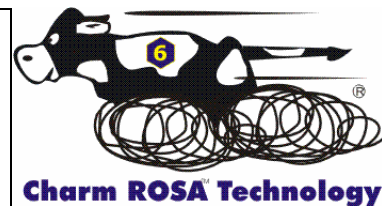


Charm SL6™ Beta-lactam Test for Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Cloxacillin and Penicillin G

Operator's Manual Validated for Raw, Commingled Bovine Milk



Introduction

The Charm SL6 Beta-lactam Test is a receptor assay utilizing ROSA™ (Rapid One Step Assay) technology. The SL6 Beta-lactam Test is made to detect all six beta-lactam drugs at or below the U.S. safe level/tolerance (Table 1). The SL6 Beta-lactam Test is designed for use by milk, intake, laboratory, field, and regulatory personnel.

Equipment

The SL6 Beta-lactam Test uses equipment (ROSA Incubator with blue metal insert at 45°C and ROSA Reader v.01-05-03 or higher) available only from Charm Sciences Inc. A 300µl pipet and a timer, if not part of the incubator, are required. Various 300µl pipet options are available from Charm Sciences.

Reagents and Supplies

Test Kit Contents	Order Code:		Material required but not Included: (Ordered Separately)	Order Code
	LF-SL6-100	LF-SL6-500		
SL6 Beta-lactam Test Strips	1 Container of 100 tests	5 Containers of 100 tests	1000 Disposable Pipet Tips, 1000µl 600 Disposable Pipet Tips (MLA Pipette), 1000µl	1MLT 1-MLT-MLA-X3
Operator's Manual	1	1	500 Disposable PolyPipets, 300µl	LF-POLY-500
SL6 Positive Control	1 Bottle	5 Bottles	5000 Disposable PolyPipets, 300µl	LF-POLY-5000

Performance Information

Sensitivity — Results of sensitivity evaluations are presented in Table 1. Drugs shown with * are detected more than 25% lower than their safe level/tolerance.

Selectivity — 60 negative milk samples were evaluated 5 times. 0 of 60 samples tested positive in all 5 experiments. The test meets 90% selectivity with 95% confidence requirements.

Cross Reactivity — There are no interferences from the following animal drugs (at 100 ppb): sulfadiazine, sulfanilamide, sulfathiazole, sulfamethazine, sulfapyridine, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline, doxycycline, enrofloxacin, gentamicin, neomycin, streptomycin, ivermectin, erythromycin, pirlimycin, tilmicosin, novobiocin, furosemide, trichlormethiazide, chlorothiazide, thiabendazole, oxytocin, phenylbutazone, dexamethasone, PABA and dipyrone. Other beta-lactam drugs detected are presented in Table 2.

Table 1: Concentration Response Information — (Displayed as % Positive)

Concentration ppb (parts per billion)	*Amoxicillin	Ampicillin	Ceftiofur	Cephapirin	Cloxacillin	Penicillin G
1						7
2	0	0			0	23
3						93
4	7	3		0	40	100
5						100
6	57	17			83	
8	97	77		7	97	
10	100	100	0		100	
12				53		
16				100		
20				100		
30			27	100		
40			73			
50			93			
			100			
Safe Level / Tolerance (ppb)	10	10	50	20	10	5
Calculated 90/95 Detection Level	7.1	9.6	37.5	18.7	8.3	4.2

Table 2: Intrafamily Cross Reactivity Levels Detected in Milk with High Confidence:

Cefadroxil	50 ppb	Oxacillin	25 ppb
Dicloxacillin	5 ppb	Ticarcillin	100 ppb

Training

Equipment setup and use is simple and can be self-taught from the manual. Proficiency samples (4 blind coded positive and negative samples) are available for retraining and procedural validation. Contact Charm Sciences 1 800 343-2170. E-mail: support@charm.com

Sample and Test Information

Raw, commingled sample must be sampled, cooled to 0-7°C, kept refrigerated, and tested within 5 days of milking. For NCIMS (National Conference of Interstate Milk Shippers) purposes, milk may **not be frozen** at any time during testing process and samples must not be more than 72 hours old. After testing, to preserve samples, Charm Sciences recommends freezing samples at -15 °C or below. Frozen samples may be stored up to 2 months.

Thaw frozen samples in luke warm water, mix well and cool to 0-4.4C before testing. Noticeable protein precipitation indicates an unsuitable sample that must be centrifuged, and cooled before testing.

Test may be performed at ambient temperatures of 7 to 35 °C in naturally circulating air.

For all NCIMS testing, please refer to the 2400 form (Appendix N Bulk Milk Screening Test Form) for the Charm SL and SL6 Beta-lactam Tests.

Precautions

- High fat samples (>7.5%) may cause invalid results.
- Invalid test strips should not be read in the ROSA Reader.
- Samples (0-7°C) should be well agitated and mixed prior to testing.
- Dust and milk debris may alter the reader optics. Keep equipment clean and wipe dust and milk off test strips before putting in the reader.
- Calibrator strips are provided to monitor ROSA Reader operation. Daily and before performing **Test of Initial Positive**, check ROSA Reader calibration by verifying calibrator strips read within 20% of their printed

averages. If not in range, **DO NOT PROCEED**, call Charm Sciences.

Charm SL6 Beta-lactam Test Procedure

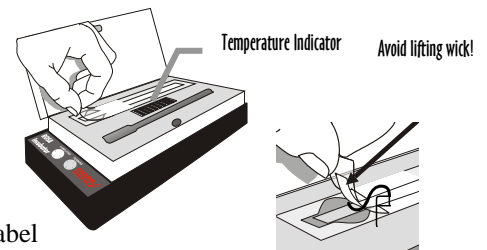
Preparation

- ROSA Incubator lid should remain closed at all times unless performing procedure.
- ROSA Incubator temperature indicator should be brown at 45 indicating 45±2°C. A daily thermometer check using a 0.5°C calibrated thermometer in the thermometer port is a NCIMS requirement. Incubator must be clean and level.
- **Mix Raw Milk Well before testing.**
- If testing reconstituted powdered milk (procedure not NCIMS approved), centrifuge (3 minutes at 1200g ±200) and use skim portion for test.
- Multiple samples may be incubated simultaneously in 2 or 4 place incubator.

Procedure

ONE

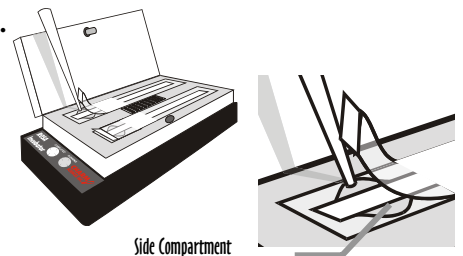
- Label test strip to identify sample.
- Open lid and place SL6 Beta-lactam Test strip in ROSA Incubator with flat side facing up. If needed, sample compartments may be re-shaped to allow strips to easily fit into incubator.
- While holding strip flat on incubator, **use tab to peel tape** back to edge of yellow label exposing sample pad. Avoid bending back the white wick and sponge under tape.



Pipet only into side compartment

TWO

- **SHAKE MILK SAMPLE THOROUGHLY.**
- **Slowly** to avoid spilling, pipet 300µl (± 15µl) milk or centrifuged sample into either **side well** of the sample pad compartment, as shown.
- Reseal tape over sample pad compartment by pressing. When testing multiple samples, complete the peel, pipet, and reseal steps on each strip before going on to the next strip.
- Close lid on incubator, and tighten latch. Solid red timer light will automatically start when the lid is closed.

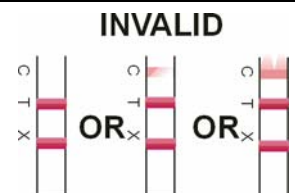


THREE

Incubate for at least 8 minutes, but not more than 10 minutes. A beeper and/or a test complete light (yellow blinking) will start at 8 minutes. Remove test from incubator before beeper stops (2 minutes), and close lid.

Visual Inspection

Be careful to hold the sample pad in the down position. Do not squeeze the sample pad. Carefully wipe any foreign matter (milk, dried milk, dust, etc.) off test strips, if present. Visually inspect results by comparing Test line (T) and the Clox line (X) to Control line (C). For visual aid and interpretation refer to **Additional Information**.



If C line is missing,
smearred or uneven, or
if milk is obscuring

either the C, X or T lines, test is INVALID. Re-test the sample.
DO NOT PUT INVALID STRIPS IN READER.

Reader Interpretation

Insert the clean and visually valid test strip into ROSA Reader. Slide completely into slot.



Read results on SLBL channel of ROSA Reader making sure that SLBL channel is set for three-line mode^φ. If desired, enter Samp# and/or Oper# and press ENTER*. A numerical value (**Read:=**) and an interpretation (**Result:**) are displayed on screen in 5 seconds. Results are in memory, and may be subsequently recalled, downloaded to printer or computer.

^φ Refer to Charm ROSA Reader (v.01-05-03 or higher) Operating Instructions for set up and operation. In two-line mode, results will always be positive.

* If strip is not fully inserted, the Reader will display an error message. Reinsert and press Enter.



Negative sample - If Reader sends a negative number or zero (**Read: =**), the reader will display **Result: Negative**. Report as “Not Found”.

Initial Positive sample - If Reader sends a positive number (**Read: =**), the reader will display **Result: Positive**. Sample must be immediately retested (see **Retest of Initial Positive**).

Negative results are stable and positive results gradually increase (become more positive) over several hours. Test results do not significantly change up to ten minutes at room temperature after completing the incubation. Refrigerate (0-4.4°C) or freeze finished tests (-15 °C or below) for permanent record. Negative results are stable refrigerated for 1 hour and frozen at -20°C, while positive results may become more positive.

Retest of Initial Positive

Test “**Initial Positive**” samples in duplicate along with one positive control and one negative control.

1. Check ROSA Reader calibrator strips and verify reader performance (see Precautions).
2. Negative control must be visually negative and yield a **Result: Negative** on Reader with a reading less than (more negative than) -400.
3. Positive Control must be visually positive and yield a **Result: Positive** on Reader with a reading greater than +400.
4. If these conditions are not met, rerun assay. If still not acceptable, **DO NOT PROCEED**, contact Charm Sciences.
5. If these conditions are met and *either* of the retested samples tests **Result: Positive**, sample is a “**Beta-lactam Presumptive Positive Test**”. Repeat steps 1-5 to confirm as “**Beta-lactam Screening Test Positive**” sample.

Note: All NCIMS testing must be performed in accordance with the current PMO.

Performance Monitoring

1. Test one negative control and one positive control each day of testing, prior to testing samples to verify performance of test strips and equipment. Rotate performance monitoring among all SL6 Test users.
2. Refer to “**Test of Initial Positive**” conditions 1.-4. for performance specifications.

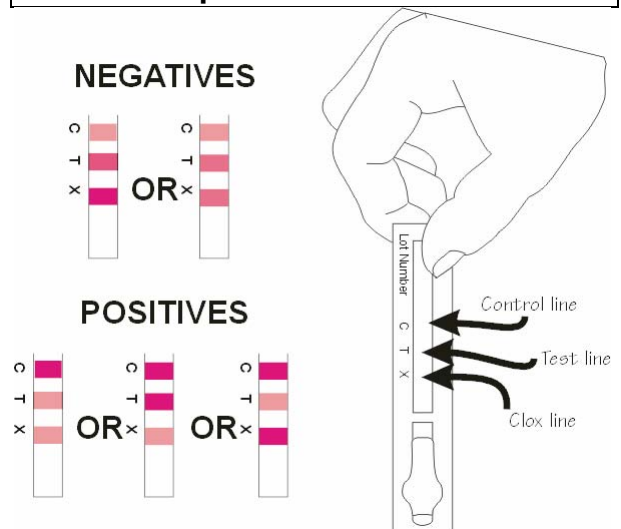
For 24-hour technical assistance call (978) 687-9200.

Additional Information

The SL6 Beta-lactam Test may be visually interpreted without a reader.

The visual procedure is not accepted for use in NCIMS Milk Monitoring Programs.

Visual Interpretation



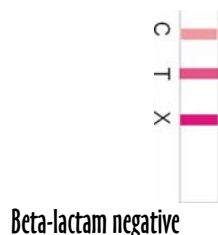
- **Negative sample** – Control line (C) is **the same as or lighter/less than both** the Clox line (X) and the Test line (T).
- **Positive sample** – Control line (C) is **darker/more than either** the Clox line (X) or the Test line (T).

When needed, positive samples can be quantitated and specific identification made of beta-lactam drugs using Charm HPLC Receptorgram. Contact Charm Sciences for more information.

Test Strips and Standards

Test Strip Storage — Store refrigerated at 0-4.4 °C in tightly closed moisture resistant container. Remove from container the number of strips to be used in one day. Strips may be kept at room temperature during daily use for up to 12 hours. Return unused strips to a separate tightly sealed moisture resistant container and use these strips the following day.

Negative Control —Use qualified beta-lactam negative commingled raw milk. Beta-lactam negative milk will be SL6 Beta-lactam Test strong negative—Control line (C) is lighter than both the Test line (T) and Clox line (X) (see illustration). Qualified negative raw milk will read less than (more negative than) –400 on the ROSA Reader. Negative control may be frozen. Refer to positive control for frozen storage times and conditions.



SL6 Positive Control —The Charm SL6 Beta-lactam Test uses a combined 5ppb (0.0089 IU/ml) Penicillin G and 10ppb Cloxacillin Standard as the SL6 positive control for performance monitoring. Drug standards are manufactured from USPC standards.

Preparation: Reconstitute contents of bottle with 10 ml negative control (qualified commingled raw milk), shake well, and allow to stand refrigerated for 10 minutes before use. Store refrigerated. Reconstituted positive control may be used for up to 48 hours. Positive controls will read greater than +400 on the ROSA Reader. Re-make/re-test control if it tests out of range. **Do not proceed** and contact Charm Sciences, Inc. if still out of range.

For longer storage of reconstituted positive controls, freeze 1 ml aliquots within 6 hours of reconstitution. Frozen aliquots may be stored up to 2 months at -15 °C or below. If frost free freezer then store in styrofoam box in the freezer. To use frozen positive controls, thaw with lukewarm tap water, shake well. Thawed positive control is stable for 24 hours at 0-4.4 °C. Discard thawed aliquot. Do not refreeze.

Principle

The Charm SL6 Beta-lactam Test uses receptors with binding to beta-lactam drugs. As milk flows through the device, a line forms in the X (clox) and T (test) position when the sample contains no beta-lactams. A weaker intensity X or T line forms when beta-lactam is present in the sample. The X and T lines are compared to the C (control) line, which is designed to discriminate beta-lactam close to safe levels. If both the X and T lines are darker than or equal to the C line, the sample is negative. If either the X or T line is lighter than the C line, or the X or T line does not form, the sample is positive. If the C line does not form the test is invalid and must be repeated. A ROSA Reader will convert the line comparison into a reading.

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U.S. Patent s 5,985,675; 6,319,466; 6,475,805; Additional Patents Pending



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