TwinSensor40°C

Rapid test for detection of β-lactam and tetracycline antibiotics

Introduction:

TwinSensor is a rapid test in dipstics form that allows you to detect simultaneously the presence of β-lactam and tetracycline antibiotics in cow, goat, buffalo and sheep milk.\

Reaction mechanism:

Twinsensor is a competitive test involving two receptors in one single operation. The test requires the use of two components. The first component is a microwell containing predetermined amounts of both receptors and antibodies linked to gold particles. The second is a dipstick made up of a set of membranes with specific capture lines.

The control line has red colour; it is visible all the time and serves for comparison. The other two are the specific "test" lines placed on both sides of the control line. The line for ß-lactam antibiotics is located below the "control" while the line relating to tetracyclines is located above it. When the reagent from the microwell is re-suspended with a milk sample, both receptors will bind the corresponding analytes if present during the first 3-minute incubation at 40°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 antibiotics, a colour development occurs at the specific capture lines, indicating the absence of the targeted analytes in the milk sample. On the contrary, the presence of antibiotics in the sample will not cause the coloured signal to appear at the specific capture lines.

Table 1 – ## Limits of detection:

Antibiotics		**Limits of detection ppb (µg/l)**
β-lactam antibiotics	Penicillin-G	2 - 3
	Ampicillin	3 – 5
	Amoxicillin	3 – 5
	Oxacillin	12 - 18
	Cloxacillin	6 – 8
	Dicloxacillin	6 – 8
	Nafcillin	30 – 50
Cefalosporins	Cefacetrile	30 - 40
	Cefalexin	>750
	Cefalonium	3 – 5
	Cefazolin	18 – 22
	Cefoperazone	3 – 4
	Cefquinome	20 - 30
	Ceftiofur	10 – 15
	Cephapirin	6 – 8
Tetracyclines	Chlortetracycline	30 – 40
	Doxycycline	10 – 15
	Oxytetracycline	50 – 60
	Tetracycline	80 – 100

Directions for use:

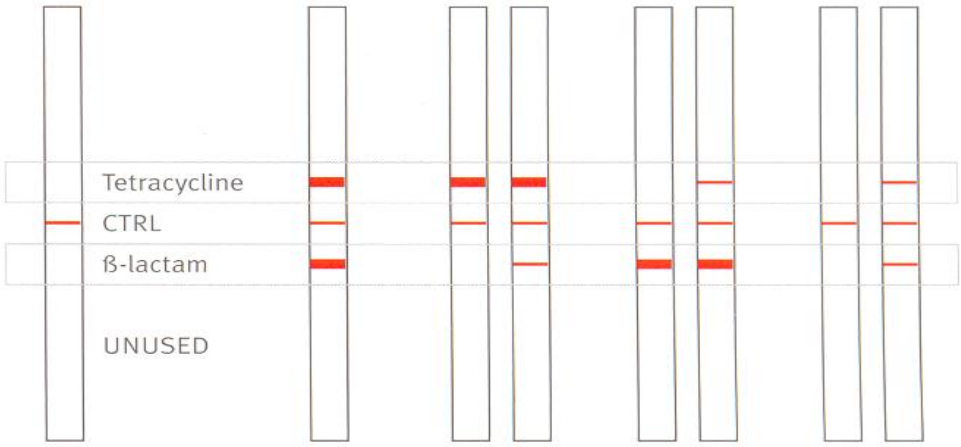
This procedure is described to easily run one single sample or a set of many samples. The optimal temperature of the milk sample is 4˚C-20˚C.

1. Connect the incubator and wait until the temperature of the heating block is stabilized at 40^0C.
2. Open the package containing the microwells with reagent and take out so many microwells as there are milk samples to be tested.
3. Place a tip on the minipipet and transfer 200µl of milk in the microwell. With the minipipet and the tip mix the milk in the microwell until receiving homogenous mixture with a rose colour.
4. Use a new tip for every next sample.
5. Place the microwells in the incubator and start the first 3-minute incubation.
6. During the incubation, open the same pot as before, take out as many dipsticks as there are analyses in progress and close the pot. We recommend you to write identification numbers of the samples directly on the white cellulose paper of the dipsticks.
7. When the 3 minutes are over (you will hear a sound-signal), dip the corresponding dipstick into each of the micro-wells so that the arrow points downwards and start the second 3 minutes incubation. Leave the microwells in the incubator during the second incubation as well.
8. After the end of the second incubation (you will hear a sound-signal), take the dipsticks out of the microwells. There are 1, 2 or 3 red lines on the dipstics. Read the result visually or instrumentally directly.
9. If you are not planning to perform any other test make sure that the pots are hermetically closed. Put them in a fridge at a temperature ranging from 2 to 8^0C.

When you have more collected samples you can test them in series, avoiding any delays when you mix milk and reagent or when dipping or taking the strip out. **Make sure that you use the same incubation time and the same temperature for each of the samples**. If you have more than 8 samples to test we recommend you to divide the series of a maximum of 8 samples.

Visual interpretation of results:

Comparing the colour intensity of the test lines with the control line allows visual interpretation of results. The bottom (under the control line) “Test” line is for β-lactam antibiotics and the upper one (above the control line) for tetracyclines.



- If the test lines are more distinct (visible) than the control line, the milk is negative which means that the milk sample contains fewer antibiotics than the value indicated in the table (that means there are no residues of β-lactam and tetracycline antibiotics in quantities above the permitted MRL limits).
- If the test lines are less sharp or as distinct as the control line, the milk is positive, which means that the milk sample contains as many or more antibiotics than the value indicated in table
- If the test lines are not visible, the milk sample contains much far β-lactam and tetracycline antibiotics.

If you hesitate, regard the sample as positive and confirm your interpretation by performing a second visual reading within the next 4 minutes.

Composition of a kit for performing 96 analyses:

- 96 microwells
- 96 dipsticks
- 1 minipipet of 200µl + 96 disposable tips
- "Positive Standard" – milk powder that after the reconstitution contains 4ppb Penicillin G and 100ppb Oxytetracycline. The "Positive Standard" has a reddish colour.
- "Negative Standard" – milk powder that after the reconstitution does not contain antibiotics. The "Negative Standard" has a greenish colour.
- 1 information notice
- 1 certificate of analyses

General remarks:

- At reception, store the kit in a dry place and at a low temperature between 2 and 8^0C;
- Before opening, let the plastic pots reach room temperature and avoid exposure of the product to moisture and light.
- The Standards are in glass vials or in individual plastic pots. The Positive Standard in the glass vial has to be re-hydrated with 1 ml of Pure water and the Negative Standard in the plastic pots has to be re-hydrated with 200µl of Pure water each. Mix vigorously to avoid clots. The reconstituted vial can be stored in the freezer at -20^0C. Do not freeze more than one time.
- Avoid using clotted milk with Twinsensor.
- The best temperature to perform the test is 40^0C±3^0C.
- **Read the result within a 15-minute time-frame after the start of the second incubation. Do not do it after the 15 minutes.**
- Instrumental reading may be done with Readsensor. When drying, the colour intensities of the lines will become sharper.
- When positive result is recorded, the test results should be confirmed.

Dilution of milk powder:

Mix 10g of milk powder with 90ml warm (40^0C) distilled water in a container. Shake vigorously for optimal dissolution.