KENYA STANDARD

DKS 2147: 2007
ICS 67.100

Raw whole goat milk — Specification

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TECHNICAL COMMITTEE REPRESENTATION

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Ministry of Health — Food Safety Unit
Ministry of Agriculture, Livestock and Fisheries — Directorate of Livestock Resources and Market Development
Ministry of Agriculture, Livestock & Fisheries - Directorate of Veterinary Services and Livestock Production
Egerton University — Department of Dairy and Food Science Technology
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In order to keep abreast with the progress in the industry, Kenya standards shall be regularly reviewed. Suggestions for improvement of published standards are welcome and should be addressed to the Managing Director, Kenya Bureau of Standards.

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Raw whole goat milk — Specification

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Foreword

This Kenya standard was prepared by the Technical Committee on Milk and Milk Products under the guidance of the Standards Project Committee and it is in accordance with the procedures of the Kenya Bureau of Standards.

This standard only covers raw whole goat's milk.

This standard covers the quality, microbiological, contaminants and labeling requirements of raw whole goat milk.

This standard has also provided a method for determining adulteration of goat's milk with cow's milk

The standard intends to promote the production of safe and high quality goat milk.

Raw whole goat milk — Specification

1 Scope

This Kenya Standard specifies the requirements and methods of sampling and test for raw whole goat's milk.

2 Normative references

The following publications contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the edition indicated was valid. For undated references, the latest edition of the normative document referred to applies.

AOAC 922.08, Hypochlorite and chloramins in milk, colorimetric method
AOAC 942.17, Arsenic in foods Molybdenum blue method
AOAC 947.05; Acidity of milk, trimetric method
AOAC 960.27, preservatives in milk
AOAC 962.16, Beta-lactam Antibiotics in milk
AOAC 972.17 Official method; copper in milk and milk products, colometric method
AOAC 980.21, Aflatoxin M1 in milk and cheese-thin layer chromatographic method
AOAC 999.10, Lead, Cadmium, Copper, Iron, and Zinc in foods, Atomic Absorption Spectrophotometry after dry ashing
AOAC 960.40, Official method; Copper in, milk and milk products- Colometric method
AOAC 942.17, Arsenic in foods molybdenum blue method
AOAC 970.52; Organochlorine and organophosohorous pesticides residues in milk
AOAC 974.17, Aflatoxin M1 in milk and cheese-thin layer chromatographic methods
969.19; moisture in cheese, method iii (Distilation method)
AOAC 982.14, 15, 16, 17 and18, Beta-lactam Antibiotics in milk
AOAC 962.14, Beta-lactam Antibiotics in milk
CAC/MRL 1; Maximum Residue Limits (MRLs) for pesticides
CAC/MRL 3 Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for Residues of Veterinary Drugs in Foods
KS CODEX STAN 193, Codex general standard for contaminants and toxins in foods
KS ISO 1211:2010 (IDF1:2010); Milk -- Determination of fat content -- Gravimetric method (Reference method)
KS ISO 2446, Milk — Determination of fat content (Routine method)
KS ISO 3890-1:2009 (IDF 75-1:2009); Milk and milk products -- Determination of residues of organochlorine compounds (pesticides) -- Part 1: General considerations and extraction methods
KS ISO 3890-2:2009 (IDF 75-2:2009); Milk and milk products -- Determination of residues of organochlorine compounds (pesticides) -- Part 2: Test methods for crude extract purification and confirmation

KS ISO 4831:2006; Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms - Most probable number technique)
KS ISO 4832; 2006; Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique.
KS ISO 4833-1; 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

KS ISO 5738:2004 (IDF 76:2004); Milk and milk products -- Determination of copper content -- Photometric method (Reference method

KS ISO 5764, Milk - Determination of freezing point - Thermistor cryoscope method (Reference method)

KS ISO 6785, Milk and milk products -- Detection of Salmonella spp

KS ISO 6611, Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 degrees C

KS ISO 6731, Milk, cream and evaporated milk - Determination of total solids content (reference method)

KS ISO 6732; Milk and milk products -- Determination of iron content -- Spectrometric method (Reference method)

KS ISO/TS 6733:2006 (IDF/RM 133:2006); Milk and milk products -- Determination of lead content -- Graphite furnace atomic absorption spectrometric method

2 Definitions

For the purposes of this standard, the following definition shall apply.

2.1
raw whole milk
isthe normal, clean and fresh secretion obtained by practically emptying the udder of a healthy goat, properly fed and kept, but excluding that got during the first seven days after kidding and free from colostrum

3 Principal compositional requirements

3.1 Chemical

3.1.1 When tested in accordance with KS ISO 2446; raw whole milk shall contain not less than 3.5 % milk fat and not less than 8.5 % milk solids not fat.

3.1.2 It shall not contain added water, preservatives, or other added substances, nor shall any proportion of a natural constituent be removed when tested in accordance to AOAC 960.27, preservatives in milk

3.1.3 Adulteration of goat's milk with cow's milk
This practice shall not be allowed.
Adulteration of goat milk with Cow's milk shall be tested in accordance to the RIDA Quick CIS
Test method given in Annex A

3.1.4 Hypochlorite's and chloramines in goat's milk

Pasteurized goat milk shall contain no Hypochlorite's and chloramines when tested in accordance to AOAC 922.08

3.1.5 Density of the milk at 20 ^{0}C shall be within the following range:

1.028 g/mL — 1.032 g/mL

3.1.6 When tested according to the KS ISO 5764, the freezing point depression of milk shall be a -0.48 ^{0}C — 0.568 ^{0}C; Indicating the absence of added water.

3.1.7 Raw milk shall have a pH of 6.6 — 6.8.

3.1.8 The milk shall comply with the rapid tests described in Annexes A — H.

Table 2 — Physico-chemical requirements for raw whole goat milk

SL No	Parameter	Requirement	Test method
i)	pH	6.5 - 6.8	KS ISO 5546
ii)	Total acidity (expressed as % of lactic acid),max	0.20	KS ISO/TS 11869
iii)	Density of the milk at 20 ^{0}C	1.028 g/mL - 1.032 g/mL	KS ISO 5764
iv)	freezing point depression of milk (to indicate the absence of added water)	±0.480 ^{0}C to ±0.568 ^{0}C	AOAC 961.07/ KS ISO 5764

4 Microbiological limits

4.1 Total plate count

The plate shall be incubated for 48 h at 32 ^{0}C. The counts shall be taken and graded as follows:

Table 1 — Microbiological load in raw goat whole milk

Grade	Bacterial counts per mL
i)	0 – 400 000
ii)	400 001 – 1 000 000

4.2 Coliform plate count

The plate shall be incubated for 24 h at 37 ^{0}C. The counts shall be taken and graded as follows:

Table 2 — Coliform counts in raw whole milk

Grade	Counts per mL
i)	0 – 1 000
ii)	1 001 – 40 000

Pathogens

Microbes	Limits	Test method
S. aureus	Nil	KS ISO 6888-1
E. coli	Nil	KS ISO 4833
L. monocytogenes	Nil	KS ISO 4833

Salmonella spp	Nil	KS ISO 6785

5 Contaminants

The products covered by this standard shall comply with the maximum levels of CODEX STAN 193 and the maximum residue limits for pesticides and veterinary drugs established by the Codex Alimentarius Commission (CAC).

5.1 Heavy metals

Heavy metal limits for pasteurized goat milk shall be as given in Table 4.

Table 4 — Limits for heavy metal contaminants pasteurized goat milk

SL No	Heavy metal	MRL (max.)	Test method
i)	Arsenic (AS)	0.1 mg/kg	AOAC 942.17
ii)	Lead (Pb)	0.02 mg/kg	KS ISO 6733
iii)	Mercury (Hg)	1.0 mg/kg	AOAC 999.10
iv)	Copper (Cu)	5.0 mg/kg	AOAC 960.40/ KS ISO 5738
v)	Zinc (Zn)	50 mg/kg	AOAC 999.10
vi)	Tin (Sn)	250 mg/kg	AOAC 999.10
vii)	Cadmium as Cd,	1.5 mg/kg	AOAC 999.10
viii)	Iron (fe),	0.5 mg/kg	KS ISO 6732

5.2 Mycotoxin residues

Pasteurized goat milk shall not have more than 0.015 ppb aflatoxin M1 content when tested according to KS ISO 14501 or AOAC 980.21.

5.3 Total antibiotic residues

Pasteurized goat milk shall not have more than 10.0 ppb total antibiotic residues as (beta lactam) content when tested according to AOAC 962.16 and when analyzed by the appropriate approved methods as given in the Food, Drugs and Chemical Substances Act, Cap. 254 of the Laws of Kenya and the CODEX guidance.
The milk shall not contain any antibiotics.

5.4 Veterinary drug residues

In addition to the maximum veterinary drug residue limits in table 6 below; the products covered by the provisions of this standard shall conform to those maximum limits for veterinary drug residue limits established by the Codex Alimentarius Commission for these products in codex Stan 193;

When analyzed by the appropriate approved methods as given in Table 5, pasteurized goat milk shall not contain any veterinary drug residues.

Table 5 — Maximum veterinary drug residue limits for pasteurized goat milk

S/N	Parameter	Requirements/ MRL	Test method
i)	ChloramPhenical	ND	AOAC 972.17
ii)	Nitrofunas (including metabolites)	ND	AOAC 960.63
iii)	Ronidazole	ND	AOAC 969.56
iv)	Metronidazole	ND	AOAC 991.17
v)	Fenbendazole	100 ppb	AOAC 991.17
vi)	Albendazole	100 ppb	AOAC 991.17
vii)	Phenylbutazone	ND	AOAC 991.17

5.5 Pesticide residues

In addition to the maximum pesticide residue limits in table 6 below; the products covered by the provisions of this standard shall conform to those maximum limits for pesticide residue limits established by the Codex Alimentarius Commission for these products in codex Stan 193;

Pasteurized goat milk shall not contain pesticide residues in excess of the residue limits given in Table 6.

Table 6 — Maximum pesticide residue limits for pasteurized goat milk

SL No	Parameter	Requirement	Test method
i)	ORGANOCHLORINE Group	0.01 ppm	KS ISO 3890
ii)	ORGANOPHOSPHOROUS Group	0.01 ppm	AOAC 960.40

6 HYGIENE

6.1 It is recommended that the products covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of KS 1552:2016 and other relevant Kenya standards and

regulations. The products should comply with any microbiological criteria established in accordance with KS CAC/GL 21.

6.2 It shall be transported in sanitized containers. The containers shall be made of approved food grade materials and shall comply with the relevant Kenya standards. Where no immediate processing or marketing is undertaken after milking, the milk shall be rapidly cooled to 4 C

[3)] Guide to maximum pesticide limits in products.

[4)] Code of hygienic practice for milk and milk and milk products.

ANNEX A
(Normative)

A.1 Determination of goat's milk adulteration with cow's milk using RIDA Quick CIS Test

A.1.1 Principle

RIDA Quick CIS Test is an immunochromatographic test for the detection of cow's milk in milk or cheese of other species (sheep and goat).
The detection of an addition cow's milk to sheep and goats milk or to sheep and goats cheese is carried out by determining bovine igG (antibody class), which is a component of cow's milk. Adulteration with cow's milk in ultra-heated products cannot be detected since the antibodies are destroyed by ultra-high heating.
The detection of cow's milk in ultra-high heated products can be done using RIDA Screen casein test adulteration

A.1.2 Apparatus and Reagents

The test is strip format. The apparatus and reagents include;
Reaction strips
Pipette
Test tubes
10ml buffer

A.1.3 Sample Preparation

Milk samples are used directly in the test without preliminary treatment and the result can be read off after ten minutes

A.1.4 Detection Limit

RIDA QUICK CIS test is capable to detect adulterations or mixtures up to 0.5 % cow's milk in sheep and goats milk. A positive result indicates that the sample has a cow milk content of 0.5 % or more.

Annex B
(normative)

Organoleptic test and temperature

B.1 General

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

B.2 Procedure to be adopted on the receiving platform

B.2.1 Foreign matter

Presence of the foreign matter such as pieces of dung, hair or any other foreign material should be checked.

B.2.2 Odour

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.

B.2.3 Colour

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

B.2.4 Flavour/Taints

Examine the milk for the following taints:

B.2.4.1 Those due to developed acidity. This is the most important factor to be examined when grading milk by organoleptic test.

B.2.4.2 Those due to feed, or exposure of milk to air or contamination from containers.

Annex C

(normative)

Determination of insoluble matter

C.1 Sediment test

C.1.1 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.

The test is carried out by allowing a measured quantity of milk to pass through a fixed area of a filter disc and comparing the sediment left with the prepared standards.

C.1.2 Apparatus

C.1.2.1 Sediment tester, with filtering surface 24 mm in diameter.

C.1.2.2 White lintine cotton discs, 32 mm in diameter, expected filtration area 28 mm in diameter.

C.1.2.3 Sampling dipper, of 500 mL capacity for sampling from milk cans or weighing vats.

C.1.2.4 Sieves, two, one coarse corresponding to 425-micron sieves.

C.1.2.5 Sediment disc ratings, showing 0.0 mg, 0.2 mg, 0.5 mg, 1.0 mg, 2.0 mg, 3.0 mg sediment or higher concentration as required per 500 mL of milk.

C.1.3 Preparation of standard sediment discs

Since sediment encountered in different localities may vary in composition, density, colour and other physical characteristics, thus giving rise to variations in the appearance, sediment discs shall be prepared as follows:

Make a uniform mixture of oven-dried (100 ^{0}C) materials which meet the following screening specifications:

i) cow dung passing through 53 parts fine sieve;

ii) cow dung passing through 10 parts coarse sieve but retained on the fine one;

iii) garden soil passing through 27 parts sieve;

iv) charcoal passing through the fine 8 parts sieve;

v) charcoal passing through the coarse sieve but retained on the fine one.

Total: 100 parts.

Accurately weigh 0.1 g of the above mixture and transfer to a 100 mL flask using 50 % sugar solution to wash all fine particles down into the flask. Make the volume up to the mark with more of the sugar solution after most of fine particles have been wetted by shaking the half-filled flask thoroughly several times. After the volume is made up to the mark, shake the contents of the flask vigorously every 5 min for sufficient time (for 30 min to one hour) to saturate particles thoroughly.

When particles have been thoroughly wetted, it will be noted that the sugar solution will hold them evenly in suspension and the mixture is ready for use in making the standard discs.

On the basis of 0.1 g per 1 000 mL, 10 mL of the sugar solution contains 1 mg of the sediment. Make test discs with one of the usual sediment testers using varying volumes of the sediment suspension.

After forcing the milk through the disc, run through a small quantity of filtered skimmed milk to obtain a more even distribution of the sediment on the disc.

Remove the discs from the tester, mount them permanently by spraying with a strong disinfectant, such as corrosive sublimate. Below each mounted standard disc on paper, note the quality of dried material that the dirt or filth on the disc represents.

C.1.4 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.

C.1.5 Interpretations

For the purpose of comparisons, 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, pieces 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 in sanitary 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 D

(normative)

Determination of pH

D.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 milk the pH ranges from 6.6 to 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.

D.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 to 6.8, colour changes from yellow to purple) and bromothymol blue (pH range 6.0 to 7.6, colour changes from straw yellow to bluish-green) are commonly used as indicators. Booth narrow and wide range ready-made indicator papers are available over the pH range 2.0 to 10.5.

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

D.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 over 6.9 should be regarded with suspicion as indication of some diseases of the udder or of late lactation milk.

Annex E

(normative)

Clot on boiling (COB) test

E.1 General

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

E.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

E.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 are indicative of a positive test.

E.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 COB test has an acidity generally above 0.17 % (as lactic acid) and is not suitable for distribution as liquid milk or for processing.

Annex F
(normative)

Alcohol test

F.1 General

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 so much as an index of developed acidity. The test aids in detecting abnormal milk, such as a colostrum, milk from animals in late lactation, milk from animals suffering from mastitis and milk in which the mineral balance has been disturbed.

F.2 Apparatus

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

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

F.3 Reagent

F.3.1 **Ethyl alcohol**, 62 % by weight or 75 % by volume (density 0.867 5 g/mL at 27 ^{0}C).

F.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.

F.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 G
(normative)

Alizarin-alcohol test

G.1 General

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

GF.2 Apparatus

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

F.2.2 Measure for alcohol, for 5 mL.

G.3 Reagent

F.3.1 Alizarin solution

0.2 % in ethyl alcohol (6.8 % by weight or 75 % by volume, density 0.867 5 g/mL at 27 ^{0}C).

G.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.

G.5 Interpretation

G.5.1 The general interpretation of the results is as indicated for the alcohol test (see Clause 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 (% lactic acid)

Lilac	-	up to 0.14
Pale red	-	0.14 to 0.17
Reddish-brown to brown	Small flakes	0.17 to 0.20
Brownish-yellow	Large flakes	over 0.20

G.5.2 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 alizarin-alcohol 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 H
(normative)

Ten-minute resazurin test

H.1 General

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 easily brought about so that the fairly 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 bacterial 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 down graded 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.

H.2 Apparatus

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

H.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.

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

Overall length	300 mm
External diameter	7.5 mm to 8.5 mm

Graduation	one mark only at 1 mL level
Distance of graduation from tip	140 mm to 180 mm
Internal diameter	2.3 mm to 3.0 mm

The pipettes shall also be calibrated to deliver 1 mL of water at 27 ^{0}C when the contents are blown out with the tip touching the side of the vessel, 3 s allowed for drainage and the accumulated drop then blown out. No pipette should have an error of more than ± 2 %, that is, the amount delivered should be between 1.98 mL and 1.02 mL.

H.2.4 Sterile 10 mL pipettes, straight-sided, 1.98 mL and blow-out type.

H.2.5 Sampling dippers, these shall be 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.

H.2.6 Pipette case, of metal.

H.2.7 Water-bath, maintained at 37.5 ^{0}C ± 0.5 ^{0}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.

H.2.8 Stop-watch

H.2.9 Hot air oven autoclave or steam-sterilizer

H.2.10 Wire baskets, for holding test-tubes

G.2.11 Glass marking pencil

H.2.12 Bunsen burner or spirit lamp

H.2.13 Comparator with standard resazurin disc, the comparator may be provided with artificial daylight source of illumination.

H.2.14 Sterile 50 mL, measuring flask or cylinder.

H.2.15 Glass distiller, for preparing distilled water.

H.3 Reagent

G.3.1 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.

NOTE Resazurin powder shall conform to the following requirements:

i) it shall contain sodium resazurate equivalent to 60 % ± 3 % resazurin;

ii) apart from traces of sodium resocefate, no other dyestuff shall be present;

iii) the remaining part shall consist of sodium carbonate and/or sodium acetate and moisture only;

iv) it shall give a colourless water-clear solution on reduction in alkaline solution;

v) 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.

H.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, mix 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 ± 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 apartures. 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.

H.5 Precautions

The following precautions are necessary to get consistent results:

i) all testers should be trained to correctly match the colours in the comparator;

ii) the control and experimental test-tubes shall be of the same type and thickness of glass;

iii) control samples used shall be from the same consignment as milk tested to compensate for the natural colour of milk;

iv) resazurin solution, milk, and milk to which resazurin has been added shall not be exposed to direct sunlight in the laboratory;

v) the water-bath shall be kept covered during the test;

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vi) the temperature of the water-bath shall be checked before commencing each batch of tests.

H.6 Interpretation

The results shall be interpreted as follows:

Disc reading	**Keeping quality**
5 or higher	Satisfactory
3.5 to 1	Doubtful
0.5 to 0	Unsatisfactory

Annex I
(normative)

Half-hour methylene blue reduction (MBR) test

I.1 General

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

I.2 Apparatus

Same as in Clause G.2.

I.3 Reagent

I.3.1 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 amber-coloured 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.

I.4 Procedure

Thoroughly mix the sample of the milk by inverting and shaking the sample bottle as described in Clause 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 to 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 moisture 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 decolourisation 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 decolorized 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 de-colourization is observed. Where a tube is found not to be decolourized within 30 min, the sample conforms to the test.

I.5 Precautions

The following precautions shall be taken:

i) 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;

ii) 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;

iii) 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;

iv) the precautions against the contamination of the milk sample described in the method for carrying out the test shall be carefully observed.

I.6 Interpretation

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

