

EKOTEST

QUICK TEST FOR DETECTION OF ANTIBIOTICS AND INHIBITORS IN THE MILK



OPERATING INSTRUCTIONS

INTRODUCTION

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

TEST SENSITIVITY

The test sensitivity is sufficient to guarantee detection of any substances in suppressing concentrations for the starter culture in raw milk.

ADVANTAGES

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

APPLICATION

Ekotest is used as a control test during acceptance of raw milk or before its technological processing, as well as for pasteurized milk and in any other situations requiring fast inhibitor estimation of the milk samples.

EQUIPMENT

- Water-bath incubator
- Active substance – lyophilized / 1 g per pack / - 8 pieces
- Test-tubes – 13 pieces, with plugs and rubber rings
- Easel
- Reagent 1
- Bottle for reagent 2
- Ampoule for reagent № 2
- Instruction for test application

TEST PROCEDURE FOR ONE SAMPLE

1. 10 ml of the milk, which will be tested (**warmed in advance to 95 ° C temperature for several seconds and cooled down to room temperature**), are poured into each of two test-tubes – a test and a control one, with identical colours or numbers.
2. 1.5 ml active substance is added to each milk sample. The tubes are plugged and their contents are stirred by turning the tubes up and down.
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.
4. It is incubated for 10 minutes after reaching the necessary milk sample temperature of 44° C, and for sheep milk – 15 minutes.
5. Meanwhile, 2 drops of reagent № 1 are dropped into the control-tube and the content is stirred by turning the tube up and down. After that, reagent № 2 is added by a burette or a pipette (no less than 2 ml). The content is stirred again and drops of reagent № 2 are added till the sample becomes pale pink and the color remains the same for more than 30 seconds. The used quantity of reagent № 2 is registered.
6. Upon incubation completion, the test-tube is taken out. 2 drops of reagent № 1 are dropped into the incubated sample, too, and the content is stirred well.
7. Precisely the same quantity of reagent № 2 is added as the quantity, added to the control sample. After careful turning the tube up and down, the color of the tested sample is compared visually to the color of the control one and the result is interpreted as **follow**:

RESULT INTERPRETATION

(-) – **Negative result** – the sample is with the milk color – white. There are not inhibitors in it, i.e. the tested milk is biologically sterile and can be technologically processed.

(+) – **Positive result** – the sample is pink colored. It is either with suppressing substances in it, or the milk doesn't contain growth factors. Such samples can be additionally tested to corroborate the available inhibitor.

NOTES

1. The correct incubation temperature is approximately 44° C (± 2 °C). Deviations below 42° and above 48° will change the optimum test conditions and might decrease the test accuracy, due to the short incubation time.
2. The recommended temperature of the milk to be tested is about 20° C, for its faster tempering.
3. The milk should be tested within 24 hours and should be stored for that time at 0 - 5° C.
4. Only milk with initial acidity between 16 and 22 degrees T should be tested – for cow milk and, 16 - 24° T – for sheep milk.

5. The test results can be affected by imprecise dosage of the reagents and the test culture!
6. The reagents should be stored in a refrigerator. Before testing they should be left at room temperature for 10-15 min.

ADDITIONAL INFORMATION ABOUT INHIBITORS

Inhibitors are a wide range of alien milk components, which kill or suppress the active microorganisms in the milk. Inhibitors can be, for example:

- remainders after washing and disinfection of the milking machines;
- antibiotics and other medicinal preparations, which have been secreted or have fallen in the milk;
- available additional micro-flora;
- conservative substances in the milk.

The lack of fermentation and coagulation of the milk can be both a result of suppressing substances and of insufficient level of the microorganism growth factors, such as free Amino-acids, Fe and some vitamins of the B group. These factors are strongly affected by the season and the type of feeding, the soils, the forages and the climate as well as the animal breed and some physiological reasons.

INSTRUCTION HOW TO ACTIVATE LYOPHILIZED GROWTHS AND FURTHER APPLICATION

Obtaining activated growths:

1. Pasteurize pure and fresh cow milk, featuring acidity of 17-18 °T and containing no inhibitors or antibiotics at 98 – 100 °C and then cool it down to 43-45 °C.
2. Empty the contents of a lyophilized growth pack in 200 milliliters of milk. It is recommended first to dissolve the dry growths in a small quantity of milk, then add it to the other milk and stir thoroughly.
3. Close tightly the inoculated milk and leave it at 43-45 °C to initiate fermentation. Under these conditions the milk will coagulate for 3 to 5 hours (reaching acidity of 70 – 75 °T).
4. Now cool down the milk and store it at 4 - 6 °C for maximum 20 days.

Within 2 to 3 days the activated growths can be applied again in the above described manner. For this purpose add 1 % of the activated growth (2 - 3 % in winter) to the pasteurized milk and follow the instruction.

The life of the activated growths is about 20 days in a refrigerator, with tightly closed storage pots.

2.5 °T is adequate to 1 ° SH, or 0.0225 % milk acid.

EKOTEST HEATER BLOCK

INCUBATOR –WATER- BATH TYPE

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

It is developed by BULTEH – 2000 Ltd. in conformity with the directives for performance of quick tests for inhibitors in the milk.

This is a state-of-the-art microprocessor appliance, which is easy and reliable to operate. All settings are preset in the power-independent appliance memory. When operating, the data are displayed on an LCD display. The appliance is equipped with a timer, which, by the help of the keypad buttons, is set by the user to count the time. The sound signalization and the displayed messages considerably facilitate the operation of the appliance, even if it is done by an unskilled operator. This appliance is suitable both for laboratories and for field work (12 V powered is an option).

Technical data:

Incubation temperature – 30°-65° C – adjustable

Accuracy of temperature setting - $\pm 0.5^{\circ}$ 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 $\varnothing 16$ mm

Water amount for the thermo-bath – 350 ml

Power supply – 220 V; 50 Hz

Consumed power – for heating – 60 W
– for stabilization – 20 W

Overall sizes – 104/ 238/ 275 mm

Weight – 3.3 kg

- Warning:**
1. It is not allowed to switch on the appliance without water in it.
 2. The test-tubes should not touch the bath bottom.
 3. It is necessary to change the water on a daily basis.
 4. The use of de-ionized or boiled water is mandatory.

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 the thermo-bath and close it with the sealing cover. Turn on the unit and press the button on the rear panel. The display will indicate:

WARM UP

T = .X. °C

This means that the unit is in warm-up mode and by that time it has reached temperature .X. °C

2. Incubator operation modes and settings:

2.1. Having reached the preset temperature (factory setting – 44 °C), 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 °C

Insert the milk samples, prepared in the test-tubes in advance, into the perforated cover and dip them in the bath. If you select the START function, using the OK button, the unit will start counting down the preset time (for example, 10 min.). Once the 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 specific character of this product.

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

- SET TEMPERATURE - press the OK button to enter this mode and then using the ▲ ▼ arrows you can set temperatures within the range 0 °C - 65 °C ;
- SET TIMER - press the OK button to enter this mode and then using the ▲ ▼ arrows you can set the required sample heating time;
- SET TIMER MODE - press the OK button 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 only if there is no deviation from the preset temperature limits (for example $\pm 1^{\circ}\text{C}$). In case of such a deviation, the counter will reset and restart counting. Symbol **R**.

INDEPENDENT - the timer will count the preset time regardless of any deviations from 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 the deviation limits within the range from 0.2 °C to 5 °C as the user may find acceptable.

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

The Incubator is offered to the clients with ready factory settings for operation of the test procedure.

**List of antibiotics and drugs with inhibition in raw and pasteurized milk
detected by EKOTEST**

/ by prof. Baltadgieva and Laboratory "LB Lact "/

ANTIBIOTICS AND DRUGS	Minimal inhibition dose mg/ml
Amoxicillin	0,005
Ampicillin	0,005
Cephalexin	0,07
Cephtiofur	0,07
Sulfathiazole	0,1
Sulfadimethoxine	0,1
Gentamycin	0,3
Penicillin G	0.002
Pharmazin	0.05
Cephazolin	0.05
Oxytetracycline	0.1
Tetracycline	0.1
Bacitracin	0.1
Erythromycin	0.4
Lipomycin	0.2
Lincospectin	0.02
Pandex	0.05
Mastijet	0.05
Streptomycin	0.25