Bangladesh Standard SPECIFICATION FOR PASTEURIZED LOW FAT MILK AND STANDARDIZED MILK

(Draft for First Revision)

1. SCOPE

1.1 This standard prescribes the requirements and the method of sampling and tests for pasteurized low fat milk and standardized milk.

2. REFERENCES

2.1 The Bangladesh Standards listed in Annex A is necessary adjuncts to this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

3. TERMINOLOGY

- 3.1 For the purpose of the specification the following definitions shall apply:
- **3.2 Milk** Milk is the normal lacteal secretion derived from complete milking of healthy animal without either addition of thereto or extraction therefrom. It shall be free from colostrum and obtained by the complete milking of one or more healthy animals (cow, buffalo, sheep or goat).
- **3.3 Whole milk/Full cream milk** Whole milk/Full cream milk means any of the milk mentioned under section **3.2** or adding water¹ in Whole milk powder/Full cream milk powder which obtain through spray-drying process (comply with the requirements of BDS 860). In both cases, minimum milk fat and milk solids-not-fat content are 3.5 and 8.0 respectively.
- **3.4 Medium fat milk –** Medium fat milk which obtain from milk (see section **3.2**) having milk fat minimum 2.0 % (m/m) and not more than 3.4 % (m/m).
- 3.5 Low fat milk Low fat milk which obtain from milk (see section 3.2) having milk fat minimum 0.5 % (m/m) and not more than 1.9 % (m/m).
- **3.6** Fat free/skimmed milk Fat free/skimmed milk which obtain from milk (see section **3.2**) having milk fat less than 0.5 % (m/m).
- **3.7 Skimmed milk powder –** A product obtained by the removal of water only from skimmed milk (see section **3.6**) of cow, buffalo, sheep or goat (section **3.2**) through spray-drying process and comply with the requirements of BDS 860.
- **3.8** Reconstituted milk Milk prepared by dispersing whole milk powder/full cream milk powder (comply with the requirements of BDS 860) in water¹ in the amount necessary to re-establish the appropriate water-to-solids ratio only to achieve similar characteristics and appropriate compositional requirements of medium fat milk or low fat milk or fat free/skimmed milk (see section **3.4**, **3.5**, and **3.6** respectively).

¹Water means drinking water, which obtain from a treatment process and conform BDS 1240

- **3.9 Standardized milk** Standardized milk is the milk which fat and/or solids-not-fat (SNF) content have been adjusted to a certain pre-determined level. The standardization can be done by partially skimming of fat in the milk (see section **3.2**) with a cream separator, or by admixture with fresh or reconstituted skimmed milk/reconstituted whole milk in proper proportions to adjust prescribed milk fat and SNF content. Fat content of standardize milk shall be minimum 1.9 % (m/m) but not more than 3.4 % (m/m).
- **3.10** Toned milk Milk obtained by adding water¹ and skimmed milk powder (see section **3.5**) to whole milk. Fat content of toned milk shall be more than 2.0 % (m/m) but less than 3.5 % (m/m).
- **3.11 Pasteurized medium fat milk –** Pasteurized medium fat milk is the milk obtain through pasteurization (see section **4**) of medium fat milk (see section **3.4**) and fulfilled the requirements described in section **5** of this standard.
- **3.12** Pasteurized low fat milk Pasteurized low fat milk is the milk obtain through pasteurization (see section 4) of low fat milk (see section 3.5) and fulfilled the requirements described in section 5 of this standard.
- **3.13** Pasteurized fat free/skimmed milk Pasteurized fat free/skimmed milk is the milk obtain through pasteurization (see section 4) of fat free/skimmed milk (see section 3.6) and fulfilled the requirements described in section 5 of this standard.
- **3.14** Pasteurized Reconstituted milk Pasteurized reconstituted milk is the milk obtain through pasteurization (see section 4) of reconstituted milk (see section 3.8) and fulfilled the requirements described in section 5 of this standard.
- **3.15** Pasteurized standardized milk Pasteurized standardized milk is the milk obtain through pasteurization (see section 4) of Standardize milk (see section 3.9) and fulfilled the requirements described in section 5 of this standard.
- **3.16** Pasteurized Toned milk Pasteurized toned milk is the milk obtain through pasteurization (see section 4) of toned milk (see section 3.10) and fulfilled the requirements described in section 5 of this standard.
- **4** Pasteurization It shall refer to:
 - a) the process of heating every particle of milk at a specified temperature;
 - b) holding the milk at such temperature continuously for a specified period in standard and properly operate of equipment, and
 - c) immediate cooling in the same equipment after heating.
- **4.1 Holding or Batch method** Milk is heated to at least 65 °C and held continuously at that temperature for at least 30 minutes and then immediately cooled in the same equipment to 4.4 °C or less to prevent the growth of the souring organism (largely proteolytic spore formers).
- **4.2 High Temperature Short Time (HTST) Method** In this method milk is heated at least 75 °C and held continuously at that temperature for at least 16 seconds and then immediately cooled in this equipment to 4.4 °C or less.
- **4.3 Flash pasteurization** The temperature of milk shall be raised to not less than 80 °C and retained at this temperature for at least 10 s and immediately and rapidly cooled to 10 °C or less. ¹Water means drinking water, which obtain from a treatment process and conform BDS 1240

5. REQUIREMENTS

Physical, chemical and microbiological requirements of milk described in section 3.11 to 3.16 are as follows:

- **5.1 Description –** Milk (see section **3.11** to **3.16**) shall have white yellowish opaque colour with fresh sweetish characteristic flavour of liquid milk. It shall be free from visible dirt and extraneous matter. It shall not contain whey powder, preservatives, detergent, or any other added substances except water¹ only for pasteurized reconstituted milk.
- **5.1.1** All products shall be free from any kind of foreign fat (other than milk fat) and solids. Milk fat in any form like, cream, butter, butter oil, ghee etc. are not allowed to add in milk for standardization and reconstitution purpose.
- **5.2 Hygienic condition –** The material shall be prepared in the premises maintained in accordance with BDS 822.
- **5.3 Legal requirement –** The product shall also comply in all other aspects with the requirements of the legislations enforced in the country.
- 5.4 The material shall also comply with the requirements given in Table 1

TABLE 1: REQUIREMENT FOR PASTEURIZED LOW FAT MILK AND STANDARDIZED MILK (Clause 3.11 – 3.16)

SI No.	Characteristic	Requirement	Method of Test
(1)	(2)	(3)	(4)
(i)	Milk Fat, %, (m/m), a) pasteurized medium fat milk	>2.0 - ≤3.4	ISO 2446
	b) pasteurized low fat milk	>0.5 - < 2.0	
	c) pasteurized fat free/skimmed milk	Max. 0.5	
	d) pasteurized standardized milk	1.9 - 3.4	
	e) pasteurized reconstituted milk:		
	(e-i) pasteurized reconstituted medium fat milk	(e-i) >2.0 - ≤3.4	
	(e-ii) pasteurized reconstituted low fat milk	(e-ii) >0.5 - < 2.0	
	(e-iii) pasteurized reconstituted fat free/skimmed milk	(e-iii) Max. 0.5	
	f) pasteurized toned milk	>2.0 - <3.5	
(ii)	Solids-not-fat (SNF), percent by mass, Min.	8.0	ISO 6731
		9.0*	
(iii)	Density, g/ml at 15.5 °C	1.028-1.036	See NOTE- 1
(iv)	Lactose, percent by mass, Min.	4.4	ISO 5548
(v)	Protein, percent by mass, Min.	3.0	ISO 8968-1
(vi)	Titratable acidity (as Lactic acid per 100 ml of milk), Max.	0.18	ISO TS 22113:2012
(vii)	Total plate count, CFU per ml, Max.	30,000	ISO 4833-1
(viii)	Total coliform count, CFU per ml. Less than	10	Annex B
(ix)	Phosphatase test	Negative	Annex C
(x)	Alcohol test	Negative	Annex D

^{*} Solids-not-fat (SNF), percent by mass, Min. subject to pasteurized toned milk only

NOTE -1: Density of normal milk may be determined by hydrometer (lacto-meter)

¹Water means drinking water, which obtain from a treatment process and conform BDS 1240

5.5 Pesticides and veterinary drug residues, heavy metal and Aflatoxin M₁

5.5.1 The product may comply with the requirements described in Bangladesh Food Safety Authority's Regulation. This characteristic shall be tested only if considered as necessary.

6. Packing and Marking

- **6.1 Packing** Pasteurized low fat milk and standardized milk mentioned in (see section **3.11** to **3.16**) shall be filled in clean, sound and sanitary containers made of glass or food grade polyethylene pouches, inner laminated tetra pack, plastic containers, cans or any other suitable materials or dispensing units. The product when marketed shall be packaged in well-sealed containers in order to prevent spoilage or contamination of the product. The packaging material used shall be food grade light proof, gas proof, mechanically strong, non-toxic and do not impart any off-flavour to the milk.
- **6.1.1** All packets/containers shall be clean and free from chips, cracks and any other defects. Bottles shall be hermetically sealed with new and clean closures and cans shall be sealed with new and clean can closures. The plastic container shall be heat sealed along one or more edges and shall not leak after it is filled with the products. All containers shall be subjected to cleansing and sanitizing process before filling.
- **6.2 Labeling** All labelling information shall be in Bangla and if necessary, any other suitable languages. The following information shall appear legibly on each container or label. Labels shall be clear in readable form of normal vision, easily visible and pasted securely. Brand name and product name shall be close together in front panel preferably in similar font size. Product name shall not smaller than two font size of the brand name.
 - a) Name of the product: 'Pasteurized medium fat milk/Pasteurized low fat milk/Pasteurized fat free or skimmed milk/Pasteurized standardized milk/Pasteurized toned milk/Pasteurized reconstituted (medium fat/low fat/fat free or skimmed) milk' as applicable;

Note 2:

- (i) For Pasteurized medium fat milk/Pasteurized medium low fat milk/Pasteurized fat free or skimmed milk declaration on label shall be "Prepared from fresh milk".
- (ii) For pasteurized standardized milk and pasteurized toned milk declaration on label shall be "Prepared by adding skimmed milk with fresh milk/Reconstituted skimmed milk with fresh milk/Reconstituted skimmed milk with fresh milk/.
- (iii) For pasteurized reconstituted milk declaration on label shall be "Prepared by mixing water with milk Powder".
- b) Fat ----- percent [See item (i) of Table 1];
- c) Batch or code number;
- d) Name and address of the manufacturer/importer;
- e) Net volume of the contents in litres or milliliters;
- f) Nutritional information;
- f) Date of packing;
- g) Date of expiry;
- h) Storage and user instruction;
- i) Maximum Retail Price (MRP) and
- j) Any other requirements as specified in the current Legislations and Regulation enforced in the country.
- 6.2.1 The container or label shall be marked with the BSTI Certification Mark

NOTE: The use or BSTI Certification Mark is governed by the provisions of the Bangladesh Standards and Testing Institution Act, 2018 and the Rules and Regulations made there under. Details of conditions under which a licence for the use of BSTI Certification Mark may be granted to manufacturers or processors or importer may be obtained from the Bangladesh Standards and Testing Institution.

7. SAMPLING

7.1 The method of drawing representative samples of the material and the criteria for conformity shall be as prescribed in BDS 1009.

8. TESTS

- **8.1.1** Tests shall be carried out as prescribed by the methods specified in column 4 of Table 1.
- **8.2** Quality of regents Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (see BDS 833) shall be used where the use of water as a reagent is intended.

NOTE 3: 'Pure chemicals' shall mean chemicals that do not contain impurities, which affect the result of analysis.

Annex A (Clause 2) List of Relevant Standards

BDS and ISO No.	Title
BDS 103	Methods of rounding off numerical value
BDS 822	Code of hygienic conditions for food processing units
BDS 833	Water for laboratory use
BDS 1009	Method of sampling and test for milk and milk products
BDS 1240	Drinking water
ISO 2446:2008	Milk – Determination of milk fat content
ISO 4833-1:2013	Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by the pour plate technique
ISO 5548:2004	Caseins and caseinates – Determination of lactose content – Photometric method
ISO 6731:2010	Milk, cream and evaporated milk – Determination of total solids content (Reference method)
ISO 8968-1	Milk and milk products - Determination of nitrogen content - Part 1: Kjeldahl principle and crude protein calculation
ISO TS 22113:2012	Milk and milk products — Determination of the titratable acidity of milk fat

ANNEX B [Table 1, item (viii)] DETERMINATION OF COLIFORM COUNT

B-1 GENERAL

B-1.1 Coliform Bacteria – Coliform bacteria include all aerobic and facultative anaerobic gram negative non-spore forming bacteria which ferment lactose with the production of acid and gas. A positive presumptive test is indicated by formation of acid and gas within 48 hours at 35 °C to 37 °C in a fermentation tube containing lactose bile salt broth. Alternatively, the development of dark red colonies at least 0.5 mm in diameter in a solid medium (violet red bile agar) within 20 to 24 hours at 35 °C to 37 °C may be considered as a positive evidence of the presence of coliform bacteria. Violet red bile agar is one of the standard media used for determination of general types of coliform organisms including those of faecal origin in water, milk and other materials or sanitary importance.

B-2 APPARTUS

- B-2.1 Weighing scoop sterile with counter mass (weight)
- **B-2.2** Bacteriological transfer pipettes sterile accurately graduated, with cotton plug in the upper orifice.
- **B-2.3** Dilution bottles, sterile made of heat-resistance glass (preferably silicate glass) closed with rubber stoppers (preferably screw cap) with new friction-fit liners for making them leak-proff and of the following capacities:
 - a) 150 ml with mark at 99 ml level; and
 - b) 25 ml with mark at 9 ml level.
- **B-2.4** Petri dishes with outside dish diameter 100 mm, inside dish diameter 91 mm and depth 15 mm. The exterior and interior surfaces of the bottom should be flat and free from bubbles scratches or other defects which would interfere with counting of colonies.
- **B-2.5** Bacteriological tubes sterile 25 ml capacity with a mark at the 10 ml level, with cotton plugs.
- **B-2.6** Tubes having inverted vials Durham tubes.

B-3 REAGENT

- **B-3.1** Dilution water Dissolve 34 g of potassium dihydrogen phoshate (KH₂PO₄) in 500 ml of distilled water, adjust with 1 N sodium hydroxide solution and make up to one litre with distilled water. Dilute 1.25 ml of this stock phoshpate buffer solution with distilled water to one litre to obtain dilution water.
- **B-3.2** Medium Violet red bile agar of following composition and pH shall be used as the medium:

Yeast extract	3.0 g
Peptone	7.0 g
Sodium taurocholate	1.5 g
Lactose	10.0 g
Sodium chloride	5.0 g
Agar-agar	20.0 g
Indicator	
Neutral red	0.03 g
Crystal violet	0.002 g
Water	100 ml
Final pH	7.4 ± 0.1

B-3.2.1 Preparation and sterilization of medium - Soak the materials (B-3.20) for 3 to 5 minutes in cold water, then bring the mixture into complete solution with minimum delay by boiling above asbestos centered wire gauze, over a flame. Stir continuously and efficiently to avoid charring. Adjust the solution to pH 7.4 ± 0.1 at 5 $^{\circ}$ C with sodium hydroxide solution. Filler through cotton pad till clear filtrate is obtained. Fill into bacteriological tubes to 10 ml mark. Sterilize in an autoclave 121 $^{\circ}$ C for 15 minutes.

B-4 PROCEDURE

B-4.1 Dilution - Weigh 11 g of the material from the samples for bacteriological examination using a sterile spatula and suspend in 99 ml of dilution water at 45 °C. Agitate mildly, soak for one to three minutes and then agitate vigorously to avoid churming out the fat. Prepare dilutions of this and add on millilitre of suitable dilutions in triplicate to the sterile petri dishes.

- **B-4.2** Pouring plates Melt the medium (see B-3.2.1) in bacteriological tubes and keep at 48 °C to 50 °C. Introduced this medium aseptically at 42 °C to 44 °C into the petri dishes and mix by rotating and tilting dishes without spreading over the edges, spread the mixture evenly over the bottom of the plate. Allow to solidify, after solidification of medium in plate, add cover layer of the medium.
- **B-4.3** Incubation Invert plates and incubate at 35 °C to 37 °C for 24 hours.
- B-4.4 Counting Count the dark red colonies which have a diameter of 0.5 mm or over.
- B-4.5 Computation Compute the coliform count per gram from the dilutions used (see B-4.1)
- NOTE 4: In case of doubt regarding the colonies developed on violet red bile agar representative colonies are picked and transferred to lactose bile salt broth in tubes having inverted vials. Production of acid and gas in confirmatory for coliform organisms.
- NOTE 5: All precautions shall be observed to prevent microbiological contamination throughout the test.

Annex C [Table 1, Item (ix)] Phosphatase Test

- **C-1** Alkaline phosphatase is an indigenous milk enzyme. The enzyme activity is destroyed at pasteurization temperature and has been adopted as an index of the efficiency of pasteurization. Since milk is a proven vector for a number of pathogenic bacteria, including *Salmonella*, *Campylobactor* and *Listeria*, the test is very great significance to the dairy industry as a means of policing the thoroughness of heat treatments or the addition of raw milk to heated or unheated products. In the following method, a solution of disodium p-nitrophenyl phosphate in a buffer of pH 10.2 is used as substrate. The compound, colourless in solution, is hydrolyzed by alkaline phosphatase of milk to liberate *p*-nitrophenol, which under alkaline condition gives an intense yellow colouration to the solution. The liberated *p*-nitrophenol is measured by direct comparison with standard colour discs in a Lovibond comparator. The test does not apply to sour milk and milk preserved with chemical preservatives.
- **C-2** Reagents/Apparatus All reagents should be of analytical grade.
- **C-2.1** Buffer solution 1.5 g of sodium bicarbonate and 3.5 g of anhydrous sodium carbonate dissolved in water and made up to one litre. Store in a refrigerator and discard after 1 month.
- **C-2.2** Disdium p-nitrophenylphosphate The solid substrate must be kept in the refrigerator.
- **C-2.3** Buffer-substrate solution Weigh accurately 0.15 g of subtrate (disodium p-nitrophenyl phosphate) into a 100 ml measuring cylinder and make up to 100 ml with buffer solution. The solution should be stored in refrigerator and protected from light. The solution should give a reading of less than the standards marked 10 on comparator disc APTW or APTW 7 when viewed through a 25 mm cell (distilled water is used as blank). The solution must be discarded after one week.
- **C-2.4** A lovibond comparator with stand for work in reflected light.
- **C-2.5** A lovibond comparator disc APTW or APTW 7.
- C-2.6 Two fused glass cells of 25 mm depth.
- C-2.7 A water bath or incubator capable of being maintained at $37.5 \,^{\circ}\text{C} \pm 0.5 \,^{\circ}\text{C}$.
- **C-2.8** One ml pipette and 5 ml pipette.
- **C-2.9** One litre graduated flask.

- C-2.10 100 ml measuring cylinder.
- **C-2.11** Test tubes, nominal size 150/16 mm with rubber stoppers.
- **C-3** Procedure Into a test tube pipette 5 ml of buffer substrate solution, stopper and bring the temperature to 37 °C. Add 1 ml of test milk to it shake and replace stopper, incubate at 37 °C for 2 hours. Incubate one blank prepared from boiled milk of the same type as that undergoing the test with each series of sample. Remove the tubes after 2 hours and the content should be well mixed. Place the boiled milk blank on left hand side of the comparator stand and test sample on the right. Take reading in reflected light by revolving the disc until the test sample is matched. Record readings failing between two standards by affixing a plus or minus sign to the figure for the nearest standard.
- **C-4** Interpretation The test is considered satisfactory if it gives a reading of 10 μg or less of p-nitrophenyl per ml of milk. Properly pasteurized milk gives no discernible colour.

Precautions Note 6: All glassware must be cleaned before use. Cleaning should be done by soaking in Chromic acid solution prepared by slowly adding 4 volumes of concentrated H_2SO_4 to 5 volumes of 8 % potassium dichromate. After cleaning in chromic acid glassware must be rinsed in warm water and distilled water and finally dried. Glassware used for the test must not be used for any other purpose and must be kept apart from other apparatus in the laboratory.

Precautions Note 7: A fresh pipette must be used for each sample of milk. Pipettes must not be contaminated with saliva.

Precautions Note 8: The sample of milk should be examined as soon as possible after arrival at the laboratory. If not examined immediately it must be kept at a temperature between 3 °C and 5 °C until examined. The sample must be brought to room temperature immediately before being tested.

Annex D
Table -1, Item (x)
Alcohol test

D-1 Alcohol Test

The test is quick and simple. It is based on instability of the proteins when the levels of acid and/or rennet are increased and acted upon by the alcohol. Also increased levels of albumen (colostrum milk) and salt concentrates (mastitis) results in a positive test.

D-2 Procedure

The test is done by mixing equal amounts of milk and 68 % of ethanol solution in a small bottle or test tube. 68 % Ethanol solution is prepared from 68 ml 96 % (absolute) alcohol and 28 ml distilled water. If the tested milk is of good quality, there will be no coagulation, clotting or precipitation, but it is necessary to look for small lumps. The first clotting due to acid development can first be seen at 0.21 - 0.23 % Lactic acid. For routine testing 2 ml milk is mixed with 2 ml 68 % alcohol.