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EKOTEST

FAST TEST FOR FINDING ANTIBIOTICS AND INHIBITORS IN MILK

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EKOTEST

1. Introduction

The test for finding suppressing substances in milk is designed for quick laboratory estimation for suppressing substances presence, including antibiotics in medium.

2. Test sensibility

The test sensibility is sufficient to guarantee finding any substances in suppressing concentration for starter culture in milk raw material.

3. Advantages

- Test fastness – within 10 – 12 minutes.
- Use of cheap reagents in small quantity.
- Possibility for simultaneous testing of 6 samples.

4. Application

Ekotest is used as a controlling test in milk material acceptance or before its technological processing, although for pasteurized milk, and in any situation requiring fast inhibitor estimation for milk samples.

5. Equipment

- Water-bath incubator
- Active substance – lyophilized / 1 g per pack / - 8 pieces
- Transfer pipettes - 5 pieces
- Test-tubes – 13 pieces, with plugs
- Easel
- Bottles of reagents – 2 pieces
- Ampulla for reagent № 2
- Instruction

6. Test procedure – (for one sample)

6.1. 10 ml of milk, which will be tested (**in advance pasteurized at temperature 95 ° C for several minutes and cooled down to room temperature**) are put in each of two test-tubes – tested one and controlling one, with identical colours or numbers.
6.2. Add 1.5 ml active substance with transfer pipette to each milk samples. The tubes are plugged and their content are mixed by turning up and down.
6.3. The test-tube is put into the incubator after reaching the necessary water temperature of 44° C, and the control-tube – in the easel.
6.4. It is incubated 10 minutes after reaching the necessary milk sample temperature of 44° C, and for sheep milk – 15 minutes.
6.5. At this time 2 drops of reagent № **1** are put into the control-tube, the content is mixed by turning up and down. After that reagent № **2** is added by burette or pipette (no less than 2 ml). The content is mixed and reagent № **2** drops are added till the sample becomes pale pink coloured and remaining the same for more than 30 seconds. The used quantity of the reagent № **2** is registered.
6.6. After the incubation the test-tube is taken out and put in easel next to the control-tube. 2 drops of the reagent № **1** are also put into the incubated sample and the content is mixed well.
6.7. The same quantity of reagent № **2**, as the added to the controlling sample is added to the tested one. After carefully turning up and down the colour of the tested sample is compared visually to the colour of the controlling one and the result is interpreted according to item 7.

7. Results interpretation

(-) – Negative result – the sample is with the milk colour – white. There are not inhibitors in it, i.e. the tested milk is biologically sterling and may be technologically processed.
(+) – Positive result – the sample is pink coloured. There are suppressing substances in it, i. e. there is a shortage of growing factors in milk material. Such samples may be additionally tested for corroborating the available inhibitor.

8. Notes

- The right incubating temperature is approximately 44° C (± 2° C). Deviations below 42° and above 48° change the optimum test conditions and would decrease the test accuracy, because of the small incubation time.
- It is advisable the milk which will be tested to be with temperature around 20° C, for its faster tempering.
- It is advisable to be tested only milk with acidity between 16 and 22 degrees T – for cow milk and 16 - 24° T – for sheep milk.
- The test results may be affected by imprecise dosage of the reagents.
- Reagents are to be stored in a refrigerator. Before testing they are to be left on room temperature for 10-15 min.

9. Additional information about inhibitors

Under inhibitors you have to understand large range of alien milk components which kill or suppress the active micro-organism in milk. Inhibitors are for example:

- remainders after washing and disinfection of the milk machines;
- antibiotics and other medicine preparations which are secreted in milk;
- presence of side micro-flora;
- conservative substances in the milk etc.

The lack of fermentation and coagulation of milk may be due besides suppressing substances to insufficient level of the micro-organism growing factors, like free Amino-acids, Fe and some vitamins of B group. These factors are strongly affected by the season and the type of feeding, soils, forages and climate and also the breed of animals and some physiological reasons.

Instruction on how to activate lyophilized growths

Obtaining of activated growths:

1. Pasteurize pure and fresh cow milk featuring acidity of 17 -18 ºT and containing no inhibitors or antibiotics milk at 98 – 100 ºC and then cool it down to 43-45 ºC.
2. Empty the contents of a lyophilized growth pack in 200 milliliters of milk. It is advisable that the dry growths be dissolved in advance in a small amount of milk before adding them to the other milk and then stir it thoroughly.
3. Close tightly the inoculated milk and leave it at 43-45 ºC to initiate fermentation. Under these conditions the milk will coagulate in 3 to 5 hours (reaching acidity of 70 – 75 ºT).
4. Now cool down the milk and store it at 4 to 6 ºC no more than 20 days.

2.5 ºT are adequate to 1 º SH, or 0.0225 % milk acid.

Instruction for preparing of 1 l of 0.1 N solution of Sodium Hydroxide (NaOH) Reagent Nº 2

1. Insert the ampoule with concentrated solution into the volumetric flask.
2. Put a piercing rod into the upper funnel.
3. Press, carefully, piercing the upper and lower membranes.
4. Wash the ampoule and the piercing rod deeply with distilled water. Measure up to **1 l** and homogenize.
5. Transfer the solution from the flask into empty bottle with label **" Solution Nº 2"**, for storage.

NOTE:
Causes burns! In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye-face protection. In case of accident or if you feel unwell, seek medical advice immediately.

EKOTEST HEATER BLOCK –

INCUBATOR – TYPE WATER- BATH

It is designed for incubation of the tests for presence of antibiotics and other suppressing substances in milk.

It is developed in BULTEH – 2000 Ltd. according to the directives for performance of a fast test for inhibitors presence in milk.
This is a modern microprocessor appliance which is easy and reliable to operate. All settigs are input in the power undependable appliance memory. When operating, data are displayed on an LCD display. The appliance is equipped with a timer which is set by keypad. Sound signalization and displayed messages considerably facilitate the operation of the appliance, also if it is operated by unskilled operator. This appliance is suitable for both laboratories and the open field (12 V powered – an option).

Technical data:
Incubation temperature – 30°-65° C – adjustable
Accuracy of temperature setting - ± 0.5° C
Timer range – 0-12 h, accuracy 1 sec, adjustable
Sound signalization when the set time and temperature are reached
Capacity – 6 test-tubes with Ø16 mm
Water amount for thermo-bath – 350 ml
Power – 220 V; 50 Hz
Used power – for heating – 60 W
– for stabilization – 20 W
Size – 104/ 238/ 275 mm
Weight – 3.3 kg

Warning:1. It is prohibited to switch on the appliance without water in it.
2. It is not allowed the test-tubes to touch the bath bottom.
3. It is necessary to change water daily.
4. It is obliged deionized or boiled water to be used.

This incubator can be used for applications different from the above test as a laboratory heater unit.

1. Preparing the unit for operation

Pour the required amount of de-ionized water, about 350 ml, in a heating pot and close it using the sealing cover. Turn on the unit and press the switch on the rear panel. The display will indicate:

WARM UP
T = .X. ºC

Which means that the unit is in warm-up mode and by that time it has reached temperature .X. ºC

2. Incubator operation modes and settings:

2.1. Having reached the preset temperature (factory setting – 44 ºC), the unit will produce a sound signal and the display will indicate:

► START 00:10:00 (provided the preset time is 10 min)
SET T= 44 ºC

Insert the milk samples, prepared in test-tubes in advance, into the perforated cover and dip them in the bath. If you select the START function using the OK switch the unit will start counting down the preset time (for example 10 min). Once heating is completed a sound signal will alert you to take out the samples for further analysis. If you test sheep milk, set the timer to 15 min due to the specifics of this product.

2.2. If, using the OK switch you select the SET function from the main menu, you will enter the unit setting modes:

- SET TEMPERATURE - press the OK switch to enter this mode and then using the ▲▼ arrows you can set temperatures within the range 0 ºC to 65 ºC ;
- SET TIMER - press the OK switch to enter this mode and then using the ▲▼ arrows you can set the required sample heating time;
- SET TIMER MODE - press the OK switch to enter this mode and then using the ▲▼ arrows you can select one of the three available time counting options:

RELOAD TIMER - this option will count the time if only there is no violation of the preset temperature limits (for example ± 1ºC). In case of such a violation the counter will reset and restart counting. Symbol **R**.

INDEPENDENT - the timer will count the preset time regardless of any violations of the preset time limits. Symbol **I**.

PAUSE TIMER - by selecting this option the timer will count the preset time and once the temperature goes beyond the preset limits it will make a pause only to resume counting after the temperature has returned within the preset range. Symbol **P**.

- SET TEMP. LIMITS - by using the ▲▼arrows you can set deviation tolerances within the limits 0.2 ºC to 5 ºC as the user may find it possible.

Press the OK switch to confirm any of the above settings. Press the MODE switch to get out of the SET mode without saving them. The latest saved TIME and TEMPERATURE settings will appear at any subsequent re-starting of this unit.