



EAST AFRICAN COMMUNITY



EDICT



# OF GOVERNMENT

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EAS 68-4 (2006) (English): Milk and milk products – Enumeration of colony-forming units of yeasts and/or moulds – Colony-count technique at 25 °C



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**EAS 68-4:2006**  
**ICS 67.100.10**

## **EAST AFRICAN STANDARD**

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**Milk and milk products — Methods of microbiological examination**

**Part 4:**  
**Swab tests**

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## **Foreword**

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to achieve this objective, the Partner States in the Community through their National Bureaux of Standards, have established an East African Standards Committee.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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## Milk and milk products — Methods of microbiological examination

### Part 4: Swab test

#### 1 Scope

This standard deals with the test intended for checking sanitization of the surface of containers and equipment with which milk and milk products can come into direct contact.

#### 2 Principle of the method

Assessing of the bacteriological condition of the surface under test is carried out by means of a swab technique and a total bacterial count of the solution in which the swab is dipped.

#### 3 Sterilization of instruments, medium and solutions

All instruments shall be sterilized as follows:

**Glass instrument** — This shall be sterilized by heating for 2 h at a temperature of 170 °C, or by autoclaving at a temperature of 121 °C for 15 min.

#### 4 Buffered rinse

The solution shall be prepared by diluting one part of the following full strength Ringer's solution with 3 parts of distilled water:

Sodium chloride	(NaCl)	9.00 g
Potassium chloride	(KCl)	0.42 g
Calcium chloride	(CaCl <sub>2</sub> )	0.24 g
Sodium bicarbonate	(NaHCO <sub>3</sub> )	0.20 g
Distilled water	(H <sub>2</sub> O)	1000 ml

Ready prepared tablets may be used, provided that the concentration in the solution used shall be the same as the diluted solution described above.

#### 5 The medium

Dissolve in distilled water the following components and make up to 1 000 ml by adding water:

a) Yeast extract	2.5 g
b) Tryptone	5.0 g
c) Dextrose	1.0 g
d) Agar	15 g

The pH of the solution shall be 7.0

Ready prepared mediums may be used, provided that the proportion of components is respected.

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## 6 Apparatus

### 6.1 Swab

The swab consists of non-absorbent cotton and wooden stick. The sterilized cotton fabric should be twisted around one end of the stick and have the length of about 20 mm. The length of the stick should exceed the length of the test tube. The test tube shall be plugged with cotton wool.

### 6.2 The test tube

Shall be 16 mm in diameter and about 150 mm long.

## 7 Preparation for the test

Pour an amount of the solution into the test tube so that 5 ml will remain after sterilization. The quantity required shall be determined by a preliminary trial. Immerse the swab in the solution, plug the test tube with cotton wool and sterilize as required in 4.3 of Part 1.

## 8 The swabbing procedure

Press the swab with a rolling motion against the side of the test tube. Remove the swab from the test tube and rub the cotton end of it exerting pressure against the surface under test, so that every part of the surface is rubbed 3 times to and fro. The total surface tested shall be equal to about 10 000 mm<sup>2</sup> and care shall be taken that in each rubbing this entire surface be covered. Replace the swab in the solution, mix and exert pressure as before. Repeat the cycle of immersion, swabbing and pressure four times, each time in a different place, covering each time a surface of 10 000 mm<sup>2</sup>.

After the swabbing break or cut off the swab with sterilized scissors over the cotton wool and let the portion of the swab covered with cotton fall into the solution and plug the test tube.

A total of 50 000 mm<sup>2</sup> shall be swabbed or, if the surface of the tested equipment is smaller than 50 000 mm<sup>2</sup>, then the whole surface is swabbed. Keep the test tubes with the swabbing solution until the time of the test at a temperature not exceeding 10 °C.

## 9 Microbiological examination

The test shall be performed as soon as possible after the swabbing but not later than 24 h after it. Agitate the test tube vigorously during 15 min, withdraw with the aid of a pipette 2 portions (1 ml each) of the rinse solution, and introduce each portion to a petri dish having an inside diameter of about 90 mm.

Add 10 ml — 12 ml of the liquefied medium at the temperature of about 45 °C to each dish. Mix thoroughly until the rinse solution is uniformly spread throughout the medium. Immediately after solidification, invert the dishes and transfer them to incubator.

## 10 Incubation and counting

Incubate for 48 h ± 3 h at a temperature of 32 °C ± 1 °C and count the colonies. If no immediate count is possible, store the dishes in the refrigerator until, the following day. Count the colonies with the aid of a magnifying glass or other suitable device (for example, the Quebec apparatus).

In parallel with the incubation of the inoculated medium, perform the sterility test by incubating uninoculated medium. If this control test reveals the development of tiny bacteria whatsoever, the results of the swab test are not reliable.



## 11 Calculation of results

Express the results of the test by the number of colonies of bacteria per mm of the swabbed surface

$$N = \frac{5(N_1 + N_2)}{2 \times 50\,000} = \frac{N_1 + N_2}{20\,000}$$

from the following formulas:

$$n = \frac{5(N_1 + N_2)}{2 \bullet S}$$

If the whole surface of the equipment has been swabbed use the formula:

where

$n$	=	number of colonies per mm <sup>2</sup>
$N_1$	=	number of colonies in the first dish
$N_2$	=	number of colonies in the second dish
$S$	=	the area of the whole surface in mm <sup>2</sup>

If it is impossible to determine the surface of the equipment, relate the formula to the equipment and describe in the test certificate the shape of the equipment.



