

SCHEDULE 3

PART V

the phosphatase test for pasteurised semi-skimmed milk and skimmed milk

Examination of sample

1. The sample of milk shall be examined as soon as possible after arrival at the testing laboratory. If it is not examined immediately on arrival at the testing laboratory, it shall be kept at a temperature of between 3°C. and 5°C. until examined. The sample shall be raised to room temperature immediately before being tested.

Precautions

2. 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 milk.
 - (d) The test shall not be carried out in direct sunlight.
 - (e) Distilled or de-ionised water shall be used throughout.

Reagents

- 3.—(1) Whenever possible, reagents of analytical quality shall be used.
- (2) 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 shall be kept in a refrigerator.
 - (c) Buffer-substrate solution: 0.15 g. of the substrate shall be placed in a 100 ml. measuring cylinder, and made up to 100 ml. with the buffer solution. The solution shall be stored in a refrigerator and protected from light. It 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. The solution shall not be used for more than one week.

Apparatus

4. The following apparatus shall be used:
- (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 capable of being maintained at 37.5°C. \pm 0.5°C.
 - (e) A pipette to deliver 5.0 ml.
 - (f) A supply of 1.0 ml. straight-sided pipettes of an accuracy equal to that of N.P.L. grade B.
 - (g) A 1,000 ml. graduated flask.

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- (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.

Care of apparatus

5.—(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 dried.

(3) If after treatment in accordance with sub-paragraph (2) above, 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 of carrying out the test

6. 5 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. 1 ml. of the milk to be tested shall be added, the test tube stopper replaced and the contents well mixed by shaking. The test tube shall then be incubated for exactly 2 hours at 37°C. One blank prepared from boiled milk of the same type as those undergoing the test shall be incubated with each series of samples. (Where the sample consists of highly coloured milk, such as homogenised milk or milk from Channel Island cows, a separate blank of such milk shall be prepared). After incubation the test tube shall be removed from the water bath and its contents shall be well mixed. The blank shall be placed on the left hand ramp of the stand and the test sample on the right. 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). If artificial light is needed for matching, a 'daylight' type of illumination must be used. The disc shall be revolved until the test sample is matched. Readings falling between two standards shall be recorded by affixing a plus or minus sign to the figure for the nearest standard.