TwinSensor RT

A Rapid Test For **ß-lactams and Tetracyclines** Detection **in MILK**

I. Introduction

Twinsensor RT (KIT088) is a rapid qualitative test that allows you to simultaneously detect the presence or absence of both ß-lactam and Tetracycline molecules in a milk sample <u>without</u> incubator (*i.e.* at room temperature).

Twinsensor RT is a competitive test involving specific receptors and antibodies 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.

When the reagent from the microwell is re-suspended with a milk sample, both receptors and antibodies will bind the corresponding analytes if present during the first 4-minute incubation at room temperature. Afterwards, when the dipstick is dipped into the milk, the liquid starts running vertically on the dipstick and passes through capture zones. If the sample is free of antibiotics, a color development occurs at the 'test' lines, indicating the absence of the targeted analytes in the milk sample. On the contrary, the presence of antibiotic(s) in the sample will impair the reagent to color the 'test' line. In any case, milk has to migrate at least to the upper part of the dipstick after the second incubation.

Limits of detection:

β-LACTAMS (ppb)			TETRACYCLINES (ppb)		
Penicillins	**EU MRL**	**LOD**		**EU MRL**	**LOD**
Penicillin G	4	1 - 2	Tetracycline	100	80 - 100
Ampicillin	4	3 - 4	Oxytetracycline	100	40 - 60
Amoxicillin	4	3 - 4	Chlortetracycline	100	30 - 40
Oxacillin	30	14 - 20	Doxycycline	Prohibited	10 - 15
Cloxacillin	30	10 - 14			
Dicloxacillin	30	7 - 8			
Nafcillin	30	> MRL*			
Cephalosporins	**EU MRL**	**LOD**			
Ceftiofur	100	7 - 10			
Cefquinome	20	20 - 30			
Cefazolin	50	7 - 10			
Cephapirin	60	2 - 3			
Cefacetrile	125	< MRL*			
Cefoperazone	50	0.5 - 1			
Cefalexin	100	850 - 1100			
Cefalonium	20	1 - 2	* To be determined		

Table 1: *Limits of detection of Twinsensor RT Milk Assay.*

II. Summary of the protocol

- Add 200 µl of milk into one reagent microwell and mix gently to homogeneity

- Incubate 4 min at room temperature

- Dip one dipstick into each microwell

- Continue incubating for 6 min at room temperature

- Remove the dipstick and stop the reaction by removing the sample pad

- Read the results

III. Composition of kits

Twinsensor RT Milk Kits contain everything needed to perform 96 measurements.

- 12 tubes each with 1 strip of 8 reagent microwells and 8 dipsticks
- 1 micropipette of 200µl and disposable tips
- 1 empty tray for microwell strips
- The user manual
- The certificate of compliance

Additional equipment/material needed but not included:

- Timer/Watch
- Optional: 1 ReadSensor (refer to the ReadSensor manual)

IV. General Remarks

1. Store the kit at a temperature between 2°C and 8°C in a controlled fridge.

2. Avoid repeated temperature variation. Do not expose to temperature over 35°C for longer period than a day and over 25°C for 7 days (shipment included).

3. Avoid exposure of the reagents to moisture and light.

4. Do not mix components included controls of kits from different batches.

5. Do not use the kit after the expiration date (see expiry date on the box / 12 months after production date).

6. Avoid touching the membrane on the dipstick.

7. Avoid leak of reagent during sample addition and mixing step.

8. Empty one tube before opening another tube.

9. Close accurately the tube after use.

10. The temperature to perform the test is 18 to 25°C (room temperature);

11. The timing to perform the test is 4 minutes + 6 minutes. This timing must be respected, no extra time (second) is allowed (the reading process must be done within 2 min).

12. When drying, the color intensities of the lines will become sharper;

13. When a positive result is recorded, the test result should be confirmed by performing at least two more tests.

Limitation of use:

1. Avoid to expose the dipstick to direct air flow like air conditioner, open windows, … during migration

2. Respect recommended temperature for sample (4°C to 8°C)

3. Do not use milk outside milk type limitation described into chapter V

4. Do not use assay in environment where relative humidity is outside normal range (20%-80% at 20°C)

5. Do not use the assay with room temperature outside recommended range (18°C to 25°C)

6. Do not leave dipstick outside the tube for a long period before to be use

Potential interferences:

Currently no formal interference studies have been performed. Any foreigner molecules may lead incorrect results.

V. Milk type

The current kit is only compatible with **raw cow milk**.

- Raw commingled cow's milk should be selected and at 4°C-8°C before testing.
- The cooled raw milk should be tested within 48 hours after milking.

- The milk sample must be liquid and homogeneous. There can be neither clots nor sedimentation phases. Any protein precipitates or lipid segregation will be indicating that the milk is not suitable for the test.

- No frozen, transformed or powder milk can be tested.
- High fat samples may impact the results.
- Level of somatic cells or bacterial presence and pH of milk may have an impact on result.

If the sample is not respecting these limitations, Unisensor will not warranty the trueness of results recorded.

VI. Directions for use

Before starting

1. Read the instruction carefully.
2. Choose a clean and dry place to perform the test. Wash and dry your hands.
3. Reader (Optional) must be clean.
4. Before opening the reagents, take the kit out of the fridge and wait until the temperature of the reagents reaches the room temperature (from 20 min to 30 min).
5. If instrumental reading is chosen for result interpretation, switch on the « ReadSensor » (Optional; see ReadSensor manual). Make sure the check /calibration was performed according to the user manual of ReadSensor.
6. Check expiry date before to use.

Testing steps

1. Identify all samples with a number.
2. Open a tube and take out as many microwells as there are milk samples to be tested. Empty one tube before opening another one. Be careful to leave the protecting film on the unused microwells. Immediately put the remaining reagents back into the white tube without damaging the dipstick, close the tube and make sure it is tightly sealed.

Testing more than 6 samples at a time is not recommended. In the case of many samples have to be analysed, try to perform the tests in cascade to avoid any delays occurring during the manipulations. For more than 1 sample, consider using a multichannel pipette. Make sure you have the same incubation time for each sample.

3. Place the microwell(s) in the empty tray for microwell strips.
4. For each sample, place a new disposable tip on the micropipette. Quickly transfer 200µl of milk into one microwell. Mix as quick as possible by aspirating and deliberating sample to ensure that freeze dried reagent and milk are mixed together. The initial incubation of 4 minutes begins.

WARNING: when reagents and milk are in contact, the reaction starts. Make sure the time for sample preparation and handling is minimal

5. During this first incubation, take out as many dipsticks as there are analyses in progress. Close the tube and be sure that it is tightly sealed.
6. After the initial incubation, dip the corresponding dipstick into each microwell before re-starting the timer for 6 minutes. The second incubation / vertical migration of 6 minutes begins.

7. Immediately when the incubation with the dipstick(s) are over, gently remove the sample pad without damaging the central membrane.

8. Proceed to the interpretation by visual observation or with the ReadSensor (as described below) within 2 minutes.

9. If you are not planning to performed any other test in the next 8h, put the tube back for storing into the fridge (2°C to 8°C).

10. Never store back wet used dipstick into the tube containing unrevealed dipstick or in closed container.

VII. Interpretation of the results

Visual interpretation:

1. If the milk has not migrated correctly* (see Figure 1) **and** if the control line is not visible (with or without the presence of a test line), do consider the test as not valid; don't interpret the result and directly run another test.

     *If lines are not straight, blurred, or uneven the test is invalid.

2. If the test is valid, examine one test line at a time and compare the color intensity to that of the control line.

    o If the test line is darker in color than the control line, the result is NEGATIVE. Given the sensitivity of our test, the milk sample contains no antibiotics or antibiotics at a lower level than the value (limit of detection) mentioned in table 1.

    o If the test line's color intensity is similar, lighter (absent) compared to the control line, the result is POSITIVE. Given the sensitivity of our test, the milk sample contains antibiotics at or above the value (limit of detection) mentioned in table 1.

3. When you have interpreted one test line, do the same for the other line.

4. Write down your assessment on each dipsticks;

NOTE

1. If you hesitate, consider the sample as POSITIVE and confirm your interpretation by performing at least two more tests.

2. Dried dipsticks can be archived as a permanent record if the sample pad has been removed just after the end of the test. Allow the strip to dry before storage. Please note that the color intensity can vary with drying. Never store back wet used dipstick into the tube containing unrevealed dipstick and in closed container.
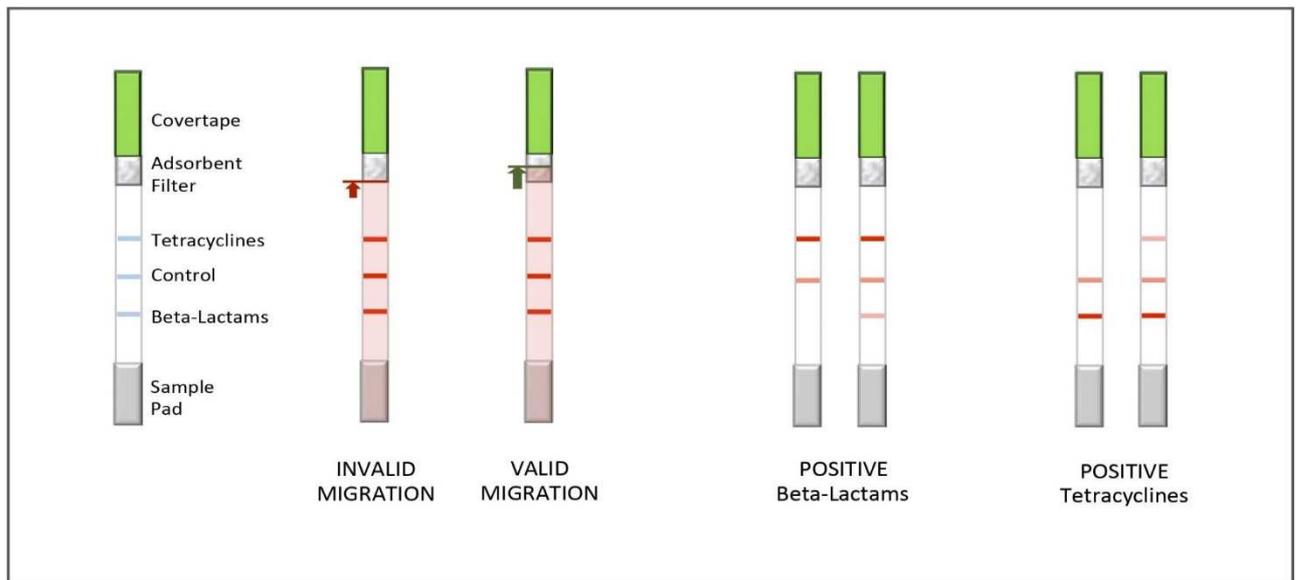

Figure 1: Visual check and interpretation.

ReadSensor interpretation (See ReadSensor manual):

1. Make sure you have entered the appropriate version of the reading method into your ReadSensor (Check Unisensor Website for the latest appropriate versions – appropriate version: see certificate of compliance).

2. If the milk has not migrated correctly* (see Figure 1) **and** if the control line is not visible (with or without the presence of a test line), do consider the test as not valid; don't interpret the result and directly run another test.

      *If lines are not straight, blurred, or uneven the test is invalid.

3. If the test is valid, the dipstick can be read by ReadSensor within 2 minutes of performing the test.