

SCHEDULE 6

TESTING OF SAMPLES

Part I

THE PLATE COUNT TEST FOR RAW MILK, BULK MILK HEAT TREATED FOR TRANSPORTATION, PASTEURISED MILK, STERILISED MILK AND ULTRA HEAT TREATED MILK

General

1. In testing samples of milk for bacterial count the following paragraphs of this Part of this Schedule shall be complied with.

Treatment of sample before testing

2. On arrival at the testing laboratory samples of milk shall be treated according to the following provisions.

- (a) Any samples of raw milk, bulk milk heat treated for transportation or “Pasteurised” milk taken at the heat treatment establishment and intended for immediate testing. All samples of milk shall be examined as soon as possible after arrival at the testing laboratory. If a sample is not examined immediately it shall be kept at a temperature not higher than 5degC. until examined, provided that any sample on which the test is not begun within 24 hours of taking such sample shall be discarded.
- (b) Any sample of “Pasteurised” milk taken at the heat treatment establishment and intended for incubation before testing. On arrival at the testing laboratory the sample shall be placed unopened in an incubator at a temperature of 6degC. +/-0.5degC. and shall be retained at that temperature for a period of 5 days, provided that, where these incubation requirements are not begun immediately after the arrival of the sample at the laboratory, the sample shall be kept at a temperature not higher than 5degC. until the start of the prescribed incubation period. Any sample on which the incubation requirements are not begun within 24 hours of taking such sample shall be discarded.
- (c) Any sample of “Sterilised” or “Ultra Heat Treated” milk taken at the heat treatment establishment. On arrival at the testing laboratory the sample shall be placed unopened in an incubator at a temperature of 30degC. +/-0.5degC. and shall be retained at that temperature for a period of 15 days.
- (d) Any sample of “Ultra Heat Treated” milk taken after it leaves the heat treatment establishment and before delivery to the consumer. On arrival at the testing laboratory the sample shall be placed unopened in an incubator at a temperature of 30degC. +/-0.5degC. and shall be retained at that temperature for a period of 24 hours.

Culture medium

3. Bacterial count. The medium to be used shall be yeastrel milk agar, either compounded in the laboratory or prepared in accordance with the manufacturer’s directions from a granular desiccated medium and shall have the following composition:—

Yeastrel	3.0 grams
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Peptone	5.0 grams
Agar	15.0 grams
Milk (fresh or spray-dried, skim or whole milk)	10.0 ml.
Distilled water	to 1 litre

Where the medium is compounded in the laboratory it shall be filtered through a pulp-paper filter.

The medium shall be sterilised either by autoclaving at 121degC. for 20 minutes or in a steamer for 30 minutes on three successive days.

The final reaction of the medium at room temperature shall be pH 7.2.

Dilutions

4. Quarter-strength Ringer's solution shall be used. The composition of full-strength Ringer's solution shall be —

Sodium chloride	9.00 grams
Potassium chloride	0.42 grams
Anhydrous calcium chloride	0.24 grams
Sodium bicarbonate	0.20 grams
Distilled water	1,000 ml.

Add 1 part of the above solution to 3 parts of distilled water. Fill into test tubes or bottles and sterilise by autoclaving at 121°deg. for 15 minutes. The quantity to be filled into the container before sterilisation must be predetermined to allow for evaporation losses during sterilisation.

Alternatively, sterile tubes and bottles may be filled aseptically with measured quantities of sterile quarter-strength Ringer's solution.

Technique of tests

5.—(1) The sample shall be mixed thoroughly by shaking and the container opened with aseptic precautions. For the colony plate count the dilutions specified in sub-paragraphs (2) to (5) below shall be used. For each sample being tested not less than two plates shall be prepared. The Petri plates shall be incubated as specified in sub-paragraphs (2) to (5) below.

(2) Any sample of raw milk, bulk milk heat treated for transportation or "Pasteurised" milk taken at the heat treatment establishment and intended for immediate testing. For raw milk and milk which has been heat treated elsewhere, 1 ml. of 1 in 1,000 dilution shall be plated. For milk pasteurised on the premises, 1 ml. of 1 in 100 dilution shall be plated. All plates shall be incubated at 30degC. +/-0.5degC. for 72 hours.

(3) Any sample of "Pasteurised" milk taken at the heat treatment establishment and intended for incubation before testing. 1 ml. of 1 in 1,000 dilution shall be plated and the plates incubated at 21degC. +/-0.5degC. for 25 hours.

(4) Any sample of "Sterilised" or "Ultra Heat Treated" milk taken at the heat treatment establishment. 1 ml., undiluted, shall be plated and the plates incubated at 30degC. +/-0.5degC. for 48 hours.

(5) Any sample of “Ultra Heat Treated” milk taken after it leaves the heat treatment establishment and before delivery to the consumer. 1 ml. of 1 in 10 dilution shall be plated and the plates incubated at 30degC. +/-0.5degC. for 48 hours.

Examination of cultures

6. All colonies (including “pin-point” colonies) on each plate shall be counted and the arithmetic mean count obtained. To facilitate counting it is desirable to use a counting chamber, a suitable lens and a tally counter. The result of the count shall be recorded as the number of bacteria per ml. and shall be to the nearest 10, 100 or 1,000 according to whether a dilution of 1 in 10, 1 in 100 or 1 in 1,000 has been used.

General precautions

7.—(1) The sterility of the media and apparatus shall be tested by carrying out a blank test using sterile water in place of milk when each batch of samples is examined.

(2) Before the dilutions are prepared, the milk shall be thoroughly mixed by inverting the bottle 25 times.

(3) Each dilution shall be thoroughly mixed without vigorous shaking.

(4) In the preparation of the dilutions a separate sterile pipette shall be used for each dilution and for transferring the dilution to the Petri plate or bile salt broth.

(5) The pipettes shall be straight-sided, of the “blow-out” type and plugged with cotton wool.

(6) Not more than 15 minutes shall elapse between the dilution of the milk and its admixture with the agar medium or bile salt broth.

(7) The melted agar shall be cooled to 45degC. before it is poured into the Petri plates.

(8) If Petri plates are stacked in the incubator, so far as possible no stack shall consist of more than six Petri plates.

(9) The temperature of the incubator shall be frequently checked by means of a thermometer conforming to the British Standards Institution’s specification and adjusted if necessary.

(10) Bile salt broth tubes showing any air in the Durham tube shall not be used to carry out the test.

(11) Distilled water—water prepared with a glass still or water of equal quality shall be used.