unisensor
DIAGNOSTIC ENGINEERING

# OPERATING PROCEDURE


3LITE

## PRODUCT REFERENCE
LITE001

English

Version 1.2 • March 23, 2020.

# CONTENTS

# CHAPTER 1 - INTRODUCTION

3LITE (LITE001) is a rapid qualitative test that allows you to simultaneously detect and discriminate the presence or absence of ß-lactam, Sulfonamide and Tetracycline molecules in a milk sample.

## Summary of the protocol

1. **Add 100 µl of milk into one reagent microwell and mix to homogeneity;**
2. **Incubate 3 min at 40°C;**
3. **Dip one Dipstick into each microwell;**
4. **Continue incubating for 3 min at 40°C;**
5. **Remove the dipstick and stop the reaction by removing the sample pad;**
6. **Read the results**

## Reaction mechanism

**3LITE (LITE001)** is a competitive test involving two receptors and generic monoclonal 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.

For a valid test, the upper red control line has to be visible after the second incubation (see **Figure A**).

The other three are specific "test" lines placed below the control line. The line for **ß-lactam** antibiotics (penicillins and cephalosporins) is located below the **sulfonamide** line while the line related to **tetracyclines** is located above it.

When the reagent from the microwell is reconstituted with a milk sample, both receptors and monoclonal antibodies will bind the corresponding analytes if present during the first 3-minutes 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 color development occurs at the specific capture lines, indicating the absence of the targeted analytes in milk sample. On the contrary, **the presence of antibiotics in the sample** will not cause the colored signal to appear at the specific capture lines.

# CHAPTER 2 - COMPOSITION OF THE KIT

3LITE Kits contain everything needed to perform 480 measurements.


### TEST TUBES

30 tubes each with 2 strips of 8 reagent microwells and 16 dipsticks.


### NEGATIVE CONTROLS

1 tube with 1 strip of 8 negative control microwells. The negative control contains a dye to give it a very pale green colour.


### POSITIVE CONTROLS

1 tube with 1 strip of 8 positive control microwells. For ease of recognition, the positive control contains a dye to give it a very pale red colour.

## ADDITIONAL MATERIAL NEEDED

- HeatSensor (40°C incubation, refer to the HeatSensor User Manual) with its 3mm dipstick adapter;
- Readsensor 2 (optional, refer to the Readsensor 2 User Manual) with its 3mm dipstick adapter;
- Readip (optional, refer to the Readip User Manual) with its 3mm dipstick adapter;
- 1 micropipette - 100µL (we recommend the use of LILPET-100 from MICROLIT Company);
- Tips;
- Distilled water;
- 3LITE Certificate of Analysis and Operating Procedure can be downloaded in the download center on the Unisensor website (www.unisensor.be).

# CHAPTER 3 - GENERAL REMARKS

- If an instrumental reading is chosen for result interpretation, the Readsensor 2 or Readip must be switched on before the analysis (see Readsensor 2 or Readip User Manual);
- 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 tubes reach room temperature and avoid exposure of the product to moisture and light;
- The milk sample must be liquid and homogeneous. There can be neither clots nor sedimentation phases. The ideal temperature of the milk sample, before the analysis, is between 4 and 8°C;
- Use the entire content of one tube before opening the next one;
- Do not mix components of kits from different batches;
- Avoid the loss of reagents during sample addition and mixing step;
- The best temperature to perform the test is 40°C ± 1°C. Any other type of incubator than HeatSensor is not appropriate to perform the 3LITE assay. The use of other incubators than indicated in this document is the responsibility of the client and does not bind Unisensor (refer to the HeatSensor User Manual for setting at right temperature and timing);
- The timing to perform the test is 3 minutes + 3 minutes; no extra time is allowed;
- After the migration step, take out the dipsticks immediately, remove the sample pad and read the result immediately. Do not attempt to interpret the result after 3 minutes;
- When drying, the color intensities of the lines will become sharper;
- When a positive result is recorded, the test result should be confirmed.

## Limitation of Use

- The room temperature should be between 18°C and 24°C.
- Avoid to expose dipsticks to direct air flow like air conditioner, opened windows, … during migration;
- Respect recommended temperatures for milk samples storage (4°C to 8°C);
- Do not use other milks than fresh raw cow milk;
  - *Milk should be tested within 48 hours after milking*
  - *No frozen, transformed or powder milk can be tested*
  - *High fat samples may have an impact on the results*
  - *Level of somatic cells or bacterial presence may have an impact on results*
- To make 3LITE measurements reliable, pH of the milk must be between 6.5 and 7.2.
- Do not use 3LITE in environment where relative humidity is outside the normal range (20%-80% at 20°C);
- Do not leave dipsticks outside the tube for a long period before use.

# CHAPTER 4 - OPERATING PROCEDURE

## Preparation

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 2 samples, you should use a multichannel pipette. With more than 8 samples we recommend to run series of maximum 8 samples.

### 1. Site preparation

Choose a clean and dry place to perform the test and wash and dry your hands before starting.

### 2. Start the incubator

Start the **HeatSensor** (refer to the HeatSensor User Manual), select the UNI Method and wait until the temperature has stabilized at 40°C.

Place the adapter for 3mm dipsticks on the top of the HeatSensor.


### 3. Take the kit out of the fridge

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 carefully the operating procedure.

Determine how many samples are to be tested and write an identification number on each milk sample tube;

### 4. Open one plastic tube

Take out as many microwells as there are milk samples to be tested (the positive and negative controls included if necessary).

- *If you do not intend to use all the 8 microwells, leave the sealing plastic foil on the unused ones.*
- *Put back unused microwells immediately into the white tube without damaging the dipsticks; close and make sure it is tightly sealed.*
- *The plastic tubes should always be well closed after reagents have been taken out.*

# CHAPTER 4 - OPERATING PROCEDURE


## 5. Microwell(s) on HeatSensor

Place the microwell(s) in the heating block which shows 40°C;


## 6. Transfer 100 µl of milk

**Immediately** transfer with a new tip 100 µl of milk into each of the microwells, then mix by pipetting up and down 5 to 10 times.

***Warning**: when reagents and milk are in contact, the reaction begins.*

**Then, IMMEDIATELY push the START(RUN) button.**
The 3-minutes countdown starts.

## 7. Place dipsticks into the HeatSensor

During that time, open the same tube as before, take out as many dipsticks as there are analyses in progress and close the tube. Lay the dipsticks on a clean surface and identify them according to the milk sample IDs. Place the dipsticks into the HeatSensor incubator above the corresponding microwells.

**When the 3 minutes are over, dipsticks automatically fall into the microwells. The migration step of 3 minutes begins.**

Make sure that each dipstick falls correctly into their corresponding microwell.

## 8. Sample pad removal

When the 3 minutes are over, i.e. after the sound-signal, press START (STOP)* again and take the dipsticks out of the microwells to lay them down on a sheet of paper. **Immediately** remove the sample pad and proceed to the dipstick interpretation.

*If you are not planning to perform any other test within the day, put everything back into the bag and store it in a fridge at a temperature ranging from 2 to 8°C.*

# CHAPTER 5 - RESULTS

## FIGURE A: Visual interpretation of the results


Make visual readings as follows:

1. Check whether the top control line is present. If it is not, consider the analysis as invalid and do not start (or continue) any interpretation;
2. When the top control line can be seen, interpret the three test lines as follows:
3. Examine one test line at a time and compare the intensity of the line color of the test line with the intensity of the line color of the control line. Start with the bottom line of ß-lactam antibiotics for example;

    - *If the test line is darker than the control line, the result is NEGATIVE, which means that, given the sensitivity of our test, the milk sample contains no antibiotics or antibiotics at a lower level than the value stated in the enclosed table A;*
    - *If the test line is lighter than the control line, the result is POSITIVE, which means that, given the sensitivity of our test, the milk sample contains antibiotics at or above the detection values stated in the enclosed table A.*

4. When you have interpreted one test line, do the same for the other lines;
5. If you hesitate, consider the sample as POSITIVE
6. Write down your assessment on each of the dipsticks;
7. Dipsticks can be archived as a permanent record if required, by allowing dipstick to dry before storage. Note: line color intensity will darken on drying.

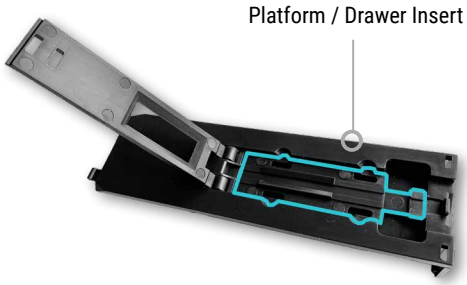
### NOTE

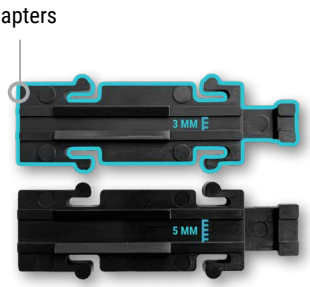
If you are equipped with a **ReadSensor 2** or **Readip**, you should read the dipstick with the 3 mm dipstick adapter (reference: APP110 for ReadSensor 2 - APP113 for Readip) within a 3-minute time frame after the end of the test. See the ReadSensor 2 (or Readip) User Manual.

# CHAPTER 5 - RESULTS

## USING READSENSOR 2

| MATERIALS NEEDED | |
|---|---|
| APP110 | Platform / Drawer Insert |
| | 5 MM Adapter |
| | 3 MM Adapter |




### 1. Device Calibration

Place the **5 mm** adapter in the drawer and use the APP105 calibration dipstick provided with the device.

Refer to APP088 User Manual for detailed calibration procedure.

### 2. 3LITE Test Reading

Place the **3 mm** adapter in the drawer and refer to APP088 User Manual for detailed procedure.

## HOW TO REMOVE THE READSENSOR 2 ADAPTER?

- Remove the insert from the drawer using its two front lugs;
- Pull forward on both lugs (the drawer detaches and tilts);
- Move forward to detach the back of the insert;

- Replace the drawer insert;
- Slide the insert on its drawer with a slight inclination to clip the back of the latter;
- Press down to snap the front of the insert.

# CHAPTER 5 - RESULTS

## TABLE A: Limits of detection*

| B-LACTAMS (ppb) | | SULFONAMIDES (ppb) | | TETRACYCLINES (ppb) | |
|---|---|---|---|---|---|
| **PENICILLINS** | | **Sulfadiazine** | 5-7 | **Tetracycline** | 80 - 100 |
| **Penicillin G** | 2.5-3.5 | **Sulfapyridine** | 0.5-1 | **Oxytetracycline** | 60-70 |
| **Ampicillin** | 3-4 | **Sulfathiazole** | 7.5-8.5 | **Chlortetracycline** | 30-40 |
| **Amoxicillin** | 3-4 | **Sulfamethoxazole** | 320-360 | **Doxycycline** | 20-30 |
| **Oxacillin** | 13-15 | **Sulfamethazine** | 1-2 | | |
| **Cloxacillin** | 7-9 | **Sulfamethoxypyridazine** | 2-3 | | |
| **Dicloxacillin** | 4-6 | **Sulfadimethoxine (Sdm)** | 10-15 | | |
| **Nafcillin** | 50-70 | **Sulfacetamide** | 300-600 | | |
| **CEPHALOSPORINS** | | **Sulfamerazine** | 2-3 | | |
| **Ceftiofur** | 16-20 | **Sulfamonomethoxine** | 8-12 | | |
| **Cefquinome** | 16-20 | **Sulfaquinoxaline** | 13-17 | | |
| **Cefazolin** | 15-19 | **Sulfachloropyridazine** | 5-10 | | |
| **Cephapirin** | 3-5 | **Sulfaguanidine** | 15-25 | | |
| **Cefacetrile** | 12-16 | **Sufamethizole** | 220-260 | | |
| **Cefoperazone** | 3-4 | **Sulfasalazine** | 250-350 | | |
| **Cefalexin** | 700-800 | **Sulfanilamide** | ND | | |
| **Cefalonium** | 3-5 | **Sulfachloropyrazine** | 75 | | |

ND: Not Detected below 1000ppb
*To be confirmed by ongoing validation

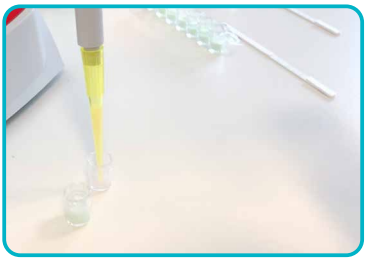
# CHAPTER 6 - NEGATIVE AND POSITIVE CONTROLS RECONSTITUTION

## 1. Take a microwell

Take the strip of 8 microwells out of the tube. Separate carefully one microwell and pay attention not to unseal unused microwells. Put the unused microwells back in the tube, close and make sure it is tightly sealed.

## 2. Reconstitute with water

Add 100µL of distilled or deionized water at ambient temperature.



## 3. Mix

Mix gently by pipetting up and down 10 times (avoid making air bubbles).

Check that the sample is properly dissolved.

Use the 100µL of control solution like any other milk samples (see CHAPTER 4: Operating Procedure).

# Technical support

**Rue Louis Plescia, 8 | LIEGE SCIENCE PARK | 4102 OUGREE | BELGIUM**
Tel.: +32 (0) 4 252 66 02
support@unisensor.be | www.unisensor.be

Version 1.2 • March 23, 2020.