Operator's Manual:

Charm® Sulfa Test for Raw Commingled Cow Milk

Note New Sulfamethazine Positive Tablet for Positive Control

Kit Information

Introduction

The Charm Sulfa Test is an immunoreceptor assay utilizing ROSA® (Rapid One Step Assay) lateral flow technology. The milk sample interacts with colored beads in the lateral flow test strip and alters the binding capacity at the test and control zones. The color intensity of the test and control zones is read visually or by the ROSA Reader. Extra-label use of sulfonamides (sulfa drugs) in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine) is prohibited in the U.S. The Charm Sulfa Test may be used to test at the U.S. Safe Level/Tolerance (Table 1) or at the EU MRL (maximum residue limit) of 100 µg/kg total sulfonamides and CODEX MRL of 25 µg/l sulfadimidine (sulfamethazine). The test is designed for use by dairy, intake, laboratory, field, and regulatory personnel.

Kit Contents and Materials Needed

Supplied with Kits	Disposables	Equipment
• SULF Test Strips • Sulfamethazine Positive Tablet • Operator's Manual	• Pipet Tips or • ROSA-Pipets	• ROSA Incubator (56°C) • ROSA Reader (optional) • Printer (optional) • 300 µl Pipet (optional)

Charm ROSA equipment available only from Charm Sciences Inc.
For details and ordering information see Order Codes and Kit Contents.

Sensitivity and Selectivity

Sulfa drug-free raw milk yields negative results at least 90% of the time with 95% confidence.

Table 1. Sensitivity – Detection Levels in Cow Milk at 0 to 7°C

Sulfa Drug	Detection Level † (ppb*)	US Safe Level / Tolerance (ppb)	Sulfa Drug	Detection Level † (ppb*)	US Safe Level / Tolerance (ppb)
Sulfacetamide	25	None	Sulfamethizole	1	10
Sulfachlorpyridazine	3	10	Sulfamethoxazole	2	None
Sulfadiazine	2	10	Sulfamethoxypyridazine	20	None
Sulfadimethoxine	1	10	Sulfapyridine	10	10
Sulfadoxine	15	None	Sulfaquinoxaline	3	10
Sulfaethoxypyridazine	25	None	Sulfathiazole	1	10
Sulfamerazine	3	10	Sulfisoxazole	15	None
Sulfamethazine (Sulfadimidine)	6	10			

† Positive 90% of the time with 95% confidence.
*parts per billion, µg/kg or µg/L

Storage

Store SULF Tests strips and Sulfamethazine Positive Tablet refrigerated (defined as 0 to 7°C or 0 to 4.4°C for US certified labs). See Reagents and Storage for details.

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Interferences and Cross Reactivity

There are no known interferences from drugs at 100 ppb in the following animal drug families: aminoglycosides, amphenicols, beta-lactams/cephalosporins, fluoroquinolones, macrolides/lincosamides, nitrofurans, tetracyclines, or chlorothiazide, dapsone, dexamethasone, dipyrone, flunixin, furosemide, ivermectin, novobiocin, oxytocin, PABA, phenylbutazone, sulfaguanidine, sulfanilamide, trichlormethiazide, and thiabendazole. Some other sulfa drugs (sulfonamides) are detected. There are no interferences from somatic cells at 10^6 SCC/ml or bacteria at 3 x 10^5 CFU/ml.

Training

- Equipment setup and use can be self-taught from the manual.
- Proficiency samples (blind coded positive and negative samples) are available for validation.
- For questions contact your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

Reagents and Storage

Test Strips

- Store test strips refrigerated in tightly closed supplied container.
- To open test strip container, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.
- In high humidity, limit condensation by opening container after it has warmed to room temperature (20 to 30 minutes from the time the container is removed from refrigerator).
- Inspect desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if blue beads turn purple or pink.
- Remove from container the number of test strips to be used in one day. Keep these test strips at room temperature during daily use for up to 12 hours. Unused test strips should be discarded.
- Use supplied plastic lid to immediately reseal container tightly and return to refrigerated storage.

Negative Control

- Use sulfa drug (sulfonamide) negative raw, commingled milk as a Negative Control for performance monitoring and to reconstitute Sulfamethazine Positive Tablet.
- Qualified Negative Control should yield a strong visual negative (T line is darker than C line). See Visual Interpretation.
- Negative Control may be stored up to 72 hours refrigerated.
- See Retest of Initial Positive for Negative Control performance specifications.

Positive Control

- The Charm Sulfa Test uses a Sulfamethazine Positive Tablet to make a 10.0 ± 1.0 ppb sulfamethazine Positive Control for performance monitoring.
- Store Positive Tablets refrigerated in the closed zip-lock moisture resistant foil bag provided.
- Reconstitute one Positive Tablet with 5.0 ml of Negative Control (see above). Shake well. Allow to stand refrigerated or on ice for 5 minutes. Mix before use.
- Reconstituted Positive Control may be stored for up to 48 hours refrigerated.
- See Retest of Initial Positive for Positive Control performance specifications.

Long-Term Sample and Control Storage

- Mix sample and freeze (at least 0.5 ml) aliquots in clean vials.
- Controls should be frozen within 6 hours of preparation.
- Freeze aliquots at -15°C or below for up to 2 months.
- Thaw slowly (overnight in refrigerator or with cool water) and shake well. Thawed sample is stable for 24 hours refrigerated. Noticeable protein precipitation indicates an unsuitable sample. Discard thawed sample. Do not refreeze.

Daily Performance Check

- ROSA Calibration Strips are provided to monitor ROSA Reader operation. Daily and before performing retest of initial positive, check ROSA Reader performance by verifying that Calibration Strips read within range. Enter performance mode in ROSA Reader by sequentially pressing ESC, 5, ENTER. If not in range, contact Charm Sciences.
- Verify performance of test strips and equipment each day by running one Negative Control and one Positive Control prior to testing actual samples.
- Refer to Retest of Initial Positive for control performance specifications.

Precautions

- High fat samples (greater than 6.0%) may cause invalid results. Do not read invalid test strips in the ROSA Reader.
- Debris on test strips may alter the ROSA Reader optics. Keep equipment clean and wipe dust and milk off test strips before inserting in ROSA Reader.
- ROSA Incubator must be clean and level. ROSA Incubator temperature must be 56°C ± 1°C. The temperature indicator should match ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched, unless performing procedure.
- ROSA Incubators may take more than 10 minutes to reach proper temperature, depending on ambient temperature.

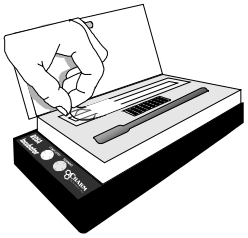
Sample and Test Information

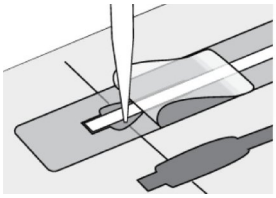
- Raw, commingled milk must be refrigerated or cool (0 to 15°C) for testing.
- Test refrigerated samples within 5 days of milking.
- To preserve samples or controls after testing, freeze samples at -15°C or below. See Long-Term Sample and Control Storage.
- Test may be performed at ambient temperatures of 10 to 30°C in naturally circulating air.

Sulfa Test for Milk – Procedure

Check that ROSA Incubator temperature is 56 ± 1°C.

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

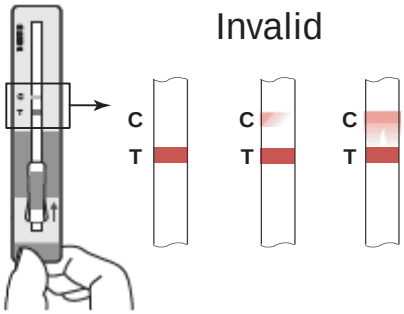




Step 1	• Mix all samples well before testing.
Step 2	• Label test strip(s) with sample identification (test strip(s) may be placed in incubator to avoid crushing sample compartment).
Step 3	• Place test strip in ROSA Incubator. • Holding test strip flat in ROSA Incubator, use tab to expose sample compartment by peeling tape to "Peel to Here" line. Avoid lifting the test strip and sponge under tape.
Step 4	• Using 300 µl pipet, draw up sample, avoiding foam and bubbles. • Holding pipet vertically, slowly pipet 300 µl (± 15 µl) sample into sample compartment at ROSA Incubator indicator line (as shown).
Step 5	• Reseal tape over sample compartment. • When performing multiple tests in a ROSA Incubator: o Peel, pipet and reseal before starting next test strip. o Complete all test strips within 1 minute.
Step 6	• Close lid on ROSA Incubator and latch. Timer starts and red light illuminates.
Step 7	• Incubate for 8 minutes, but not more than 10 minutes. • At 8 minutes, a beeper and alternating yellow and red blinking lights start.
Step 8	• Remove test strip(s) from ROSA Incubator. Do not squeeze sample compartment. Hold test strip with sample compartment in the down position until interpreted. • Read within 10 minutes of incubation completion. • Lower ROSA Incubator lid. Do not re-latch.

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.



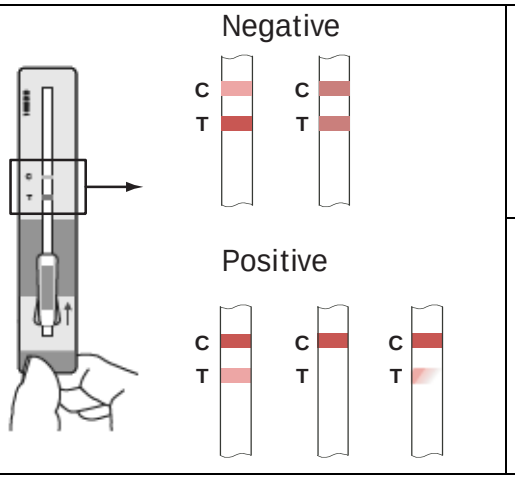
The test is INVALID if any of the following are observed:

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

Re-test INVALID samples. DO NOT INTERPRET OR PUT INVALID TEST STRIPS IN ROSA READER.

Interpretation

Visual Interpretation



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

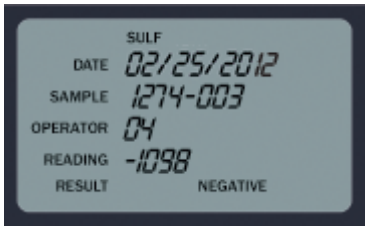
Positive - T line is clearly lighter / less than C line, or T line is absent, partially or unevenly colored. Sample should be retested. See Retest of Initial Positive.

Reader Interpretation - The ROSA Reader is an optional feature.

Within 10 minutes of completing incubation, insert the clean and visually valid test strip into ROSA Reader as shown. Slide completely into slot.

Read results on SULF 2-line channel of ROSA Reader. If desired, enter SAMPLE and/or OPERATOR and press ENTER. A numerical value (READING) and an interpretation (RESULT) are displayed on screen.

Results are stored in memory and can be recalled and downloaded to printer or computer.



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. “Initial positive” samples may be retested at the U.S. Safe Level / Tolerance of 10 ppb OR at the EU MRL of 100 µg/kg total sulfonamides and CODEX MRL of 25 µg/l sulfadimidine (sulfamethazine).
   - To test at U.S. Safe Level / Tolerance of 10 ppb, go to step 2 below.
   - To test at EU and CODEX MRL, dilute “initial positive” sample 1 part with 2 parts Negative Control; for example, 300 µl “initial positive” sample with 600 µl Negative Control and mix. Do not dilute the Negative Control or Positive Control. If using the dilution for retest of initial positive, the sensitivities of the test are 3 times the Detection Levels listed in Table 1.
2. Test “initial positive” samples or diluted “initial positive” samples (for EU and CODEX MRL) in duplicate along with one Negative Control and one Positive Control.
3. Negative Control must be RESULT NEGATIVE with READING less (more negative) than -600 on ROSA Reader.
4. Positive Control must be RESULT POSITIVE with READING greater than +400 on ROSA Reader.
5. If either control is not within range then rerun testing of “initial positive” with controls. If either control is still not within range after retest, do not continue testing - contact Charm Sciences.
6. If both controls are within range and either or both of the retested samples are RESULT POSITIVE, the sample is a “Sulfa Drug Screening Positive Test”.
7. For NCIMS confirmation purposes, the Charm II Sulfa Drug Test (Competitive Assay) must be used.

Order Codes and Kit Contents

KITS	NOT SUPPLIED WITH KIT
LF-SULF-20K: • (1) container of 20 SULF test strips • (1) Sulfamethazine Positive Tablet • (1) Operator's Manual LF-SULF-100K: • (1) container of 100 SULF ROSA test strips • (5) Sulfamethazine Positive Tablets • (1) Operator's Manual LF-SULF-100SUPK: • (1) container of 100 SULF test strips • (5) Sulfamethazine Positive Tablets • (1) Operator's Manual • (100) ROSA-Pipets LF-SULF-500K: • (5) LF-SULF-100K	1-MLT-100 (box of 100 disposable pipet tips, 200-1000 µl) 1-MLT-BG (bag of 1,000 disposable pipet tips, 200-1000 µl) 1-MLT-X1 (2 boxes of 100 disposable pipet tips, 200-1000 µl) 1-MLT-X4 (10 boxes of 100 disposable pipet tips, 200-1000 µl) LF-ROSA-PIPET-500 (500 disposable ROSA-pipets, 300 µl) LF-ROSA-PIPET-5000 (5000 disposable ROSA-pipets, 300 µl) PIP-300UL-1STOP-M (300 µl fixed volume pipet) LF-INC4-8-56D (4 place ROSA Incubator with 8 minute timer, 56°C) LF-INC2-8-56 (2 place ROSA Incubator with 8 minute timer, 56°C) LF-ROSA-PEARL-X-NB (ROSA Pearl Reader)

Warranty Information

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