



## Operator's Manual:

# Charm MRL Beta-lactam and Tetracycline 2\*-Minute Test for Raw Commingled Cow, Goat\*, and Sheep\* Milk

\* 3 minute incubation time for goat and sheep milk

## Kit Information

### Introduction

The Charm MRL Beta-lactam and Tetracycline 2-Minute Test is an immunoreceptor assay utilizing Rapid One Step Assay (ROSA®) lateral flow technology. Beta-lactams or tetracyclines interact with colored beads in the lateral flow test strip, and the color intensity in the test and control zones is measured by the ROSA Reader or Charm EZ® System. The MRLBLTET2 test detects beta-lactam and tetracycline drugs at or below the EU/CODEX MRL (maximum residue limit). The test incubation time is 2 minutes for cow milk, and 3 minutes for goat milk and sheep milk. 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"> <li>MRLBLTET2 Test Strips</li> <li>MRLBLTET/TET Positive Tablets (4 ppb Penicillin G and 100 ppb Oxytetracycline)</li> <li>Operator's Manual</li> </ul>	<ul style="list-style-type: none"> <li>Pipet Tips or ROSA Pipets</li> </ul>	<ul style="list-style-type: none"> <li>ROSA Incubator (56°C) and ROSA Reader or Charm EZ System</li> <li>Printer (optional)</li> <li>300 µl Pipet (optional)</li> </ul>

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

### Sensitivity and Selectivity

Beta-lactam and tetracycline-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**

Beta-lactam Drug	Detection Range <sup>†</sup> (ppb)	EU / CODEX MRL (ppb*)	Beta-lactam Drug	Detection Range <sup>†</sup> (ppb)	EU / CODEX MRL (ppb*)
Amoxicillin	3 to 5	4	Cloxacillin	10 to 20	30
Ampicillin	3 to 4	4	Dicloxacillin	10 to 20	30
Cefacetrile	20 to 40	125	Oxacillin	10 to 30	30
Cefalonium	10 to 20	20	Penicillin G	2 to 3	4 / 4
Cefazolin	20 to 40	50	<b>Tetracycline Drug</b>	<b>Detection Range<sup>†</sup> (ppb)</b>	<b>EU / CODEX MRL (ppb*)</b>
Cefoperazone	1 to 3	50			
Cefquinome	15 to 25	20	Chlortetracycline	50 to 100	100 / 100
Ceftiofur and Metabolite <sup>^</sup>	40 to 70	100 / 100	Oxytetracycline	50 to 100	100 / 100
Cephapirin	15 to 25	60 <sup>§</sup>	Tetracycline	10 to 30	100 / 100

\* parts per billion or µg/L

<sup>^</sup> Ceftiofur parent drug sensitivity is at levels approximately ¼ those reported in the table.

<sup>†</sup> Positive at least 95% of the time. Detection ranges in goat and sheep milk are less than the detection ranges for cow milk.

<sup>§</sup> U.S. Tolerance is 20 ppb cephapirin.

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## Storage

Store MRLBLTET2 test strips and Positive Tablets 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: aminoglycosides, amphenicols, fluoroquinolones, macrolides/lincosamides, nitrofurans, sulfa drugs/sulfonamides, or chlorothiazide, dexamethasone, dipyrone, flunixin, furosemide, ivermectin, novobiocin, oxytocin, PABA, phenylbutazone, trichlormethiazide, and thiabendazole. Other beta-lactam and tetracycline drugs are detected; cefalexin at 1000 to 2000 ppb, cefuroxime at 100 to 300 ppb, nafcillin at 100 to 200 ppb, and piperacillin at 3 to 5 ppb. There are no interferences from somatic cells at  $10^6$  SCC/ml or bacteria at  $3 \times 10^5$  CFU/ml.

## 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](mailto:support@charm.com).

## Reagents and Storage

Test kits are not required to be shipped refrigerated.

## 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 beta-lactam negative and tetracycline negative raw, commingled milk as a Negative Control for performance monitoring and to reconstitute Positive Tablet for use as the Positive Control. Do not use pasteurized whole milk.
- Qualified Negative Control should yield strong visual negative (**BL** and **TE** lines are darker than **C** line).
- Store negative Control may be used for up to 72 hours refrigerated.
- Use 300 µl of Negative Control in Procedure.
- **See Retest of Initial Positive** for Negative Control performance specifications.

## Positive Control

- Use MRLBLTET/TET Positive Tablet to make a  $4 \pm 0.4$  ppb penicillin G and  $100 \pm 20$  ppb oxytetracycline 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.
- Store reconstituted Positive Control refrigerated for up to 48 hours.
- Use 300 µl of Positive Control in Procedure.
- See **Retest of Initial Positive** for Positive Control performance specifications

## Long-Term Sample and Control Storage

- Mix sample, aliquot 0.5 ml portions (or more) into clean vials, label, and freeze.
- Freeze controls 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 thawed sample. **Do not refreeze.**

## Daily Performance Check

- Daily and before retesting "initial positive" samples, check ROSA Reader or Charm EZ system performance using the supplied Calibration Strips. With ROSA Reader, enter performance mode in ROSA Reader by sequentially pressing ESC, 5, ENTER. With Charm EZ system, go to menu and follow on-screen instructions. Calibration Strips must test within range.
- Test one Negative Control and one Positive 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 ROSA Reader or Charm EZ system.
- This test is not for use with pasteurized whole milk.
- Debris on test strips may alter the ROSA Reader or Charm EZ system optics. Keep equipment clean and wipe dust and milk off test strips before inserting in ROSA Reader or Charm EZ system.
- ROSA Incubator or Charm EZ system must be clean and level. ROSA Incubator or Charm EZ system 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 system may take up to 3 minutes to reach proper temperature, depending on ambient temperature.
- Turning on the Charm EZ system without the SD Card or the Slide Mechanism properly inserted will result in an error message.

Charm  
EZ



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

- The Charm EZ system 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 system.
- 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](mailto:support@charm.com).

<p><b>Charm EZ</b> </p> <p>Test has previously been run (lines detected) or reader lens is dirty. <b>Check lens and run another strip.</b></p>	<ul style="list-style-type: none"> <li>• Debris on test strips may alter the Charm EZ system optics.</li> <li>• Keep equipment clean and wipe dust and milk off test strips before inserting in Charm EZ system.</li> <li>• At the beginning of each assay run the Charm EZ system checks to make sure the strip inserted has not been previously run and the optics lens is clean.</li> </ul>
<p><b>Charm EZ</b> </p> <p><b>ERROR. Door was opened during assay. Remove used strip and discard.</b></p>	<ul style="list-style-type: none"> <li>• Do not open the door on Charm EZ system door while an assay is in progress.</li> <li>• Opening the door will invalidate the results and this will result in an error message.</li> <li>• Remove the test strip and restart the assay.</li> </ul>
<p><b>Charm EZ</b> </p> <p>Flow error detected. <b>Run another test and check volume dispensed.</b></p>	<ul style="list-style-type: none"> <li>• The Charm EZ system monitors the assay progress during the incubation.</li> <li>• Damaged strips are detected by the Charm EZ system and will result in an error message.</li> <li>• Remove the strip and restart the assay.</li> </ul>

## 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**.
- Individual cow, goat, and sheep milk samples or poorly mixed samples with fat greater than 6.5% may cause invalids. Mix samples well before testing.
- Test may be performed at ambient temperatures of 10 to 30°C in naturally circulating air.

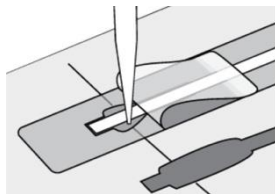
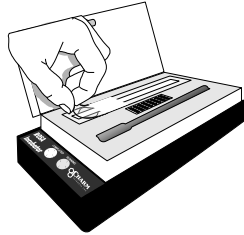
## Goat and Sheep Milk Information

- Testing raw commingled goat and sheep milk requires increasing the incubation time from 2 minutes to 3 minutes.
- To have a 3 minute incubation option added to the MRLBLTET2 channel on the Charm EZ system contact your local representative or Charm Sciences at +1.978.687.9200 or [support@charm.com](mailto:support@charm.com).

## MRLBLTET2 Test for Milk Procedure with ROSA Incubator

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

Use MRLBLTET2 test strips. Re-shape dented sample compartments to fit into ROSA Incubator.

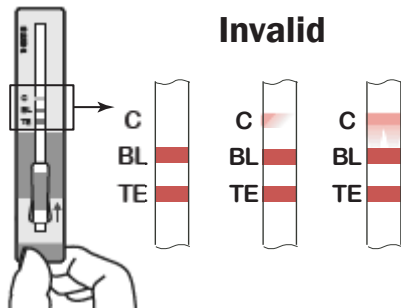


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| <b>Step 1</b> | <ul style="list-style-type: none"> <li>• <b>Mix all samples well before testing.</b></li> </ul>  |
| <b>Step 2</b> | <ul style="list-style-type: none"> <li>• <b>Label test strip(s)</b> with sample identification. Avoid crushing sample compartment.</li> </ul>  |
| <b>Step 3</b> | <ul style="list-style-type: none"> <li>• <b>Place test strip in ROSA Incubator.</b></li> <li>• Holding test strip flat in ROSA Incubator, use TAB to <b>expose sample compartment by peeling tape back to "Peel to Here" line.</b> Avoid lifting the test strip and sponge under tape.</li> </ul>  |
| <b>Step 4</b> | <ul style="list-style-type: none"> <li>• Using 300 <math>\mu\text{l}</math> pipet, draw up sample. Avoid foam and bubbles.</li> <li>• Holding pipet vertically, <b>slowly pipet 300 <math>\mu\text{l}</math></b> (<math>\pm 15 \mu\text{l}</math>) sample or Control into sample compartment at ROSA Incubator indicator line (as shown).</li> </ul> |
| <b>Step 5</b> | <ul style="list-style-type: none"> <li>• <b>Reseal tape</b> over sample compartment.</li> <li>• When performing multiple tests in a ROSA Incubator: <ul style="list-style-type: none"> <li>○ Peel, pipet and reseal before starting next test strip.</li> <li>○ Complete all test strips within 1 minute.</li> </ul> </li> </ul>                     |
| <b>Step 6</b> | <ul style="list-style-type: none"> <li>• <b>Close lid</b> on ROSA Incubator and latch. Timer starts and red light illuminates.</li> </ul>  |
| <b>Step 7</b> | <ul style="list-style-type: none"> <li>• <b>Incubate</b> for 2 minutes*, but not more than 2.5 minutes.</li> <li>• At 2 minutes, a beeper and alternating yellow and red blinking lights start.</li> </ul>   |
| <b>Step 8</b> | <ul style="list-style-type: none"> <li>• <b>Remove test strip(s)</b> from ROSA Incubator. Do not squeeze sample compartment. Hold test strip with sample compartment in the down position until interpreted.</li> <li>• Read within 3 minutes of incubation completion.</li> <li>• <b>Lower ROSA Incubator lid.</b> Do not re-latch.</li> </ul>      |

\* Incubate for 3 minutes but not more than 3.5 minutes for goat and sheep milk.

## 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.



### Invalid

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

- **C** (control) line is missing
- **C** line is smeared or uneven
- **C**, **BL** (beta-lactam), or **TE** (tetracycline) lines are obscured by sample or Control.
- Beads do not flow past **C**, **BL**, or **TE** lines.

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

## Interpretation

### ROSA Reader Interpretation

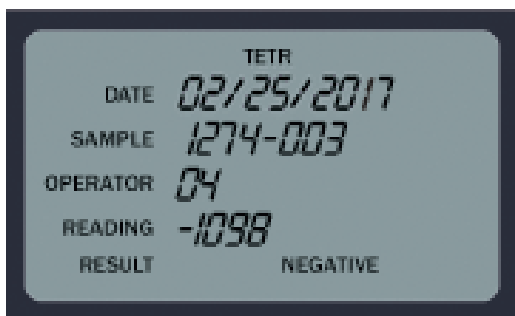


Within 3 minutes of completing incubation, insert clean and visually valid test strip into ROSA Reader as shown. Slide test strip completely into slot until it stops.

Read results on **TETR** channel of ROSA Reader in 3-line/3 minute mode\*. If desired, enter **SAMPLE** and/or **OPERATOR**. Press **ENTER** 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.

\* Refer to Charm ROSA Reader Operating Instructions for set up and operation.



**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**.

Interpretation of positive results will be: **BL** (if beta-lactam positive), **TET** (if tetracycline positive), or **BLTET** (if beta-lactam, tetracycline, or both beta-lactam and tetracycline positive). This will flash on the **SAMPLE** line and will appear on the printout as **READING**.

### Charm EZ System Interpretation (Read only mode)



Within 3 minutes of completing incubation, insert clean and visually valid test strip into Charm EZ system as shown. Slide test strip completely into slot until it stops.

If desired, enter **SAMPLE ID** and/or **OPERATOR ID**. 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.



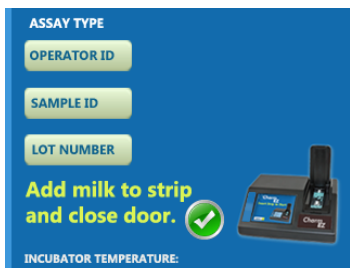
**Negative** - If **READING** is a negative number or zero, the Charm EZ system will display **RESULT NEGATIVE**. Report as "Not Found".

**Positive** - If **READING** is a positive number, the Charm EZ system will display **RESULT POSITIVE**. Sample should be retested. See **Retest of Initial Positive**.

Interpretation of positive results will be: **BL** (if beta-lactam positive), **TET** (if tetracycline positive), or **BLTET** (if beta-lactam, tetracycline, or both beta-lactam and tetracycline positive). This will flash on the **SAMPLE ID** line and will appear on the printout as **READING**.

# MRLBLTET2 Test for Milk Procedure with Charm EZ System Incubation

Use MRLBLTET2 test strips. Re-shape dented sample compartments to fit into Charm EZ system.



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

\*To have a 3 minute incubation option added to the MRLBLTET2 channel on the Charm EZ system contact your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

## Retest of Initial Positive

1. Test "initial positive" samples in duplicate along with one Negative Control and one Positive Control.
2. Negative Control must be **RESULT NEGATIVE** with **READING** less (more negative) than -400 on ROSA Reader or Charm EZ system.
3. Positive Control must be **RESULT POSITIVE** with **READING** greater than +400 on ROSA Reader or Charm EZ system.
4. If either Control is not within range, repeat testing of "initial positive" with Controls. If either Control is still not within range after retest, discontinue testing and contact Charm Sciences.
5. If both Controls are within range and either or both of the retested samples are **RESULT POSITIVE**, the sample is a "Beta-lactam and/or Tetracycline Positive Test".

## Order Codes and Kit Contents

KITS	NOT SUPPLIED WITH KIT
<p><b>LF-MRLBLTET2-20K:</b></p> <ul style="list-style-type: none"> <li>• (1) container of 20 MRLBLTET2 test strips</li> <li>• (1) MRLBLTET/TET Positive Tablet (4 ppb Penicillin G and 100 ppb Oxytetracycline)</li> <li>• (1) Operator's Manual</li> </ul> <p><b>LF-MRLBLTET2-100K:</b></p> <ul style="list-style-type: none"> <li>• (1) container of 100 MRLBLTET2 test strips</li> <li>• (5) MRLBLTET/TET Positive Tablets (4 ppb Penicillin G and 100 ppb Oxytetracycline)</li> <li>• (1) Operator's Manual</li> </ul> <p><b>LF-MRLBLTET2-500K:</b></p> <ul style="list-style-type: none"> <li>• (5) LF-MRLBLTET2-100K</li> </ul>	<p><b>1-MLT-96</b> (box of 96 disposable pipet tips, 200-1000 µl)</p> <p><b>1-MLT-768</b> (8 boxes of 96 disposable pipet tips, 200-1000 µl)</p> <p><b>1-MLT-BG</b> (bag of 1,000 disposable pipet tips, 200-1000 µl)</p> <p><b>1-MLT-MLA-X3</b> (5 boxes of 200 disposable pipet tips for MLA pipet, 1000 µl)</p> <p><b>LF-ROSA-PIPET-500</b> (500 disposable ROSA pipets, 300 µl)</p> <p><b>LF-ROSA-PIPET-5000</b> (5000 disposable ROSA pipets, 300 µl)</p> <p><b>PIP-300UL-1STOP-M</b> (300 µl fixed volume pipet)</p> <p><b>PIP-100-1000UL-1STOP</b> (100 to 1000 µl pipet)</p> <p><b>LF-INC4-3-56D</b> (4 place ROSA Incubator with 3 minute timer, 56°C)</p> <p><b>LF-INC2-3-56</b> (2 place ROSA Incubator with 3 minute timer, 56°C)</p> <p><b>LF-INC4-2-56D</b> (4 place ROSA Incubator with 2 minute timer, 56°C)</p> <p><b>LF-INC2-2-56</b> (2 place ROSA Incubator with 2 minute timer, 56°C)</p> <p><b>LF-ROSA-PEARL-X-NB</b> (ROSA Pearl-X Reader)</p> <p><b>LF-ROSA-EZ</b> (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.