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OFFICIAL METHOD NOTIFICATION

issued under regulation 3 of the Food and Drugs Regulations

The following method of analysis or examination of Ultra Heat Treated Milk or U.H.T. for Colony Count has been designated by the Minister as the official method:

OFFICIAL METHOD OF ANALYSIS OR EXAMINATION OF ULTRA HEAT TREATED MILK OR U. H. T. MILK FOR COLONY COUNT

1. Apparatus: The following apparatus shall be used:

(a) McCartney bottles of 1 fluid ounce 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 standard iridium-platinum loop of 4 mm. internal diameter made from wire conforming to British Standard Wire Gauge 19 and containing 10 per cent iridium. The loop, when used as directed, should transfer about 0.01 ml. of milk to the molten medium in a tube or a McCartney bottle;

(d) an incubator capable of operating at a preselected temperature within the range 30°C. to 37°C. and of maintaining the preselected temperature 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 thermo-regulator capable of maintaining a temperature of between 3°C. and 5°C.

2. Culture Medium: A culture medium prepared as follows should be used:

(a)

Yeastrel	3g.
peptone	5g.
agar	15g.

(If New Zealand agar is used 12g. is normally sufficient).

Fresh whole milk	10 ml.
Distilled water	1,000 ml.

(b) the Yeastrel and peptone shall be dissolved in the distilled 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. When phenol red is used, a brightness screen must be employed with Lovibond phenol red disc 2/IJ. The agar and the milk shall then be added to the broth and autoclaved at 121°C. for 25 minutes. 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. 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. The hot medium shall then be filtered through paper pulp in a Buchner funnel;

(c) the pulp shall be prepared by mashing up small pieces of filter paper in water and boiling. 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. The hot pulp shall then be poured on to the filter paper and a filter pump applied to suck through the excess water, which shall then be poured away. The pulp should be firmly packed down just before the last of the water is sucked through. At this 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. The filter when ready for use should have a total depth of about 1.5 mm. (A pulp layer of suitable depth 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. With ordinary grade filter paper 1 g. of the dry paper will be required for every 20 sq. cm. of filtering area);

(d) the flask and funnel shall be thoroughly hot before filtering commences and these and the medium shall be kept hot during filtering. 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) the reaction of the filtrate shall be tested at 50°C. and adjusted if necessary to pH 7.0. 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 6 x 5/8 in. test-tubes or in 1 ounce McCartney bottles and autoclaved at 121°C. for 15 minutes; and

(g) the final reaction of the medium at room temperature shall be pH 7.2.

3. Alternative Medium: A dehydrated medium may be used provided that on reconstitution with distilled water and fresh milk it has the same composition as that given in paragraph *2(a)* and has been shown to give similar results.

4. Sampling: A sample consisting of at least one aseptically sealed container shall be taken from each batch of U.H.T. Milk and delivered unopened to the testing laboratory.

5. Incubation of sample: On arrival at the laboratory the sample shall be placed unopened in the incubator at a temperature of between 30°C. and 37°C. and retained at that temperature for twenty-four hours.

6. Mixing of sample prior to examination: At the end of the twenty-four hour incubation period, the sample shall be removed from the incubator and shall be mixed thoroughly by inverting the container and shaking it.

7. Method of carrying out the test:

(a) After the sample has been thoroughly mixed as described in paragraph 6, it shall be opened with aseptic precautions as follows:

(i) if the sample is contained in a carton, one of the corners or edges of the carton shall be thoroughly swabbed with alcohol and the excess burnt off. The carton shall then be opened by cutting off this corner or edge using a pair of sterile scissors;

(ii) if the sample is contained in a bottle, the closure and neck of the bottle shall be thoroughly swabbed with alcohol and the excess burnt off. The closure shall then be removed by means of a sterile opener;

(iii) if the sample is a container other than a carton or bottle a suitable surface of the container shall be thoroughly swabbed with alcohol and the excess burnt off. A hole in that sterile surface shall then be punched using a sterile tool.

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

(c) With as little delay as possible, a loopful of milk from the test-tube sample shall be transferred to a sterile test-tube or a 1 ounce McCartney bottle containing about 5 ml. of melted yeastrel milk agar medium at 45°C. to 50°C. The loop, after being flame-sterilised and cooled, shall be lowered into the milk about 1 inch below the

surface and a loopful of milk withdrawn and transferred to the molten medium in the tube or McCartney bottle. The contents of the tube or bottle shall then be carefully mixed, the tube or bottle placed in a sloping position and the medium allowed to set. The tube or bottle shall then be incubated in a sloping position at a temperature of between 30°C. and 37°C. for forty-eight hours and at the end of that time it shall be examined for the presence of colonies.

8. Counting of colonies: Colonies shall be counted within four hours of the expiry of the incubation period.

9. Interpretation: The test shall be deemed to be satisfied by a sample if the number of colonies is found to be less than 10. If there is any doubt about the result, the test should be repeated using the sample in the McCartney bottle placed in the refrigerator.