



Operator's Manual: Charm® QUAD2-G Test for Macrolides and Gentamicin in Raw Commingled Cow Milk

Kit Information

Introduction

The Charm QUAD2-G Test is an immunoreceptor assay utilizing ROSA® (Rapid One Step Assay) lateral flow technology. Macrolide and gentamicin drugs interact with colored beads in the lateral flow test strip, and the color intensity in the test and control zones is measured by the Charm EZ®. The Charm QUAD2-G Test detects macrolides and gentamicin at or below the EU/CODEX or Chinese MRLs (maximum residue limits). The test is designed for use by dairy, intake, laboratory, field, and regulatory personnel.

Kit Contents and Materials Needed

Supplied with Kits	Disposables	Equipment
<ul style="list-style-type: none"> QUAD2-G Test Strips ROSA Multi-Antimicrobial Positive Tablet Operator's Manual 	<ul style="list-style-type: none"> Pipet Tips or ROSA-Pipets 	<ul style="list-style-type: none"> Charm EZ Printer (optional) 300 µl Pipet (optional) ROSA Incubator (5 minute 56 °C) (optional)

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

Sensitivity and Selectivity

Macrolide and gentamicin free raw milk yields negative results at least 99% of the time.

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

Macrolide Drug	Detection Range † (ppb*)	EU/CODEX MRL (ppb*)	China MRL (ppb*)
Erythromycin	10 to 30	40	40
Lincomycin	25 to 50	150	150
Pirlimycin	50 to 100	100	100
Tilmicosin	20 to 40	50	50
Tylosin	10 to 25	50/100	50
Aminoglycoside Drug	Detection Range † (ppb*)	EU/CODEX MRL (ppb*)	China MRL (ppb*)
Gentamicin	100-150	100/200	200

† Positive at least 95% of the time.

* parts per billion, µg/kg or µg/L

Storage

Store QUAD2-G test strips refrigerated (defined as 0 to 7°C or 0 to 4.5°C for US certified labs). See **Reagents and Storage** for details.

Interferences and Cross Reactivity

There are no known interferences from drugs at 100 ppb in the following animal drug families: amphenicols, beta-lactams/cephalosporins, fluoroquinolones, nitrofurans, tetracyclines, sulfa drugs/sulfonamides, or chlorothiazide, dexamethasone, dihydrostreptomycin, dipyrone, flunixin, furosemide, ivermectin, neomycin, novobiocin, oxytocin, PABA, phenylbutazone, streptomycin, trichlormethiazide, and thiabendazole. There are no interferences from somatic cells at 10⁶ SCC/ml or bacteria at 3 x 10⁵ CFU/ml.

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Training

- Equipment setup and use can be self-taught from the manual.
- Proficiency 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 in test strip container. Beads inside desiccant packets should be blue. Do not use test strips if blue beads have turned purple or pink.
- Remove from container the number of test strips to be used in one day; use supplied plastic lid to immediately reseal container tightly and return to refrigerated storage.
- Keep removed test strips at room temperature during daily use for up to 12 hours. Unused test strips should be discarded.

Negative Control

- Use macrolide and gentamicin negative raw commingled milk as a Negative Control for performance monitoring and to reconstitute Positive Tablets for use as the Positive Controls.
- Qualified Negative Control should yield negative result on Charm EZ.
- Store Negative Control refrigerated for up to 72 hours.
- See **Retest of Initial Positive** for Negative Control performance specifications.
- Use 300 μ L of Negative Control in **Procedure**.

Positive Control

- The Charm QUAD2-G test uses a ROSA Multi-Antimicrobial Positive Tablet to make a Positive Control for performance monitoring.
- Store Positive Tablets refrigerated in the closed zip-lock moisture resistant bag provided.
- Add one ROSA Multi-Antimicrobial Positive Tablet to 5.0 ml Negative Control (see above). Shake well. Allow to stand refrigerated or on ice for 5 minutes. Mix before use.
- Store reconstituted Positive Control refrigerated for up to 48 hours.
- See **Retest of Initial Positive** for Positive Control performance specifications.
- Use 300 μ L of Positive Control in **Procedure**.

Long-Term Sample and Control Storage

- Mix sample, aliquot 0.5 ml portions (or more) into clean vials, label, and freeze.
- Freeze reconstituted Positive Control within 6 hours of preparation.
- Freeze aliquots at -15°C or below for up to 1 month.
- Thaw frozen aliquots slowly (overnight in refrigerator or with cool water) and shake well. Store thawed sample or Controls refrigerated and use within 24 hours of thawing. Noticeable protein precipitation indicates an unsuitable sample.
- Discard any unused thawed sample. **Do not refreeze.**
- Thawed samples should be centrifuged at 1200 ± 200 g for 3 minutes prior to use. Cool and use skim portion as test sample. Draw skim milk into pipet by puncturing fat layer with pipet tip and drawing skim milk from underneath fat.
- Thawed Positive Control does **not** need to be centrifuged prior to testing.

Daily Performance Check

- Daily and before retesting “initial positive” samples, check Charm EZ performance using the supplied Calibration Strips. On Charm EZ, go to menu and follow on-screen instructions. Calibration Strips must test within range.
- Test one Negative Control prior to testing actual samples to verify performance of test strips and equipment. See **Retest of Initial Positive** for performance specifications for Controls.
- If Calibration Strips or Controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance.

Precautions

- High fat samples (greater than 6.5%) may cause invalid results. Do not read invalid test strips in the Charm EZ.
- Debris on test strips may alter the Charm EZ optics. Keep equipment clean and wipe dust and milk off test strips before inserting in Charm EZ.
- ROSA Incubator or Charm EZ must be clean and level. ROSA Incubator or Charm EZ temperature must be $56 \pm 1^\circ\text{C}$. 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.
- The Charm EZ may take up to 3 minutes to reach proper temperature, depending on ambient temperature.
- Turning on the Charm EZ without the SD Card or the Slide Mechanism properly inserted will result in an error message.
- If testing frozen milk, reconstituted powdered milk, or certain individual cow samples containing precipitates, centrifuge (3 minutes at $1200 \pm 200 \text{ g}$) and use skim portion below fat layer as the milk sample for testing.
- Low fat skimmed samples will yield increased sensitivity.
- Not for use with pasteurized whole milk.

Charm
EZ



This strip is not
Charm EZ compatible.
Remove strip and
insert correct type.



- The Charm EZ is not compatible with all Charm test strips.
- Older, non-compatible test strips will result in an error message when inserted into the Charm EZ.
- For a complete list of Charm EZ compatible tests contact your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

Charm
EZ



Test has previously been
run (lines detected) or
reader lens is dirty. Check
lens and run another strip.

- Debris on test strips may alter the Charm EZ optics.
- Keep equipment clean and wipe dust and milk off test strips before inserting in Charm EZ.
- At the beginning of each assay run the Charm EZ checks to make sure the strip inserted has not been previously run and the optics lens is clean.

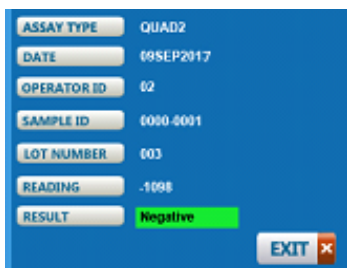
<p>Charm EZ </p> <p>ERROR. Door was opened during assay. Remove used strip and discard.</p>	<ul style="list-style-type: none"> • Do not open the door on Charm EZ while an assay is in progress. • Opening the door will invalidate the results and this will result in an error message. • Remove the test strip and restart the assay.
<p>Charm EZ </p> <p>Flow error detected. Run another test and check volume dispensed.</p>	<ul style="list-style-type: none"> • The Charm EZ monitors the assay progress during the incubation. • Damaged strips are detected by the system and will result in an error message • Remove the strip and restart the assay.

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.
- After incubation, test strip results are stable at room temperature for 10 minutes. Over several hours positive results will become more positive while negative results will remain stable. If refrigerated, results are stable for 1 hour. If frozen at -15°C or below, results are stable for 1 week.

QUAD2-G Test for Milk Procedure with Charm EZ Incubation

Use QUAD2-G test strips. Re-shape dented sample compartments to fit into Charm EZ.

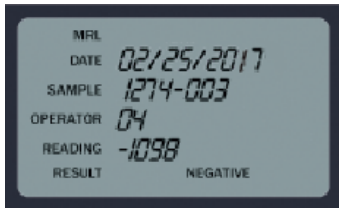
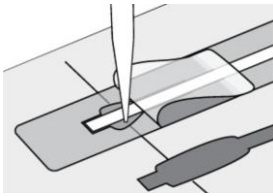
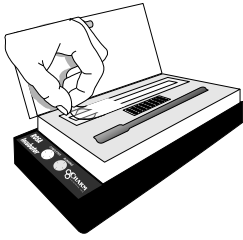


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| Step 1 | <ul style="list-style-type: none"> Power up Charm EZ and wait for Insert Strip to Start Screen. |
| Step 2 | <ul style="list-style-type: none"> Mix all samples well before testing. |
| Step 3 | <ul style="list-style-type: none"> Label test strip(s) with sample identification; test strip(s) may be placed in Charm EZ to avoid crushing sample compartment. |
| Step 4 | <ul style="list-style-type: none"> Place test strip in Charm EZ. The Charm EZ will automatically read the test strip and adjust the Assay Type and incubator to the required temperature. Wait for incubator temperature to turn green. |
| Step 5 | <ul style="list-style-type: none"> Tap the touch screen to enter the Operator ID, Sample ID or Lot Number information. |
| Step 6 | <ul style="list-style-type: none"> Holding test strip flat in Charm EZ, use tab to expose sample compartment by peeling tape to "Peel to Here" line. Avoid lifting the test strip and sponge under tape. |
| Step 7 | <ul style="list-style-type: none"> Using 300 μL pipet, draw up sample, avoiding foam and bubbles. Holding pipet vertically, slowly pipet 300 μL (\pm 15 μL) sample into sample compartment at indicator line (as shown). |
| Step 8 | <ul style="list-style-type: none"> Reseal tape over sample compartment. |
| Step 9 | <ul style="list-style-type: none"> Close door on Charm EZ. This will start the 5 minute timer. Do not open the door while the assay is in progress. |
| Step 10 | <ul style="list-style-type: none"> Assay Results will appear on the screen automatically at the end of the incubation period. Remove test strip(s) from Charm EZ to return to the Insert Strip to Start Screen. |

QUAD2-G Test for Milk Procedure with ROSA Incubator

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

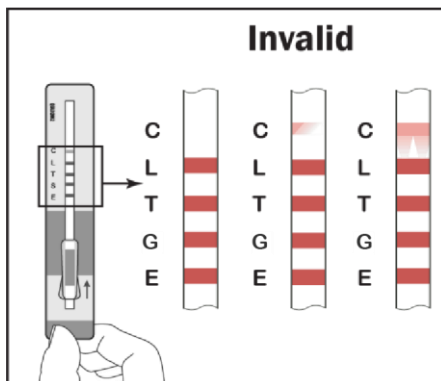
Use QUAD2-G test strips. Re-shape dented sample compartments to fit into ROSA Incubator.



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|---------------|--|
| Step 1 | <ul style="list-style-type: none"> Mix all samples well before testing. |
| Step 2 | <ul style="list-style-type: none"> Label test strip(s) with sample identification. Avoid crushing sample compartment. |
| Step 3 | <ul style="list-style-type: none"> Place test strip in ROSA Incubator. Holding test strip flat in ROSA Incubator, use TAB to expose sample compartment by peeling tape back to "Peel to Here" line. Avoid lifting the test strip and sponge under tape. |
| Step 4 | <ul style="list-style-type: none"> Using 300 μL pipet, draw up sample. Avoid foam and bubbles. Holding pipet vertically, slowly pipet 300 μL ($\pm 15 \mu\text{L}$) sample or Control into sample compartment at ROSA Incubator indicator line (as shown). |
| Step 5 | <ul style="list-style-type: none"> Reseal tape over sample compartment. When performing multiple tests in a ROSA Incubator: <ul style="list-style-type: none"> Peel, pipet and reseal before starting next test strip. Complete all test strips within 1 minute. |
| Step 6 | <ul style="list-style-type: none"> Close lid on ROSA Incubator and latch. Timer starts and red light illuminates. |
| Step 7 | <ul style="list-style-type: none"> Incubate for 5 minutes, but not more than 6 minutes. At 5 minutes, a beeper and alternating yellow and red blinking lights start. |
| Step 8 | <ul style="list-style-type: none"> 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 5 minutes of incubation completion. Lower ROSA Incubator lid. Do not re-latch. |

Visual Inspection

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



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

- C (Control) line is missing
- C line is smeared or uneven
- Any lines are obscured by sample or Control
- Beads do not flow past C line

Re-test INVALID samples. DO NOT INTERPRET OR PUT INVALID TEST STRIPS IN CHARM EZ.

Interpretation



Within 5 minutes of completing incubation, insert clean and visually valid test strip into Charm EZ as shown. Slide test strip completely into slot until it stops.

If desired, enter **SAMPLE** and/or **OPERATOR**. Close door to read. A numerical value (**READING**) and an interpretation (**RESULT**) are displayed on screen.

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



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

Positive - If **READING** is a positive number, the Charm EZ will display **RESULT POSITIVE** with information about the drug or family that tested positive:

LNC = Lincomycin/Pirlimycin

TYL = Tylosin/Tilmicosin

GEN = Gentamicin

ERY = Erythromycin

Positive samples should be retested. See **Retest of Initial Positive**.

Retest of Initial Positive

1. Test "initial positive" samples in duplicate along with one Negative Control and one Positive Control.
2. Negative Control must yield **RESULT NEGATIVE** with **READING** less (more negative) than -600 on Charm EZ.
3. Positive must yield **RESULT POSITIVE** with **READING** greater than +600 on Charm EZ.
4. If Negative Control or Positive Control are not within range, repeat testing of "initial positive" with Negative Control and Positive Control. If Negative Control or Positive Control are still not within range after retest, discontinue testing and contact Charm Sciences.
5. If Negative Control and Positive Control are within range and either or both of the retested samples are **RESULT POSITIVE**, the sample is a "Lincomycin/Pirlimycin, Tylosin/Tilmicosin, Gentamicin, and/or Erythromycin Positive Test".

Order Codes and Kit Contents

KITS	NOT SUPPLIED WITH KIT
<p>LF-QUAD2-G-20K:</p> <ul style="list-style-type: none"> (1) container of 20 QUAD2-G test strips (1) ROSA Multi-Antimicrobial Positive Tablet (1) Operator's Manual <p>LF-QUAD2-G-100K:</p> <ul style="list-style-type: none"> (1) container of 100 QUAD2-G test strips (5) ROSA Multi-Antimicrobial Positive Tablets (1) Operator's Manual <p>LF-QUAD2-G-500K:</p> <ul style="list-style-type: none"> (5) LF-QUAD2-G-100K 	<p>1-MLT-100 (box of 100 disposable pipet tips, 200-1000 µL)</p> <p>1-MLT-BG (bag of 1,000 disposable pipet tips, 200-1000 µL)</p> <p>1-MLT-X1 (2 boxes of 100 disposable pipet tips, 200-1000 µL)</p> <p>1-MLT-X4 (10 boxes of 100 disposable pipet tips, 200-1000 µL)</p> <p>LF-ROSA-PIPET-500 (500 disposable ROSA-pipets, 300 µL)</p> <p>LF-ROSA-PIPET-5000 (5000 disposable ROSA-pipets, 300 µL)</p> <p>PIP-300UL-1STOP-M (300 µL fixed volume pipet)</p> <p>PIP-100-1000UL-1STOP (100 to 1000 µL pipet)</p> <p>RW (1 bottle of Reagent Water, 30 mL)</p> <p>LF-ROSA-EZ (Charm EZ System)</p>

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.