under hygienic conditions in a clean and securely closed container suitable for transporting cream):-

(i) the cream shall be put into containers in which it is to be supplied to the consumer or to a catering establishment; and

(ii) those containers shall be securely closed.

(b) Paragraph (a) of this subparagraph shall not apply in relation to clotted cream sold to the consumer by a shop in a securely closed container in circumstances where, under hygienic conditions, that clotted cream has been delivered by the cream

processor to the shop in another container and transferred within the shop into the container in which it is sold to the consumer.

8. Any sample of the cream procured (after heat treatment with a view to pasteurisation) in accordance with Schedule 3, Part I, as indicated in a subparagraph of column (1) of this paragraph shall satisfy the test or tests set out in the corresponding subparagraph of column (2) of this paragraph:

(1)	(2)
(a) Sample taken not later than the day following heat treatment and before departure from premises where heat treatment took place.	(a) Whichever of- (i) the coliform test prescribed in Schedule 3, Part II, and (ii) the phosphatase test prescribed in Schedule 3, Part III is applied and, if both tests are applied, both of them.
(b) Sample taken before delivery to the consumer or to a catering establishment, in circumstances where subparagraph *(a)* of this column does not apply.	(b) The phosphatase test prescribed in Schedule 3, Part III.

PART II-

STERILISATION.

1. The cream shall be sterilised, that is to say it shall be separated from milk and heated in a hermetically sealed container in which it is to be supplied to the consumer or to a catering establishment-

(a) to a temperature of not less than 108°C and retained at that temperature for not less than 45 minutes, or

(b) to such other temperature for such other period as has equivalent effect to subparagraph (a) of this paragraph in relation to the rendering of the cream free from viable microorganisms and their spores and cooled as soon as practicable thereafter.

2. There shall be installed in suitable places in the apparatus used for sterilisation such thermometers or temperature calibrated pressure gauges as are necessary to ascertain that sterilisation has been correctly carried out.

3. Any sample of the cream procured in accordance with Schedule 3, Part I, after heat treatment with a view to sterilisation and before delivery to the consumer or to a catering establishment shall satisfy the colony count test prescribed in Schedule 3, Part IV.

PART III

ULTRA HIGH TEMPERATURE METHOD

A. Requirements applicable in all cases.

1. The cream shall be heat treated by the ultra high temperature method, that is to say it shall be separated from the milk and shall be heated-

(a) to a temperature of not less than 140°C and retained at that temperature for at least 2 seconds, or

(b) to such other temperature for such other period as has equivalent effect to subparagraph (a) of this paragraph in relation to the rendering of the cream free from viable microorganisms and their spores.

2. The apparatus in which the cream is heat treated shall be provided with a device which shall automatically divert the flow or prevent the onward flow of any cream which has not been heated to the appropriate temperature required by paragraph Al.

3.(1) Indicating and recording thermometers shall be installed in suitable places in the apparatus in which the cream is heat treated in order to record the temperature to which the cream is heated.

(2) The records of the recording thermometers shall give clear readings and they shall be dated and preserved for a period of not less than 12 months.

4.(1) Cream which is treated by the ultra high temperature method shall be put into sterile containers in which it is to he supplied to the consumer or to a catering establishment.

(2) Such containers shall be filled and securely sealed at the premises at which the heat treatment took place and the process is carried out, and in respect of that filling and sealing such aseptic precautions as are necessary to ensure the protection of the cream from risk of contamination shall be used.

5. Any sample of the cream procured in accordance with Schedule 3, Part I, after treatment with a view to use of the ultra high temperature method and before delivery to the consumer or to a catering establishment shall satisfy the colony count test prescribed in Schedule 3, Part IV.

B. Additional requirements applicable when the heat treatment of the cream is by direct application of steam.

1. Unless the cream is to be concentrated immediately after heat treatment as part of a continuous process, any treatment using direct application of steam shall be carried out in such a way as to ensure that an amount of water equivalent to that added to the cream in the form of steam shall be extracted from the cream by a process of evaporative cooling so that the percentage by weight of the total solids content of the cream shall be the same after treatment as before treatment.

2.(1) The equipment used shall be provided with control apparatus which shall ensure compliance with the requirement specified above.

(2) Before the equipment is initially used or after any operational change (that is to say any change in the site, lay-out or construction of equipment used, or any change in the steam supply or in the particular temperature used for treating the cream) the control apparatus shall be calibrated in relation to the particular temperature to be used for treating the cream so as to determine the input and output temperatures.

(3) Records of the input and output temperatures and the particular temperatures used for treating cream shall be kept with such equipment.

(4) In this paragraph and paragraph B3-

(a) 'input temperature' means the temperature of the cream immediately before the application of the steam; and

(b) 'output temperature' means the temperature of the cream at the point of leaving the evaporative cooling expansion vessel being part of the equipment in which the cream is treated.

3.(1) Indicating and recording thermometers shall be installed in suitable places in the apparatus to indicate the temperature to which the cream is heated, the input temperature and the output temperature and to record continuously the temperature to which the cream is heated and both the input and output temperatures or one of them and the difference between them.

(2) The records of the recording thermometers shall give clear readings and they shall be dated and preserved for a period of not less than 12 months.

4. The apparatus used for treatment of cream by direct steam injection shall be so constructed as to ensure that water is separated from the steam and does not enter the cream heating equipment, and so that only pure steam and the internal surfaces of the equipment come in contact with the cream.

5. The steam shall be dry and saturated and produced in such manner as shall ensure that it is wholesome and free from all impurities and there shall be automatic and continuous control to any entrained water droplets carried over from the boiler shall be separated from the steam before it enters the cream heating equipment.

6.(1) The treatment shall be carried out in such a way as to ensure that no external matter other than steam enters the cream and that there is no adulteration of the cream at any time before, during or after the heat treatment process.

(2) The steam shall be produced from water which is wholesome, free from pollution and contains no additives other than the following permitted boiler feed water treatment compounds:-

Potassium Alginate
Sodium Alginate
Potassium Carbonate
Sodium Carbonate
Sodium Hydroxide
Sodium Dihydrogen Orthophosphate
Disodium Hydrogen Orthophosphate
Trisodium Orthophosphdte
Penta Sodium Triphosphate
Sodium Polyphosphate
Tetrasodium Diphosphate
Sodium Silicate
Sodium Metasilicate
Sodium Sulphate
Magnesium Sulphate
Neutral or Alkaline Sodium Sulphite
Unmodified Starch
Sodium Aluminate
Polyoxyethylene Glycol (Minimum Molecular weight 1,000).

(3) The equipment shall be constructed so as to enable samples of the steam to be taken immediately before it is applied to cream.

SCHEDULE 3.

Regulation 4(1)(b) and Schedule 2

PART I-
PROVISIONS AS TO SAMPLING

Taking of sample

1. A sample (or a reasonable number of samples) of cream may be taken at any time before the cream has been delivered to the consumer or to a catering establishment, except that any sample to which it is proposed to apply the coliform test prescribed in Part II of this Schedule may only be taken from premises where heat treatment of the cream has taken place and not later than the day following that heat treatment.

2.(1) where the cream has been heat treated with a view to sterilisation or use of the ultra high temperature method, a sample shall consist of one sealed container of the cream.

(2) where the cream has been heat treated with a view to pasteurisation, then-

(a) if the cream is in containers not exceeding one litre capacity, a sample shall consist of one such closed container;

(b) if the cream is in containers exceeding one litre capacity-

(i) prior to the taking of the sample the cream shall be thoroughly mixed,

(ii) a sample (consisting of no less than 20g) shall be taken from well below the surface of the cream,

(iii) the instruments used for mixing and sampling shall be sterile,

(iv) the sample shall be transferred as soon as possible after it is taken into a sterile bottle which shall be immediately closed,

(v) the part of the stopper of the sterile bottle which comes into contact with the cream shall be sterile, and

(d) testing shall commence not later than the morning after the day of arrival of the sample at the testing laboratory.

5. In the case of cream heat treated with a view to sterilisation or use of the ultra high temperature method, the sample shall be delivered intact to the testing laboratory.

PART II

THE COLIFORM TEST FOR PASTEURISED CREAM

A. Preparation of dilution of sample for coliform test

Apparatus

1.The apparatus shall consist of-

(a) a supply of 10 ml straight sided pipettes and

(b) a supply of dilution tubes or flasks with rubber stoppers to fit or tightly fitting covers.

Diluent

2.The diluent shall be 2.0 per cent by weight aqueous sodium citrate solution.

Method

3.(1) A 1-in-10 dilution shall be made by placing the cream sample intact into a water bath maintained at a temperature of not less than 35°C and not more than 40°C for a period of not less than 20 minutes and not more than 30 minutes.

(2) The sample shall be well mixed and l0±0.1g weighed into a dilution flask containing not less than 89 ml and not more than 91 ml of 2.0 per cent by weight sodium citrate solution at a temperature of not less than 35°C and not more than 40°C and the contents thoroughly mixed.

B. The test

Apparatus and sterility requirement

1. (1) The apparatus to be used shall be-

(a) a supply of 10 ml straight sided pipettes of an accuracy equal to that of NPL grade B, and

(b) culture medium tubes which comply with British Standard 3218:1982 or 625:1959, norminal size 150/16 (each culture medium tube containing an inverted Durham tube conforming to British Standard 3218:1982 or 625:1959,35/8).

(2) The medium, diluent and all glass-ware, stoppers, covers and caps shall be sterile.

Culture Medium

2.(1)

(a) The culture medium shall be Double Strength Brilliant Green Lactose Bile Broth (BGLBB) prepared according to the following formula:-

Peptone 20 g
Lactose 20 g
Bile Salts 40 g
Brilliant Green 0.0266 g
Distilled or de-ionised water- 1,000 ml.

(b)

(i) Because of difficulties in standardising the bile salts and brilliant green a complete dehydrated medium should be purchased.

(ii) The solid components should be added to 1 litre of distilled or de-ionised water in accordance with the manufacturer's instructions.

(iii) The pH should be adjusted if necessary so that after sterilisation it is approximately 7.4.

(iv) The broth should be mixed well, distributed in 10 ml amounts into the culture tubes fitted with Durham tubes and sterilised at 121°C for 15 minutes.

(2) The medium shall have a final pH of 7.4 approximately.

(3) Other media may be used provided that they give similar results.

Method

3.(1) After mixing, a 10 ml portion of a 1-in-l0 dilution of the cream shall be transferred using a pipette to each of 3 culture tubes containing about 10 ml of culture medium.

(2) The culture tubes shall be incubated at 30°C±1°C for 48±2 hours and examined for gas production.

Interpretation

4.The sample shall be regarded as satisfactory if 2 out of 3 tubes are found to be free from gas after incubation at 30°C±1°C for 48±2 hours.

PART III-

THE PHOSPHATASE TEST FOR PASTEURISED CREAM

Precautions

1.The following precautions shall be taken:-

(a) a sample which shows evidence of taint or souring shall not be tested;

(b) all glassware shall be clean immediately before use;

(c) a fresh pipette shall be used for each sample of cream;

(d) the test shall not be carried out in direct sunlight; and

(e) distilled or de-ionised water shall be used throughout.

Apparatus

2.The apparatus to be used shall be-

(a) a Lovibond all purposes comparator complete with stand for work in reflected light;

(b) a Lovibond comparator disc A.P.T.W. or A.P.T.W.7;

(c) two fused glass cells, 25 mm depth;

(d) a water bath or incubator maintained at 37°C±0.5°C;

(e) a supply of pipettes suitable to deliver 15 ml;

(f) a supply of 1.0 ml straight-sided pipettes of any accuracy equal to that of N.P.L. grade B;

(g) a 1,000 ml graduated flask;

(h) a 100 ml measuring cylinder;

(i) a supply of test tubes conforming to British Standard 3218:1982, nominal size 150/16, with rubber stoppers to fit;

(j) glass filter funnels; and

(k) Whatman number 40 filter apers.

Reagents

3. Whenever possible, reagents of analytical quality shall be used.

4.The buffer-substrate solution shall be prepared as follows:-

(a) buffer solution: 3.5 g of anhydrous sodium carbonate and 1.5 g of sodium bicarbonate shall be dissolved in distilled or de-ionised water, and made up to one litre;

(b) substrate: disodium p-nitrophenyl phosphate (the solid substrate being kept in a refrigerator);

(c) buffer-substrate solution:-

(i) 0. 15g of the substrate shall be placed in a 100 ml measuring cylinder, and made up to 100 ml with the buffer solution and mixed;

(ii) the buffer-substrate solution shall be stored in a refrigerator and protected from light;

(iii) the buffer-substrate solution shall give a reading of less than the standard marked 10 on the comparator disc A.P.T.W. or A.P.T.W.7 when viewed in transmitted light through a 25 mm cell in the "all purposes comparator, distilled or de-ionised water being used for comparison;

(iv) the buffer-substrate solution shall not be used for more than one week.

5.Other solutions required are-

(a) 30% weight/volume aqueous solution of zinc sulphate,

(b) 15% weight/volume aqueous solution of potassium ferrocyanide, and (if necessary for subsequent testing)

(c) 40% weight/volume solution of magnesium chloride in distilled or de-ionised water.

Care of apparatus

6.(1) New glassware shall be cleaned and free from contamination from substances which may interfere with the test.

(2) After use, each test tube shall be emptied, rinsed in water, well washed in hot water containing soda, rinsed in warm water, rinsed in distilled or de-ionised water and finally air dried.

(3) If after treatment in accordance with subparagraph (2) of this paragraph a test tube does not appear to be clean, the treatment shall be repeated with the addition that after being rinsed in warm water it shall be soaked in 50 per cent commercial hydrochloric acid and then rinsed again in warm water before being rinsed in distilled or de-ionised water and finally dried.

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

Method

7.(1) 15 ml of the buffer-substrate solution shall be transferred to a test tube using a pipette and the test tube shall be stoppered and brought to a temperature of 37°C±10.5°C.

(2) 2 g of the cream to be tested shall be added, the test tube stopper replaced and the contents well mixed by shaking.

(3) The tube shall then be incubated for 120 minutes at 37°C±0.5°C.

(4) One blank prepared from boiled cream of the same type as the sample or series of samples undergoing the test shall be incubated with each sample or series of samples.

(5) After incubation the test tube shall be removed from the water bath and its contents shall be well mixed.

(6) 0.5 ml of the zinc sulphate solution shall be added to each test tube.

(7) The stopper shall then be replaced and the tubes shaken

vigorously then left to stand for 3 minutes.

(8) 0.5 ml of potassium ferrocyanide solution shall be added to each test tube and mixed thoroughly.

(9) The contents of each test shall then be filtered through Whatman No.30 filter paper and the clear filtrate of each collected in a clean test tube.

(10) The blank shall be placed on the left hand ramp of the stand and the test sample on the right.

(11) Readings shall be taken in reflected light by looking down on to the two apertures with the comparator facing a good source of daylight (preferably north light).

(12) If artificial light is needed for matching, a "daylight" type of illumination must be used.

(13) The disc shall be revolved until the test sample is matched.

(14) Readings falling between two standards shall be recorded by affixing a plus or minus sign to the figure for the nearest standard.

Interpretation and consideration of re-activation

8.The test shall be deemed to be satisfied by cream which gives a reading of 10 µg or less of p-nitrophenol/ml of cream.

9.Where the sample gives a reaction of above 10 µg further examination of the same product sample shall be carried out as follows:

(a) there shall be transferred into each of two clean test tubes log of cream.

(b) there shall be added to one tube (the control) nothing and to the other the following quantities of magnesium chloride solution according to the butterfat content as illustrated in the following table.

Fat% of cream	ml of magnesium chloride to l0g of sample
48	0.25
35	0.35
18	0.50

other Percentages by extrapolation;

(c) the test tubes shall be Stoppered, and the contents mixed by inversion and incubated at 3°C±0.5°C for 60 minutes

(d) the test tubes shall be inverted Occasionally during incubation;

(e) both test tubes shall be removed and 2.0g±0.1 g shall be transferred from each to two clean test tubes;

(f) the test shall proceed as outlined in the method detailed in Paragraph 7.

10. If the intensity of the colour of the filtrate from the tube with magnesium is higher than the control, then the following procedure shall be used:

(a) the filtrate shall be diluted with buffer solution 1 in 4 and again compared with the filtrate of the control;

(b) if the colour is equal to or more intense than that of the undiluted control, the original Positive Phosphatase result shall be declared void as reactivation has taken place and the original test shall be deemed to have been Satisfied, but otherwise the original Positive Phosphatase result shall stand.

PART IV

THE COLONY COUNT TEST FOR STERILISED CREAM AND CREAM HEAT TREATED BY THE ULTRA HIGH TEMPERATURE METHOD

Apparatus and sterility requirement

1.(1) The following apparatus shall be used:-

(a) McCartney bottles of 28 ml capacity;

(b) test tubes plugged with cotton wool or covered with closely fitting aluminium caps or stored in such a way as to prevent contamination;

(c) a standardised loop to transfer about 0.01 ml of cream to the molten medium in a tube or a McCartney bottle;

(d) an incubator operating at 37°C and maintained within ± 1°C;

(e) a water bath capable of maintaining the water at a temperature of not less than 45°C and not more than 50°C; and

(f) a refrigerator fitted with a reliable automatic thermoregulator capable of maintaining a temperature of between 3°C and 5°C

(2) The medium and all glass-ware, stoppers, covers and caps shall be sterile.

Culture medium

2. The culture medium to be used shall be prepared as follows:-

(a) yeastrel milk agar shall be made from the constituents listed below:-

Yeastrel	3g
Peptone	5g
Agar	15 g
(If New Zealand agar is used 12 g is normally sufficient)	
Skimmed milk powder (antibiotic free)	1 g
Distilled or de-ionised water	1,000 ml;

(b) (i) the yeastrel and peptone shall be dissolved in the distilled or de-ionised water in a steamer and the reaction at room temperature adjusted to pH 7.4 using phenol red as an indicator or using a pH meter;

(ii) when phenol red is used a brightness screen must be employed with Lovibond phenol red disc 2/IJ;

(iii) the agar and the milk shall then be added to the broth and autoclaved at 121°C for 25 minutes;

(iv) if shredded agar is used, it shall be wrapped in muslin and washed in running water for 15 minutes, the excess water being squeezed out before the agar is added to the broth;

(v) to ensure thorough mixing and that heat treatment of the bulk at this stage is equivalent to the final sterilisation of the tubed medium, quantities of not more than 2 litres shall be autoclaved in 3-litre conical flasks;

(vi) the hot medium shall then be filtered through paper pulp in a Buchner funnel;

(c) (i) the pulp shall be prepared by mashing up small pieces of filter paper in water and boiling;

(ii) the funnel shall be inserted into an Erlenmeyer flask fitted with a side piece and a single layer of filter paper laid on the top of the Buchner funnel to prevent the pulp being sucked through;

(iii) the hot pulp shall be poured on to the filter paper and a filter pump applied to suck through the excess water, which shall then be poured away;

(iv) the pulp should be firmly packed down just before the last of the water is sucked through, at which stage a layer of filter paper shall be laid on the filter bed, so that the hot medium can subsequently be poured on to it without disturbing the pulp;

(v) the filter when ready for use should have a total depth of about 1.5 mm;

(vi) it should be assumed that a pulp layer of suitable and approximately the same depth for any size of funnel is obtained by pulping an area of filter paper equal to four times the square of the diameter of the funnel, and that with ordinary grade filter paper 1g of the dry paper will be required for every 20 sq cm of filtering area;

(d)

(i) the flask and funnel shall be thoroughly hot before filtering commences and these and the medium shall be kept hot during filtering;

(ii) the medium shall be taken direct from the autoclave, poured on to the pulp where the filter paper is laid and the vacuum pump connected;

(e) (i) the reaction of the filtrate shall be tested at 50°C and adjusted if necessary to pH 7.0;

(ii) adjustment at this stage should not normally be necessary, and if it is needed at all frequently, the method of preparation should be checked;

(f) the medium shall be distributed in 5 ml quantities in test tubes conforming to British Standard 3218:1982 or 625:1959, nominal size 150/16, or in 28 ml McCartney bottles, and autoclaved at 121°C for 15 minutes;

(g) the final reaction of the medium at room temperature shall be pH7.2.

Other media available

3.(1) A prepared or dehydrated medium may be used in place of the medium described in paragraph 2 provided that it has been shown to give similar results to the medium described in paragraph 2.

(2) The medium described below may be used in place of the medium described in paragraph 2:-

(a) the medium should be constituted as follows:-

Yeast extract	2.5 g
Tryptone	5.0 g
Glucose	1.0 g
Skimmed milk powder (antibiotic free)	1.0 g
Agar (depending upon gel strength)	10-l5 g
Distilled or de-ionised water	1,000 ml;

(b) the pH after sterilisation should be 6.9±01 at 30°C;

(c) in all cases it is necessary to add skimmed milk powder even if the supplier considers such an addition unnecessary;

(d) the solid components should be added to 1 litre of distilled or de-ionised water in accordance with the manufacturers' instructions;

(e) the pH should be adjusted if necessary so that after sterilisation it is 6.9±0.1;

(f) the medium should be mixed well and distributed in 5 ml amounts in the tubes or Mccartney bottles and sterilised at 121°C for 15 minutes.

Incubation of sample

4. On arrival at the laboratory the sample shall be placed unopened in the incubator at a temperature of 37°C ±1°C and retained at that temperature for 24 hours.

Mixing of sample prior to examination

5. At the end of the 24-hour incubation period, the sample shall be removed from the incubator and shall be mixed thoroughly by inverting the container and shaking it.

Method of carrying out the test

6 (1) After the sample has been thoroughly mixed as described above, it shall be opened with aseptic precautions.

(2) (a) Immediately after opening the sample container, the cap from a sterile McCartney bottle shall be removed and approximately 10 ml of the sample transferred by means of a sterile pipette to the bottle, the cap replaced and the McCartney bottle put in the refrigerator.

(b) A further 10 ml (approximately) of the sample shall be transferred to a sterile test tube after removing the plug.

(c) The plug shall then be replaced.

(3) (a) With as little delay as practicable a loopful of cream from the test tube sample shall be transferred to a sterile test tube or 28 ml McCartney bottle containing about 5 ml of melted medium at 45°C to 50°C.

(b) The loop, after being flame sterilised and cooled, shall be lowered into the cream about 25 mm below the surface and a loopful of cream withdrawn and transferred to the molten medium in the tube or McCartney bottle.

(c) The contents of the tube or bottle shall then be carefully mixed, the tube or bottle placed in a sloping position (the medium being at least 12 mm from the closure) and the medium allowed to set;

(d) The tube or bottle shall then be incubated in a sloping position at a temperature of 37°C±1°C for 48 hours and at the end of that time it shall be examined for the presence of colonies

Counting of colonies

7. Colonies shall be counted within 4 hours of the expiry of the incubation period.

Interpretation

8.(1) The test shall be deemed to be satisfied by a sample if the number of colonies is found to be less than 10.

(2) If there is any doubt about the result, the test should be repeated using the sample in the McCartney bottle placed in the refrigerator.