



Operator's Manual:

Charm® SL Beta-lactam Test for Amoxicillin, Ampicillin, Cefotiofur, Cephapirin and Penicillin G Validated For Raw, Commingled Cow, Buffalo, Goat and Sheep Milk

Kit Information

Introduction

The Charm SL Beta-lactam Test is a receptor assay utilizing ROSA® (Rapid One Step Assay) lateral flow technology. The SL Beta-lactam Test detects beta-lactam drugs at or below the U.S. Safe Level/Tolerance, see Table 1.

Kit/Test Contents and Order Information

LF-SLBL-100K	LF-SLBL-500K
<ul style="list-style-type: none">• 100 SLBL Test Strips• 5 Penicillin G Positive Tablets	<ul style="list-style-type: none">• 500 SLBL Test Strips• 25 Penicillin G Positive Tablets

Equipment and Supplies Required But Not Included With Kits (order codes bolded)

The ROSA 56°C Incubator (LF-INC4-8-56D) and ROSA Pearl Reader (LF-ROSA-PEARL-X-NB) are available only from Charm Sciences. A daily hard copy print out is required for NCIMS testing and a printer (PRN-THERMAL-SYS) is available that interfaces with the reader RS-232 connector. A 300 µl pipet and a timer (the timer may be part of the incubator) are also required.

Pipet options include a fixed 300 µl pipet (PIP-300UL-1STOP-M) with disposable 200 -1000 µl pipet tips (1-MLT-X4); ROSA-Pipets (LF-ROSA-PIPET-500) are available for use by NCIMS screening labs only. Refer to Optional Materials, Disposable Supplies, and Order Codes for more order options and information.

Reagent Use and Storage Requirements

- Strips are not required to be shipped refrigerated.
- Store refrigerated at 0 to 4.4°C, tightly sealed in the supplied container.
- To open, remove and save the plastic lid.
- Lift foil tab on canister and peel the foil-seal off the container. Discard foil seal.
- Inspect desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if blue beads have turned white or pink.
- In high humidity, limit condensation by opening container after it is warmed to room temperature (20 to 30 minutes from the time the container is removed from refrigerator).
- Remove from container the number of strips to be used in one day and immediately re-seal container and return it to refrigeration. Removed strips may be kept at room temperature during daily use for up to 12 hours. Unused tests must be discarded.
- Desiccant should remain in tightly closed container at all times. Discard strips if blue beads inside desiccant packets turn white or pink.
- Always tightly seal the container with the supplied plastic foil-lined lid and return to refrigerated storage.

Charm Sciences, Inc.

659 Andover Street, Lawrence, Massachusetts, 01843-1032, USA

Tel: +1.978.687.9200 | Fax: +1.978.687.9216 | Email: info@charm.com | www.charm.com

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Performance Information

Sensitivity

Results of sensitivity evaluations are presented in Table 1 and show concentration-response information — (# Positive per 30 Tests, Displayed as % Positive).

Drugs shown with * are detected more than 25% lower than their safe level/tolerance. Sole reliance on the use of this test kit may result in the rejection of milk which the FDA considers safe for human consumption.

Selectivity

Two groups of 60 negative milk samples were evaluated in an independent laboratory; 1 of 60 and 2 of 60 samples tested positive. The test meets 90% selectivity with 95% confidence requirements.

Cross Reactivity

There are no known interferences from the following non-beta-lactam animal drugs at 100 ppb: sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline, doxycycline, gentamicin, neomycin, streptomycin, ivermectin, erythromycin, pirlimycin, tilmicosin, novobiocin, furosemide, trichlormethiazide, chlorothiazide, thiabendazole, oxytocin, phenylbutazone, dexamethasone, PABA and dipyrone.

Table 2 lists some other cross reactive beta-lactam (intra-family) drugs.

Table 2: Intra-family Cross Reactivity Levels Detected in Milk with High Confidence

Cefacetrile	30 ppb	Cefoperazone	15 ppb	Dicloxacillin	50 ppb
Cefadroxil	50 ppb	Cefquinome	30 ppb	Nafcillin	100 ppb
Cefalexin	50 ppb	Cefuroxime	15 ppb	Oxacillin	50 ppb
Cefalonium	4 ppb	Cloxacillin	50 ppb	Ticarcillin	100 ppb
Cefazolin	40 ppb				

Table 1: Concentration-Response Information —Displayed as % Positive

Concentration ppb (parts per billion)	Amoxicillin*	Ampicillin	Ceftiofur [§] (as DFC)	Cephapirin*	Penicillin G*
1					0
2	3	3			13
3					73
4	70	13		0	100
5			0		100
6	100	83			
8	100	100		50	
10	100	97	0		
12				97	
16				100	
20			0	100	
40			0		
60			23		
80			100		
100			100		
Safe Level/ Tolerance (ppb)	10	10	100	20	5
Calculated 90/95 Detection Level: [§] (ppb)	5.6	8.5	77	13.7	3.6

*The 90% sensitivity with 95% confidence level is calculated based on FDA-CVM statistical analysis of independent lab blind study results. Results with the exception of ceftiofur are based on fortified raw milk samples.

§The test sensitivity for ceftiofur was evaluated using milk containing ceftiofur and desfuroylceftiofur (DFC) related metabolites from treated animals. The ceftiofur tolerance is based on measuring the sum of ceftiofur and desfuroylceftiofur related metabolites in milk as desfuroylceftiofur.

Training

- Equipment setup and use is simple and can be self-taught from the manual. In addition, training materials and/or video is available for review of procedure, equipment use, or maintenance.
- Proficiency samples (blind coded positive and negative samples) are available for retraining and procedural validation (see Additional Test Information).
- For questions contact your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

Sample and Test Information:

- Raw, commingled milk must be cooled and refrigerated (0 to 4.4°C). Refrigerated samples should be well agitated and mixed prior to testing.
- Test at ambient temperatures of 10 to 30°C in naturally circulating air.
- Milk being tested for NCIMS purposes may not be frozen at any time during testing process. Testing of milk samples must be completed within 72 hours of sampling. Completed includes the time required to finish the initial testing, any necessary re-testing of the initial positive, confirmation, and/or producer trace back testing.

Precautions:

- High fat samples (greater than 6.5%) may cause invalid results.
- Keep equipment clean.
- Keep dust and milk off exterior of test strips.
- ROSA[®] Incubator must be clean, and the temperature indicator green (indicating 56 ± 1°C). Keep ROSA Incubator lid lowered, but not latched, unless performing procedure. A daily temperature check is required.

Daily Performance Check:

- Obtain negative raw milk for use as Negative Control and prepare Positive Control (sections below).
- Check ROSA Pearl Reader calibrator strips and verify reader performance. High and Low Calibrator strips must read within the range printed on the strips.
- Verify performance of test strips and equipment each day prior to testing actual samples. Rotate performance among all SL Test users.
- Negative Control must be RESULT NEGATIVE and with a READING less than (more negative than) -600 on the reader.
- Positive Control must be RESULT POSITIVE and with a READING greater than +400 on Reader. Re-make/retest control if it tests out of range. Do not proceed and contact Charm Sciences Inc if still out of range.
- If calibrator strips or controls are not in specifications, do not proceed with testing. Note in an official laboratory record, notify state LEO, and contact technical support at Charm Sciences.

Negative Control

- Use beta-lactam negative raw, commingled milk as a Negative Control for performance monitoring and to reconstitute Positive Tablet for Positive Control.
- Qualified raw milk should give a reading less than (more negative than) -600.

Positive Control

- The Charm SL Beta-lactam Test uses the Penicillin G Positive Tablet reconstituted to 5 ppb (0.0089 IU/ml) penicillin G as the Positive Control for performance monitoring.
- Store tablets refrigerated, closed in the supplied moisture resistant foil bag.
- Using a clean vial reconstitute 1 tablet with 5.0 ml Negative Control (qualified raw milk) as per label and shake well. Refrigerate for 5 minutes and shake before use. When re-using supplied vial, wash thoroughly with soap and water, rinse well, then dry between uses.

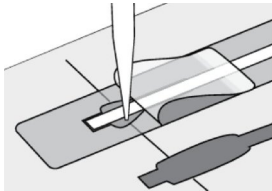
Control Storage

- Negative Control may be used for up to 72 hours refrigerated at 0 to 4.4 °C. Reconstituted Positive Control may be used for up to 48 hours refrigerated.
- For longer storage of controls, freeze 1 ml aliquots within 6 hours of reconstitution. Frozen aliquots may be stored up to 2 months at -15 °C or below. To use frozen controls, thaw slowly overnight in refrigerator or with cool tap water and shake well. Thawed controls should be centrifuged (1200g for 3 minutes) prior to use. Cool and use skim milk portion as sample for test. Draw skim milk into pipet by puncturing fat layer with pipet tip and drawing skim milk from underneath fat.
- Thawed Positive Control is stable for 24 hours refrigerated.
- Discard remaining thawed controls. Do not refreeze.

SLBL Test for Milk - Operating Instructions

Check that ROSA Incubator temperature is $56 \pm 1^\circ\text{C}$.

Use SLBL test strips. Re-shape dented sample compartments to fit into ROSA Incubator.

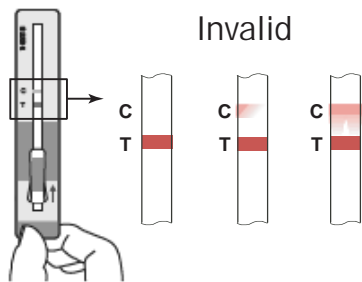


Step 1	<p>Shake milk sample(s) thoroughly before testing. Refer to 2400 form - General Requirements form.</p> <ul style="list-style-type: none"> • Allow foam/bubbles to settle for no more than 1 minute. • As many as 4 samples may be tested at one time.
Step 2	<p>Label test strip(s) with sample identification(s).</p> <ul style="list-style-type: none"> • Avoid crushing sample compartment. Strip(s) may be placed in incubator to avoid crushing sample compartment while labeling.
Step 3	<p>Place test strip in ROSA Incubator.</p>
Step 4	<p>Holding test strip flat in ROSA Incubator, use tab to expose sample compartment by peeling tape to "Peel to Here" line.</p> <ul style="list-style-type: none"> • Avoid lifting the test strip and sponge under tape.
Step 5	<p>Using 300 μl pipet, draw up sample, avoiding foam and bubbles. Note: ROSA-Pipets are available for screening testing only.</p>
Step 6	<p>Holding pipet vertically, slowly pipet 300 μl ($\pm 15 \mu\text{l}$) sample into sample compartment at ROSA Incubator etched indicator line (as shown).</p>
Step 7	<p>Reseal tape over sample pad compartment.</p> <ul style="list-style-type: none"> • When performing multiple tests in a ROSA Incubator: <ul style="list-style-type: none"> ○ Peel, pipet, and reseal (Steps 3 - 7) before starting next test strip. ○ Complete all test strips within 2 minutes of placing first strip in incubator.
Step 8	<p>Close lid on ROSA Incubator and latch. Timer starts and red light illuminates.</p>
Step 9	<p>Incubate for 8 minutes, but not more than 9 minutes.</p> <ul style="list-style-type: none"> • At 8 minutes, a beeper and alternating yellow and red blinking lights start. The beeper will stop after 30 seconds, while the lights will continue to blink, indicating over-incubation.
Step 10	<p>Remove test strip(s) from ROSA Incubator. Do not squeeze sample compartment. Hold test strip with sample compartment in the down position until interpreted.</p> <ul style="list-style-type: none"> • Read within 5 minutes of incubation completion. • Lower lid. Do not re-latch.

Inspection and Interpretation of Results

Visual Inspection

Hold the test strip vertically with sample compartment in the down position. Do not squeeze the sample compartment. Wipe foreign matter (dust, etc.) off test strips.



Inspect C (Control) line for even development before placing strip in ROSA Pearl Reader.

The test is INVALID if any of the following are observed:

- C (Control) line is missing
- C line is smeared or uneven
- Sample is obscuring either the C or T (Test) lines

DO NOT PUT INVALID STRIPS IN READER. IF INVALID THE SAMPLE MUST BE RETESTED.

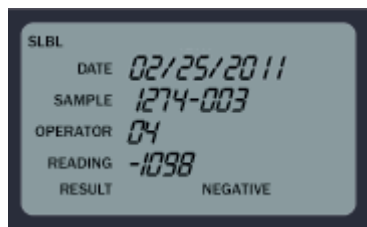
Reader Interpretation



Within 5 minutes of completing the test, insert the test strip, with valid C line, into ROSA Pearl Reader. Slide completely into slot.

Read results on SLBL channel of ROSA Reader. SLBL channel displayed in upper left of reader screen should blink slowly. Use the down arrow to enter other optional information such as SAMPLE or OPERATOR ID. If strip is not fully inserted, the reader will display an error message; re-insert strip and read again. Press ENTER. A numerical READING and a RESULT are displayed on screen in 8 seconds.

Reader results including lot number and time/date are stored in memory for later review, download, or printing, refer to ROSA Reader Manual. NCIMS policy requires a hard copy of data be printed daily.



Negative - If READING is a negative number or zero, the ROSA Reader will display RESULT NEGATIVE. Report as "Not Found".

Positive - If READING is a positive number, the ROSA Reader will display RESULT POSITIVE. Sample should be retested; see Retest of Initial Positive.

Retest of Initial Positive:

1. Test "Initial Positive" samples in duplicate along with one Positive Control and one Negative Control.
2. Negative Control must be RESULT NEGATIVE with a READING less than (more negative than) -600 on reader.
3. Positive Control must be RESULT POSITIVE with a READING greater than +400 on Reader.
4. If either control is not within range, then rerun assay. If either control is still not within range do not continue testing. Contact appropriate regulatory agency to determine milk status. Contact Charm Sciences for technical assistance with test performance.
5. If both controls are within range and either or both of the retested samples are RESULT POSITIVE, sample is a "Beta-lactam Presumptive Positive Test". In accordance with appendix N of the PMO, report all presumptive positives to appropriate regulatory agency. If acting as a confirmation lab, repeat steps 1 to 4 to confirm as "Beta-lactam Screening Test Positive" sample.

Technical Assistance:

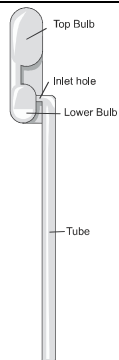
- Call 1-800-343-2170 hours 8:30 AM - 5:30 PM EST.
- For emergency 24-hour 365-day per year service call 1-978-687-9200 and follow prompts.
- E-mail: support@charm.com (monitored during 8:30 AM – 5:30PM EST).

Additional Test Information

Optional Materials, Disposable Supplies, and Order Codes

PIP-300UL-1STOP-M	300 µl fixed volume pipet, single stop	LF-ROSA-PIPET-100	100 disposable ROSA-Pipets, 300 µl
1-MLT-X1	200 disposable pipet tips, 300 -1000 µl	LF-ROSA-PIPET-500	500 disposable ROSA-Pipets, 300 µl
1-MLT-X4	1000 disposable pipet tips, 300-1000 µl	LF-ROSA-PIPET-5000	5000 disposable ROSA-Pipets, 300 µl
PRN-THERMAL-SYS	Thermal printer for data output to reader with RS-232 adapter and cable	UNKSTD-LF	4 Blind Proficiency Samples: coded, freeze-dried negative and positive samples, 8-10 ml rehydration

Use of ROSA-Pipet (for use in NCIMS screening labs only)



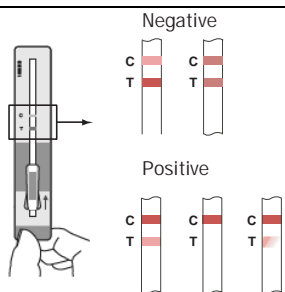
1. Hold ROSA-Pipet vertically, as shown, and squeeze top bulb completely. (Do not pre-wet.)
2. Insert tube in sample (not more than ½ inch or 1 cm) and slowly release bulb completely. Sample must overflow into lower bulb, but must not be above inlet hole.
3. Position pipet upright and vertically above the test strip at the position of the etch mark on the incubator. Put the tip in one side of strip sample compartment.
4. Squeeze top bulb slowly to dispense all sample in tube into one side of the strip sample compartment. Avoid overflowing compartment and drawing sample back into tube after it is expelled. Excess (overflowed) sample should remain in lower bulb.
5. ROSA-Pipets are single-use, disposable pipets. Do not reuse.

Post-Testing Sample and Test Strip Storage

To preserve samples or test strips for diagnostic or record keeping purposes (these are not NCIMS accepted practices):

- Samples can be preserved after testing for up to two months by freezing at -15 °C or below.
- Frozen samples must be thawed, centrifuged (3 minutes at 1200 g ± 200), and cooled before testing. Mix well to make sample homogeneous. Noticeable protein precipitation indicates an unsuitable sample for SLBL lateral flow testing.
- After incubation test strip results are stable at room temperature for up to five minutes. Over several hours positive results gradually become more positive while negative results remain stable. Freezing test strips will slow additional line development and is the best method of preserving a strip test result.

Visual Interpretation of Test Strip (not accepted in NCIMS milk programs)



Negative - T (Test) line is same as or darker/more than C (Control) line.

Positive - T line is clearly lighter/less than C line, or T line is absent, or partially or unevenly colored. Sample should be retested. See Retest of Initial Positive.

Warranty Information:

Charm Sciences, Inc. ("Charm") warrants each reagent product, including but not limited to test kits, to be free from defects in materials and workmanship and to be free from deviations from the specifications and descriptions of Charm's reagent products appearing in Charm's product literature, when stored under appropriate conditions and given normal, proper and intended usage, until the expiration of such reagent product's stated shelf life, or, if none is stated, for one year from the date of delivery of such reagent product to the end-user purchaser. THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, WHETHER STATUTORY, EXPRESS, IMPLIED (INCLUDING WARRANTIES OF TITLE, NON-INFRINGEMENT, MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE AND ALL WARRANTIES ARISING FROM COURSE OF DEALING OR USAGE OF TRADE). The warranty provided herein may not be altered except by express written agreement signed by an officer of Charm. Representations, oral or written, which are inconsistent with this warranty are not authorized and if given, should not be relied upon. In the event of a breach of the foregoing warranty, Charm's sole obligation shall be to replace any reagent product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Charm promptly of any such defect prior to the expiration of said warranty period. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Charm is willing to replace any nonconforming reagent product or part. Charm shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damages sustained by any customer from the use of its reagent products. Except for Charm's obligation set forth above to replace any reagent product that proves defective within the warranty period, Charm shall not be liable for any damages of any kind arising out of or caused by any incorrect or erroneous test results obtained while using any such reagent product, whether or not caused by a defect in such reagent product.