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## Raw goat milk — Specification

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## Foreword

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- (a) a member of International Organisation for Standardisation (ISO) and
- (b) a contact point for the WHO/FAO Codex Alimentarius Commission on Food Standards, and
- (c) the National Enquiry Point on TBT Agreement of the World Trade Organisation (WTO).

The work of preparing Uganda Standards is carried out through Technical Committees. A Technical Committee is established to deliberate on standards in a given field or area and consists of key stakeholders including government, academia, consumer groups, private sector and other interested parties.

Draft Uganda Standards adopted by the Technical Committee are widely circulated to stakeholders and the general public for comments. The committee reviews the comments before recommending the draft standards for approval and declaration as Uganda Standards by the National Standards Council.

The committee responsible for this document is Technical Committee UNBS/TC 2, *[Food and Agriculture]* Subcommittee SC 1, *[Milk and milk products]*.

This second edition



# Raw goat milk— Specification

## 1 Scope

This Draft Uganda Standard specifies requirements, sampling and test methods for raw goat milk.

## 2 Normative references

The following referenced documents referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

US EAS 39, *Code of practice for hygiene in the food and drink manufacturing industry*

US ISO 2446, *Milk – Determination of fat content*

US ISO 6731, *Milk, cream and evaporated milk – Determination of total solids content (Reference method)*

US 45, *General standard for food additives*

AOAC 999.10, *Official method for lead, cadmium, zinc, copper, and iron in foods Atomic absorption Spectrophotometry after microwave Digestion*

US ISO 14501, *Milk and milk powder – Determination of Aflatoxin M1 content – Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatograph*

US 163, *Code of hygienic practice for milk and milk products*

US 738, *General standard for contaminants and toxins in food and feed*

US ISO 707, *Milk and milk products – Guidance on sampling*

US ISO 2446, *Milk — Determination of fat content*

US ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

US ISO 4833-1, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony-count at 30 °C by the pour plate technique*

US ISO 4833-2, *Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 2: Colony-count at 30 °C by the surface plating technique*

US ISO 5538, *Milk and milk products — Sampling — Inspection by attributes*

US ISO 5764, *Milk — Determination of freezing point — Thermistor cryoscope method (Reference method)*

US ISO 6731, *Milk, cream and evaporated milk — Determination of total solids content (reference method)*

US ISO 8197, *Milk and milk products — Sampling — Inspection by variables*

US ISO 8968-1, *Milk — Determination of nitrogen content — Part 1: Kjeldahl method*

US ISO 13366-1, *Milk — Enumeration of somatic cells — Part 1: Microscopic method (Reference method)*

US ISO 22662, *Milk and milk products — Determination of lactose content by high-performance liquid chromatography (Reference method)*

### 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <http://www.iso.org/obp>

#### 3.1

**raw goat milk** normal, clean and fresh secretions extracted from the udder of a healthy mother goat (*Capra* spp.), properly fed and kept, but excluding that got or obtained during the first three (3) days after kidding

#### 3.2

##### **Foreign matter**

any foreign substance which may affect the appearance, flavour and taste of the milk

#### 3.2

##### **Colostrum**

form of milk produced by a mother goat just prior to kidding

### 4 Requirements

#### 4.1 General Requirements.

Raw goat milk shall:

- a) be obtained from healthy mother goats (*Capra* spp.);
- b) be of natural flavour without any foreign matter and adulteration;
- c) pass the goat milk authentication test when test in accordance to Annex K
- d) be free from colostrums;
- e) not be treated except by cooling;
- f) not be decomposed;
- g) not contain added water, preservatives, or other added substances, nor shall any proportion of a natural constituent be removed
- h) have a characteristic creamy white colour, free from off-flavours and taints;

- i) be free of objectionable matter;
- j) not coagulate in the clot on boiling test when tested according to Annex D;
- k) test negative to the alcohol test; when tested according to Annex E and
- l) test positive to peroxidase test

#### 4.2 Specific requirements

Raw goat milk shall comply with the limits given in Table 1

**Table 1- Chemical requirements for raw goat milk**

Characteristic	Requirement	Methods of test
Fat content, %, min.	3.5	US ISO 2446
Protein content, %, min.	3.5	US ISO 8968-1
Lactose content, %, min.	4.2	US ISO 22662
Solids Not Fat (SNF), %, min.	8.5	US ISO 6731
Density at 20 ° C, g/mL	1.028 – 1.036	Annex J
Freezing point depression, C	-0.525 – 0.550	US ISO 5764
Titrateable acidity as lactic acid, %	0.1 – 0.2	Annex I
PH	6.5 – 6.8	Annex C

## 6 Hygiene

Raw goat milk shall be manufactured and handled in a hygienic manner in accordance with US EAS 39.and US 163

#### 6.2 Microbiological requirements

Raw goat milk shall comply with limits for micro-organisms specified in Table 2

**Table 2: Microbial limits for raw goat milk**

Micro-organism	Maximum limit cfu/ ml	Test method
Total plate count	$2 \times 10^6$	US ISO 4833-1
Total Coliforms	$5 \times 10^4$	US ISO 4832

Somatic cell count	$3 \times 10^5$	US ISO 13366-1
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## 7 Contaminants

### 7.1 Heavy metals

When tested in accordance with AOAC 999.10, the level of Lead (Pb) shall not exceed 0.1 mg/kg.

### 8.2 Pesticide residues

Raw goat milk shall comply with maximum limits residues set by Codex Alimentarius Commission.

### 8.3 Veterinary drug residues

Raw goat milk shall comply with maximum tolerable residue limits for antibiotics and other veterinary drugs set by Codex Alimentarius Commission

### 8.4 Mycotoxins

When tested in accordance with US ISO 14501, the level of aflatoxin M1 shall not exceed 0.50 µg/kg.

## 9 Packing and storing

9.1 Raw goat milk collected from milking shall be kept in clean food grade containers with a good hygienic practice before and after use.

9.2 Raw goat milk from milking shall be immediately delivered to the collection center. In case of non delivery, it shall be stored at 4 °C or below and not longer than 24 hours. In case raw goat milk has to be stored longer than 24 h, it should be frozen at -18 °C or below provided the delivery time from farm to the processing factory is within 15 days.

9.3 Raw goat milk in the storage tank of the collection centre shall be kept at 4 °C or below and not longer than 24 hours prior to delivering to processing factory

## 10 Transportation

10.1 The carriage used for delivering raw goat milk from farm to collection centre shall be in a clean and safe condition for transportation. In case of non-cooling, the milk shall be immediately delivered within 2 hours.

10.2 The carriage or container used for delivery of raw goat milk from collection centre to processing factory shall keep milk temperature properly and prevent contamination during transportation

## 9 Sampling

Sampling shall be done in Accordance to US ISO 707



## **Annex A** (normative)

### **Organoleptic test and temperature**

#### **A.1 General**

Judging the quality of milk by its physical and sensory characteristics requires considerable skill, which could only be acquired by practice. Organoleptic tests are used in all dairies and an experienced person can pick out bad samples with a high degree of accuracy.

#### **A.2 Procedure to be adopted on the receiving platform**

##### **A.2.1 Colour**

Observe the colour of the milk. If abnormal in colour, it should be held over/withheld for subjection to confirmatory tests.

##### **A.2.2 Odour and taints**

Smell the milk in the container immediately after removing the lid. In case of foul or abnormal smell, hold over the milk for subjection to confirmatory tests.

## **Annex B** (normative)

### **Determination of insoluble matter (Sediment test)**

#### **B.1 Principle**

The Sediment test on raw milk reveals the extent to which visible insoluble matter has gained entrance to the milk and the extent to which such material has not been removed from milk by single service strainers.

The Sediment test represents a simple, rapid and quantitative measure of indicating the cleanliness of milk with respect to visible dirt.

Allow a measured quantity of milk to pass through a fixed area of a filter disc and compare the sediment left with the prepared standards.

## B.2 Apparatus

**B.2.1 Sediment tester**, with filtering surface 24 mm in diameter

**B.2.2 White lintine cotton discs**, 32 mm in diameter, expected filtration area 28 mm in diameter.

**B.2.3 Sampling dipper**, of 500 mL capacity for sampling from milk cans or weighing vats.

**B.2.4 Standard sediment discs**, use commercially available standard sediment discs

## B.3 Procedure

Take a milk sample from well-stirred cans or vats of milk with the sampling dipper. Measure the quantity used with reasonable accuracy. Filter the milk through a properly adjusted, firm lintine cotton disc (rough side facing milk) held in the sediment tester so that a filtration area of 28 mm is exposed. Compare the sediment disc with the prepared sediment standard discs and record the sediment score.

## B.4 Interpretation

For the purpose of comparison, it is convenient to use about five prepared standard discs so as to classify the milk with respect to its sediment content in accordance with the specific requirement of the dairy or the milk collection depot. For the former, five discs showing 0.1 mg, 0.2 mg, 0.5 mg, 1.0 mg and 2.0 mg may suffice. Under rural conditions, discs showing 0.0 mg, 0.5 mg, 2.0 mg, 5.0 mg and 7.0 mg sediment may be more convenient to start with. In either case, no attempt shall be made to estimate the degree of sediment in milk in more than five classes, for example, Excellent, Good, Fair, Bad and Very Bad. No attempt shall be made to grade as sediment any hair, flies, piece of hay or straw or any large particles of dirt. These shall be reported separately.

The presence of appreciable sediment in unprocessed milk supplies indicates careless or unsanitary dairy farm practice. However, the lack of sediment is not always indicative of ideal conditions, since visible sediment may be readily removed by straining at the dairy farm.

## Annex C (normative)

### Determination of pH

#### C.1 General

The pH value or hydrogen ion concentration gives a measure of the true acidity of milk. The relationship between pH and acidity of milk is only approximate. In normal goat milk the pH ranges from 6.5 - 6.8. The value is reduced by the development of acidity. On the other hand, the pH value of milk from a goat suffering from mastitis is alkaline in reaction, the value being over 7.0. The pH test is mainly used for the detection of abnormal mastitis in milk. The pH of milk may be determined rapidly by using the indicator strips.

## C.2 Indicator strips

Indicator paper strips or discs are made by soaking strips of absorbent paper in a suitable indicator and drying them.

A rough estimate of pH is obtained by dipping a strip of the prepared paper in milk and observing the colour. Bromocresol purple (pH range 5.2 - 6.8 colour changes from yellow to purple) and bromothymol blue (pH range 6.0 - 7.6 Colour changes from straw yellow to bluish-green) are commonly used as indicators. Both narrow and wide range ready-made indicator papers are available over the pH range 2.0 - 10.5.

Indicator paper strips shall always be kept in closed glass bottles and under dry conditions.

## C.3 Interpretation

In normal milk the pH is well below 6.9. On an average, goat milk gives a pH of 6.6. Milk of pH greater than 6.9 should be regarded with suspicion as indication of some diseases of the udder or of late lactation milk.

## Annex D (normative)

### Clot-on-boiling (C.O.B.) test

#### D.1 General

This is a quick test to determine developed acidity and the suitability of milk for processing.

#### D.2 Apparatus

**D.2.1 Test-tube**, 15.6 cm x 1.9 cm, preferably with a mark at 5 mL

**D.2.2 Water-bath**

#### D.3 Procedure

Transfer 5 mL of the sample to the test-tube and smell. Place the tube in a boiling water-bath and hold for about 5 min, and smell again for any acidic flavour. Remove the tube and rotate it in an almost horizontal position and examine the film of milk or side of the test-tube for any precipitated particles. The formation of clots is indicative of a positive test.

#### **D.4 Interpretation**

The principal features of the boiling test are speed and definiteness of results. Milk either remains unchanged or coagulates. Milk, which gives a positive C.O.B. test, has acidity generally above 0.17 % (as lactic acid) and is not suitable for distribution as liquid milk or for processing.

## **Annex E**

(normative)

### **Alcohol test**

#### **E.1 Principle**

The alcohol test is used for rapid assessment of stability of milk to processing, particularly for condensing and sterilization.

The alcohol test is useful as an indication of the mineral balance of milk and not as much as an index of developed acidity. The test aids in detecting abnormal milk, such as colostrum, milk from animals in late lactation, milk from animals suffering from mastitis and which the mineral balance has been disturbed.

#### **E.2 Apparatus**

**E.2.1 Test-tubes**, 150 mm x 19 mm, preferably with graduation marks at 5 mL and 10 mL.

**E.2.2 Measure for alcohol**, for 5 mL

#### **E.3 Reagent**

**Ethyl alcohol**, 68 % by weight or 75 % by volume (density 0.8675 g/mL at 27 °C)

#### **E.4 Procedure**

Place 5 mL of milk in a test tube and add an equal quantity of alcohol. Mix the contents of the test tube by inverting several times.

Note any flakes or clots. The presence of a flake or a clot denotes a positive test.

#### **E.5 Interpretation**

A negative test indicates low acidity and good heat stability of milk sample.

Milk showing positive is not considered suitable for the manufacture of evaporated milk, which has to be sterilized to ensure its keeping quality.

## Annex F (informative)

### Alizarin-alcohol test

#### F.1 General

This test is similar to the alcohol test and the incorporation of alizarin helps to indicate the approximate percentage of acidity.

#### F.2 Apparatus

Same apparatus as described in E.2

#### F.3 Reagent

**Alizarin solution**, 0.2 % in ethyl alcohol (6.8 % by weight or 75 % by volume, density 0.867 5 g/mL at 27 °C)

#### F.4 Procedure

Place 5 mL of milk in a test-tube and add an equal quantity of the alizarin solution. Mix the contents of the test-tube by inverting several times. Note the colour of the mixture and presence of flakes or clots. Also note whether the flakes, if any, are small or large.

#### F.5 Interpretation

The general interpretation of the results is as indicated for the alcohol test (see E.5). For acidity of 0.14 % upwards, the graduation in size of the flakes and colour is approximately as follows:

Colour	Size of flakes	Approximate acidity (percent lactic acid)
Lilac		Up to 0.14
Pale red		0.4 - 0.17
Reddish-brown to brown	Small flakes	0.17 - 0.20
Brownish-yellow	Large flakes	Over 0.20

If acidity has not developed and yet coagulation occurs, it indicates the presence of rennet producing bacteria (sweet curdling). Milk from animals suffering from mastitis is alkaline in reaction and when mixed with alizarinalcohol solution, violet or purple colour is produced. From the practical point of view, it is of little material difference whether milk clots through the production of acid or the production of rennin by bacteria as in either case it is unstable to heat.

## Annex G (informative)

### Ten-minute resazurin test

#### G.1 Principle

This test provides a rapid measure of the sanitary condition and keeping quality of milk. Resazurin reduction occurs in two stages, the first an irreversible change from the blue resazurin to the pink resorufin and the second a reversible change from the pink resorufin to the colourless dihydroresorufin. The first stage of reduction or colour change from blue to pink is fairly easily brought about so that the quality of milk is assessed in much shorter time. Taking advantage of the two-stage reduction, several procedures have been proposed for reading the end point of resazurin test.

With fresh milk the observed change in resazurin reduction is due to the bacteria present and the leucocytes content. The reduction brought about by leucocytes, however, diminishes with the age of milk. Reduction can be assumed to be brought by the leucocytes if the colour in the downgraded milk sample (for example, milk from animals suffering from mastitis) remains unchanged for a longer time than observed normally.

The test is intended as a platform test for detecting milk of poor keeping quality and shall be carried out on samples collected for bacteriological analysis.

#### G.2 Apparatus

**G.2.1 Sterile test-tube without rims**, 150 mm x 16 mm internal diameter 13.5 mm  $\square$  0.5 mm accurately marked at 10 mL. If not used directly after sterilization, they shall be kept in closed boxes protected from dust.

**G.2.2 Sterilized rubber stoppers**, for closing the test tubes. The stoppers are sterilized by immersing in a boiling water bath for not less than 10 min.

**G.2.3 Sterile 1-mL pipettes**, straight-sided, blow-out delivery pipettes for measuring the dye solution (see G.3) shall preferably comply with the following specifications:

- Overall length 300 mm
- External diameter 7.5 mm - 8.5 mm
- Graduation one mark only at 1-mL level
- Distance of graduation from tips 140 mm - 180 mm
- Internal diameter 2.3 mm - 3.0 mm

The pipettes shall also be calibrated to deliver 1 mL of water at 27  $\square$ C when the contents are blown out with the tip touching the side of the vessel, three seconds allowed for drainage and the accumulated drop then blown out. No pipette should have an error of more than  $\square$  2 %, that is, the amount delivered should be between 0.98 mL and 1.02 mL.

**G.2.4 Sterile 10 mL pipettes**, straight-sided, blowout type

**G.2.5 Sampling dippers**, sterilized by keeping in boiling water for 30 min. Water shall be changed at frequent intervals when a series of samples are to be examined.

#### **G.2.6 Pipette case of metal**

**G.2.7 Water-bath**, maintained at  $37.5 \pm 0.5$  °C, fitted with a cover to exclude light and containing a metal rack designed to hold test tubes when immersed in water. The water-bath shall preferably be thermostatically controlled. The level of water in the bath shall be maintained above the level of the milk in the tubes. The interior of the bath shall be completely dark.

#### **G.2.8 Stop watch**

#### **G.2.9 Hot air oven autoclave or steam-sterilizer**

#### **G.2.10 Wire baskets**, for holding test tubes

#### **G.2.11 Glass marking pencil**

#### **G.2.12 Bunsen burner or spirit lamp**

**G.2.13 Comparator** with standard resazurin disc. The comparator may be provided with artificial daylight source of illumination.

#### **G.2.14 Sterile 50 mL measuring flask or cylinder**

#### **G.2.15 Glass distiller**, for preparing distilled water

### **G.3 Reagents**

**Sterile standard resazurin solution.** Prepare 0.05 % (W/V) stock solution by resazurin in glass distilled, sterilized water. Preserve in tightly stoppered amber-coloured bottle in a refrigerator. Prepare a 0.005 % bench solution by diluting with sterile water. It shall be prepared fresh after every 8 h. When actually not in use, keep it in a cool dark place.

Resazurin powder shall conform to the following requirements:

- a) it shall contain sodium resazurate equivalent to 60 %  $\pm$  3 % resazurin;
- b) apart from traces of sodium resocofate, no other dyestuff shall be present;
- c) the remaining part shall consist of sodium carbonate and/or sodium acetate and moisture only;
- d) it shall give a colourless water-clear solution on reduction in alkaline solution; and
- e) at a concentration of 1 to 220 000 in fresh normal mixed goat milk of 3.4 % fat, it shall give a tinto-meter disc reading of not less than 6.

### **G.4 Procedure**

Start the test as soon as possible after a group of samples has been taken and at least within 30 min.

Shake the sample container at least 25 times, each shake being an up and down movement with an excursion of about 30 cm, the whole process of shaking not exceeding 12 s. After shaking, take 10 mL for the test in the test tube. Place the tubes in numerical order, with the thumb and fingers of the left hand, taking



care not to touch the mouth of the tube. Measure 1 mL of the resazurin solution with a sterile pipette, insert the pipette about half an inch into the mouth of the tube and expel the solution by blowing.

Replace the stopper, by inverting the tube twice in 4 s and return to the rack. When resazurin has been added to a batch of not more than five tubes, place immediately in the water-bath and note the time. The delivery jet of the pipette shall not touch the milk in the tube. Any pipette becoming contaminated shall be immediately discarded. Use a fresh sterile pipette for every group of five samples.

At the end of 10 min and 30 s, remove the tubes from the water-bath and immediately match the colour with the resazurin disc in the comparator, recording the results for the tubes in the right section. The comparator and stand are placed on a bench at such a height that the operator is able to look down on the two apertures. The disc is then revolved until the sample is matched and the disc reading noted. When the colour falls between two disc numbers, it shall be recorded as the half value; for example, a reading between 3 and 4 shall be recorded as 3.5.

Tubes giving a reading between 0 and 1, streaky pink or very pale pink are recorded as 0.5.

NOTE It is an advantage for two persons to work in a team when a number of samples are to be taken rapidly, one to take the samples and the other to handle the containers and check the identity of the samples. Similarly, at the time of reading one person to watch the tubes and another to record.

## G.5 Precautions

**G.5.1** All testers should be trained to correctly match the colours in the comparator;

**G.5.2** The control and experimental test tubes shall be of the same type and thickness of glass;

**G.5.3** Control samples used shall be from the same consignment as milk tested to compensate for the natural colour of milk;

**G.5.4** Resazurin solution, milk, and milk to which resazurin has been added shall not be exposed to direct sunlight in the laboratory;

**G.5.5** The water-bath shall be kept covered during the test; and

**G.5.6** The temperature of the water-bath shall be checked before commencing each batch of tests.

## G.6 Interpretation

The results shall be interpreted as follows:

<b>Disc reading</b>	<b>Keeping quality</b>
4 or higher	Satisfactory
3.5 - 1	Doubtful
Below 1	Unsatisfactory

## Annex H (informative)

### Half-hour Methylene Blue Reduction (M.B.R.) test

#### H.1 General

The length of time taken by milk to decolourise methylene blue is a fairly good measure of its bacterial content and hence its sanitary and keeping quality.

#### H.2 Apparatus

Same as in G.2

#### H.3 Reagent

**Methylene blue solution.** Prepare a standard solution of methylene blue by dissolving one of the good quality methylene blue thiocyanate tablets in 200 mL of cold, sterile, glass-distilled water in a sterile flask. It is preferable to allow the mixture to stand for several hours to ensure complete solution.

Depending on the nature of methylene blue tablets used, sometimes the stock solution is further diluted with 800 mL of sterile glass distilled water. A concentration of 1 part of methylene blue thiocyanate in 300 000 parts of milk is used to obtain satisfactory results. The solution shall be stored in a sterile glass-stoppered ambercoloured bottle in a dark place and at no time exposed to light. The solution remains stable in the dark for a considerable time but no stock solution more than two months old shall be used.

The amount of methylene blue required for a day's work shall be poured off from the stock bottle into a suitable glass container. On no account shall the pipette used for transferring the methylene blue solution to the tubes of milk be introduced into the stock bottle. Moreover, if at any time during the filling of the tubes methylene blue solution should become contaminated with milk carried into it by a pipette, which has inadvertently come into contact with the milk, the methylene blue solution shall be immediately discarded and replaced by a fresh stock.

#### H.4 Procedure

Thoroughly mix the sample of the milk by inverting and shaking the sample bottle as described in G.3 and then pour the milk in the test-tube up to the 10-mL mark.

While doing this, remove the stopper or cap of the bottle under aseptic conditions, the pouring lip of the bottle and the mouth of the test-tube being flamed, and then pour the milk rapidly into the tube up to the 10-mL mark. While pouring into the tube, take care to leave one side of the interior unwetted with milk. Add 1 mL of methylene blue solution to the tube from a pipette taking care that the pipette does not come into contact with any of the milk in the tube or with the wet side of the interior of the tube.

If this occurs, discard the pipette immediately. During delivery, hold the tip of the pipette against the dry side of the tube about 1 cm - 2 cm above the level of the milk and expel the methylene blue solution by blowing with the mouth or by means of a jet in the pipette. After the lapse of 3 s, blow out the solution remaining in the tip of the pipette and withdraw the pipette. Close the tube with sterile forceps or by the tips of the fingers on the extreme upper end. On no account shall the fingers come into contact with the mouth of the test-tube or end of the stopper which comes into contact with the test-tube. Invert the tube slowly once or twice so that

the whole column of contained air rises above the level of the milk and then within 5 min, place the tube in the water-bath.

Put up the following control tubes with each batch:

- i) 10 mL of mixed milk + 1 mL of tap water.
- ii) 10 mL of mixed milk + 1 mL of methylene blue solution.

The milk for the control tubes shall consist of a mixture of milk, preferably from several products, so as to have an average fat content and colour. Fit the control tubes at (i) and (ii) with stoppers and immerse for 3 min in boiling water in order to destroy the natural reducing system present in the milk.

Comparison of the experimental tubes with control tube (ii) will show when decolourization begins and comparison with control tube (i) will show when it is complete.

Inspect the tube after 30 min. Regard the milk as decolourized when the whole column of milk is completely decolourized or is completely decolourized up to within 5 mm of the surface. If a trace of colour persists at the bottom of the tube and does not extend upwards for more than 5 mm, it may be ignored. Record the time at which decolourization is observed. Where a tube is found not to be decolourized within 30 min, the sample conforms to the test.

## H.5 Precautions

**H.5.1** It is important that the methylene blue solution when not in use should be kept in the dark. It shall at no time be exposed to sunlight.

**H.5.2** It is essential that the interior of the water-bath during the progress of the tests shall be completely dark since sunlight; diffused daylight and even artificial light catalyze the reduction of methylene blue.

**H.5.3** The sterilization of the rubber stoppers for the test tubes and their subsequent satisfactory manipulation can be facilitated by employing a simple rack for holding a large number of rubber stoppers immersed in a suitable vessel of boiling water.

**H.5.4** The precautions against the contamination of the milk sample described in the method for carrying out the test shall be carefully observed.

## H.6 Interpretation

The samples, which show complete decolourization of blue colour on incubation for 30 min or less shall not be suitable for acceptance.

## Annex I (normative)

### Determination of titratable acidity

#### I.1 Principle

Bacteria that normally develop in raw milk produce more or less of lactic acid. In the acidity test the acid is neutralised with 0.1 N Sodium hydroxide and the amount of alkaline is measured. From this, the percentage of lactic acid can be calculated.

#### I.2 Apparatus

I.2.1 A porcelain dish or small conical flask

I.2.2 10-mL pipette, graduated

I.2.3 1-mL pipette

I.2.4 Burette, 0.1 mL graduations

I.2.4 Glass rod for stirring the milk in the dish

I.2.5 Phenophtalein indicator solution, 0.5 % in 50 % alcohol

I.2.6 0.1 N Sodium hydroxide solution

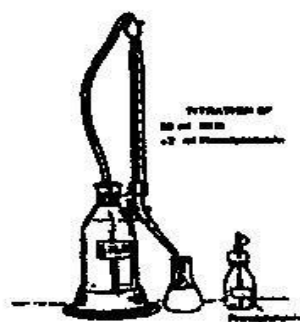


Figure 1 — Apparatus used be acidity test

#### I.3 Procedure

9 mL of the milk measured into the porcelain dish/conical flask. 1 mL phenophtalein is added and then slowly from the burette, 0.1 N Sodium hydroxide under continuous mixing, until a faint pink colour appears.

The number of millilitres of Sodium hydroxide solution divided by 10 expresses the percentage of lactic acid.

## Annex J (normative)

### Determination of density (Lactometer test)

#### J.1 Principle

Milk has a specific gravity. When it is adulterated with water or other materials are added or both misdeeds are committed, the density of milk changes from its normal value to abnormal. The lactometer test is designed to detect the change in density of such adulterated milk.

#### J.2 Apparatus

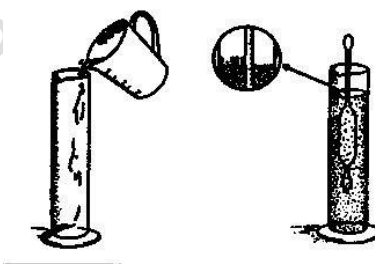
**J.2.1 Measuring cylinder** (300 mL – 500 mL)

**J.2.2 Lactometer**

#### J.3 Procedure

Mix the milk sample gently and pour it gently into a measuring cylinder (300 mL – 500 mL). Let the Lactometer sink slowly into the milk. Read and record the last Lactometer degree ( $^{\circ}\text{L}$ ) just above the surface of the milk. If the temperature of the milk is different from the calibration temperature (Calibration temperature may be =  $20^{\circ}\text{C}$ ) of the lactometer, calculate the temperature correction. For each  $^{\circ}\text{C}$  above the calibration temperature add  $0.2^{\circ}\text{L}$ ; for each  $^{\circ}\text{C}$  below calibration temperature subtract  $0.2^{\circ}\text{L}$  from the recorded lactometer reading.

EXAMPLE: Calibration temperature of lactometer  $20^{\circ}\text{C}$ .



**Figure 2 — Equipment used for determination of milk density**

Sample	Milk temperature, $^{\circ}\text{C}$	Lactometer reading, $^{\circ}\text{L}$	Correction	True reading, $^{\circ}\text{L}$
No. 1	17	30.6	-0.6	30.0
No. 2	20	30.0	Nil	30.0
No. 3	23	29.4	+0.6	30.0

For the calculations, use lactometer degrees, and for the conversion to density write 1.0 in front of the true lactometer reading that is, 1.030 g/mL

## **Annex K** (normative)

### **Method of Analysis or Identifying Goats Milk from other Dairy Milk**

#### **B.1 Equipment / apparatus**

- B.1.1 Polymerase Chain Reaction machine (thermal cycler)
- B.1.2 Water bath
- B.1.3 Micro pipettes
- B.1.4 Vertex
- B.1.5 Micro centrifuge
- B.1.6 Agarose Gel documentation machine
- B.1.7 Conical flask
- B.1.8 Ice cubes
- B.1.9 PCR tube rack
- B.1.10 Stairoform
- B.1.11 Micro centrifuge tubes
- B.1.12 Timer
- B.1.13 Agarose gel electrophoresis tank
- B.1.14 Analytical balance
- B.1.15 Microwave
- B.1.16 Casting trays and combs
- B.1.17 -20°C freezers
- B.1.18 -80°C ultra-freezer

#### **B.2 Reagents**

- B.2.1 PCR consumables
- B.2.2 DNA extraction kit (QIA prep spin mini prep form Qiagen, DNeasy Blood and tissue kit from Qiagen and Smart Helix First DNA id from Exvixon) are recommended
- B.2.3 PCR kit (New England bio labs and bioline are recommended kits for convectional PCR)

B.2.4 Agarose powder

B.2.5 Absolute ethanol

B.2.6 Nuclease free water

B.2.7 Primers for goat, cow, sheep, camel, buffalo and the expected source of the dairy animal to mixed with the goat's milk. (Primers should target the 12S rRNA region)

B.2.8 Molecular marker (depends on the expected band size of the amplicon)

B.2.9 TAE buffer (1X)

B.2.10 Gel Red dye

### B.3 Procedure

Design primers or commercially acquire them for goat, cow, sheep, camel, buffalo and any other dairy animal species you expect its milk to be mixed with the goat milk. The designed primer should target the mitochondrial cytochrome b gene (12S rRNA). The DNA extraction is done from milk samples following the extraction protocol from the above mentioned recommended DNA extraction kits. The PCR reaction mixture is set up following the PCR mini protocol that comes along with PCR kit. The reaction mixture is then transferred to the PCR machine (thermal cycler) and the conditions are set following the conditions on the PCR kit protocol. After the PCR, the amplicons are then loaded on the agarose gel stained with gel Red of a known percentage and placed in the gel electrophoresis tank containing 1X TAE (Tris Acetate EDTA) buffer and the tank is set at 100V, 300MA for 1 hour. Then the gel is then transferred to the gel documentation machine to visualise the bands of interest using the UV light lamp fixed in the gel documentation machine. The presence of the expected band size of goat only qualifies the purity and quality of the goat's milk. But the presence of other expected band size of other dairy milk animals signifies the presence of other dairy milk from other species of dairy animal and this disqualifies the purity and quality of the goat's milk.

## Bibliography

[1]

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