



A Rapid Test For β -lactams Detection in MILK

OPERATING INSTRUCTIONS

I. Introduction

Betaxpress is a rapid test allowing the detection of the presence of β -lactam molecules in a milk sample.

II. Summary of the protocol

- Add 200 μ l of milk into one reagent microwell and mix to homogeneity;
- Incubate 90 s at 45°C;
- Dip one Dipstick into each microwell;
- Continue incubating for 90 s at 45°C;
- Read the colour intensities.

III. Reaction Mechanism

Betaxpress is a competitive test involving a receptor for the recognition of β -lactam molecules present in the Milk. The test requires the use of two components. The first component is a microwell containing a predetermined amount of receptor linked to gold particles. The second is a dipstick made up of a set of membranes with two specific capture lines. For a valid test, the upper red control line has to be visible after the second incubation (see Figure A). The β -lactam "test" line is placed below the control line. When the reagent from the microwell is re-suspended with a milk sample, the receptors will bind β -lactam if present during the first 90 s incubation at 45°C. Afterwards, when the dipstick is dipped into the milk, the liquid starts running vertically on the dipstick and passes through capture zones. When the sample is free of β -lactam, a colour development occurs at the first capture line, indicating the absence of β -lactam in the milk sample. On the contrary, the presence of β -lactam in the sample will not cause the coloured signal to appear at the first capture line.

IV. Composition of kits

Betaxpress Milk Kits contain everything needed to perform 96 measurements.

- 12 pots each with 1 strip of 8 reagent microwells and 8 dipsticks;
- 1 Micropipette of 200 μ l;
- « Positive Standard » containing powder to reconstitute "positive standard" raw milk of PenG. For ease of recognition, the Positive Std is stained by a dye to give it a very pale red colour;
- « Negative Standard » containing powder to reconstitute "negative standard" raw milk. The Negative Std is stained by a dye to give it a very pale green colour;
- One instruction sheet.

V. Additional material needed

- 1 Heatsensor (45°C incubation, refer to the used Heatsensor manual).
- 1 Readsensorm (optional, refer to the Readsensorm manual).

VI. General Remarks

1. If instrumental reading is chosen for result interpretation, the Readsensord must be switched on before the analysis (see Readsensord manual);
2. At reception, store the kit in a dry place and at a low temperature between 2°C and 8°C. Before opening, let the plastic pots reach room temperature and avoid exposure of the product to moisture and light;
3. The Standard Glass vials have to be re-hydrated with 1 ml (5 X 200µl) of Pure Water. Mix vigorously (vortex) to avoid clots. The reconstituted glass vial can be stored in the freezer at -20°C. Do not freeze/thaw more than once.
4. Avoid using clotted milk with Betaxpress;
5. The best temperature to perform the test is **45°C ± 3°C** . Use the “Heatsensord” (or alternatively a water bath). Any other type of incubator is not appropriate to perform the Betaxpress assay. (Refer to the used Heatsensord manual for setting at right temperature and timing);
6. After the second incubation, read the result within a 10-minutes time-frame. Do not attempts to interpret the result after 10 minutes;
7. When drying, the colour intensities of the lines will become sharper;
8. When a positive result is recorded, the test result should be confirmed;
9. Empty one pot before opening another pot.

VII. Milk powder dilution

In an appropriate bottle, mix 10 g of milk powder with 90 ml of warm (40°C) and distilled water. For a n optimal dilution, shake vigorously.

VIII. Directions for use

This procedure is described to easily run one single sample or a set of many samples. In that case, try to perform the test in cascade and avoid any delays when mixing reagent and milk but also when adding and removing dipsticks. Make sure you have the same incubation time and temperature for each sample. You shouldn't test more than 8 samples at one time and, if there are more than 3 samples, you should use a multipipette. With more than 8 samples we recommend to share series of maximum 8 samples.

1. Choose a clean and dry place to perform the test and wash and dry your hands before starting;
2. Connect the Heatsensord (refer to the used Heatsensord manual) and wait until the temperature has stabilised at 45°C.
 - *It takes about 10 minutes for the temperature to stabilize at 45°C;*
3. Before opening the reagents, take the kit out of the fridge and wait until the temperature of the reagents reaches the ambient temperature. Meanwhile, read the directions for use attentively;
 - *There are two main components used for the test which are: dipsticks and the freeze-dried reagents in microwells. Both are stored in the white plastic pots.*
4. Determine how many samples are to be tested and write on each tube an identification number;
 - *The milk sample must be liquid and homogeneous. There can be neither clots nor sedimentation phases. The ideal temperature of the milk sample is between 4 and 20°C.*
5. Open one plastic pot and take out as many microwells as there are milk sample to be tested (the standard positive and negative milk included if necessary);
 - *To open a pot of dipsticks, take off the safety ring by pressing it down the pot, take off the ring and get the stopper off the pot with your thumb;*
 - *The pot with dipsticks should always be well closed after reagents have been taken out;*
 - *A pot with dipsticks should be emptied before another is opened;*
 - *Be careful, if you do not intend to use all the 8 microwells, leave the set of 8 caps on the unused ones and do not tear off the strip of the eight caps but leave it on the microwells that will not be used. Do not try to separate individual caps and put them immediately back into the white pot without damaging the dipsticks, close and make sure it is tightly sealed.*

6. Place the microwell(s) in the heating block which shows 45°C;
7. Place a new tip on the micropipette and transfer 200 µl of milk into each of the microwells;
8. **Warning: when reagents and milk are in contact, the reaction begins. Mix quickly AND IMMEDIATELY push the START(RUN) button. The 90 s countdown starts;**
 - *During that first incubation, the receptors detect whether or not there is β-lactam in the milk sample. It takes 90 s for the reaction to be completed.*
9. During that time, open the same pot as before, take out as many dipsticks as there are analyses in progress and close the pot. Lay the dipsticks on a clean sheet and write down the number that matches the one of the milk sample;
10. When the 90 s are over, i.e. after the sound-signal, press START (STOP)* again to stop the ringing tone and dip the corresponding dipstick into each of the microwells laid in the incubator;
 - *Make sure you put into the corresponding microwell the dipstick with the same identification number as the one of the concerned milk sample;*
11. Start the timer for the second incubation by pressing the START (RUN)* button. The 90 s countdown starts;
12. When the 90 s are over, i.e. after the sound-signal, press START (STOP)* again to stop the ringing tone and take the dipsticks out of the microwells to lay them down on a sheet of paper;
13. If you are not planning to perform any other test within the day with Betapress, put everything back into the box and store it in a fridge at a temperature ranging from 2 to 8°C.

**(Refer to the used Heatsensor manual – For DUO Heatsensor users, steps 9 to 12 are simplified)*

IX. Visual interpretation of results (see Figure A)

Make visual readings as follows:

14. First check whether the top control line is present. If it is not, regard the analysis as invalid and do not start (or continue) any interpretation.
15. When the top control line can be seen, interpret the test line as follows:
16. Compare the intensity of the line colour of the test line with the intensity of the line colour of the control line.
 - If the test line is darker in colour than the control line, the result is **NEGATIVE**, which means that, given the sensitivity of our test, the milk sample contains no β-lactams or β-lactams at a lower level than the value stated in the enclosed table A;
 - If the test line is as distinct as or lighter in colour than the control line, the result is **POSITIVE**, which means that, given the sensitivity of our test, the milk sample contains β-lactams at or above the detection value stated in the enclosed table A.
17. If you hesitate, regard the sample as **POSITIVE** and confirm your interpretation by performing a second visual reading within the next 15 minutes;
18. Write down your assessment on each of the dipsticks;
19. Dipsticks can be archived as a permanent record if required by removing the sample pad and allowing to dry before storage. N.B. Line colour intensity will darken on drying.

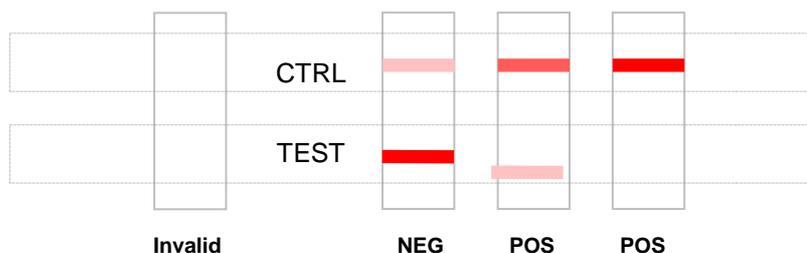


Figure A: Visual interpretation.

If you have a Readsensor, you should read the dipstick within 10 minutes of performing the test. See the user manual of Readsensor.

X. Sensitivity

In case of positive results, your milk sample is considered to contain equal or higher concentration of individual β -lactams specified in the table below.

Table A. Sensitivity of Betaxpress Milk Assay.

Group	Substance	MRL ($\mu\text{g}/\text{kg}$)	Test sensitivity ($\mu\text{g}/\text{kg}$)
Penicillins	Penicillin G	4	2-3
	Ampicillin	4	3-4
	Amoxicillin	4	3-4
	Oxacillin	30	8-10
	Cloxacillin	30	7-9
	Dicloxacillin	30	5-6
	Nafcillin	30	85-105
Cephalosporins	Ceftiofur	100	8-12
	Desfuroylceftiofur	100	60-80
	Cefquinome	20	16-20
	Cefazolin	50	10-14
	Cephapirin	60	3-5
	Desacetylcefapirine	60	16-20
	Cefacetrile	125	20-25
	Cefoperazone	50	1-2
	Cefalexin	100	700-800
	Cefalonium	20	2-3

XI. Negative and Positive standards reconstitution

1. Remove the cap and rubber stopper from the vial and add 1 ml of distilled or deionised water;
2. Replace the stopper and leave to stand for 1 minute and a half;
3. Vigorously shake the sample during 10 seconds;
4. Check that the sample is properly dissolved.

The test material is now ready to use.