

Drug Residue Testing

NCIMS Approved Drug Residue Tests



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WISCONSIN DEPARTMENT OF AGRICULTURE TRADE AND CONSUMER PROTECTION

Fall 2026 Workshop

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Covered In This Presentation:

- Types of tests approved
- Basic requirements for each test
- Basic procedures for each test
- Tests approved for other species
- Changes in the 2400 forms



NOTE: This presentation is a basic overview. Refer to the NCIMS 2400 forms for more specific information on each test.



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Confirmation With Equivalent Tests

- If you confirm a screened positive with an equivalent test, it must detect the same drugs at or below the safe level.
- Be aware that two tests that detect the same drug may not detect it at the same level.
- A confirming test that detects more drugs than the screening test can be used.
 - As long as it detects the same drugs as the screening test at or below the safe level



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Form Updates

The following 2400 forms were updated May of 2026:

Charm SL, 3SL3, and 30sec	Charm TRIO
Charm Sulfonamide	Charm Tetracycline

- Adds the Charm EZ Protect + Reader
- Clarifies the proper use of the Negative Control Qualifier
- All labs need to replace their old forms with these new forms.

- Footer

FORM NCIMS 2400n-1 Charm SL-SL3-BL30SEC Rev. 05/2026



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Tests approved for other species



In addition to cows (Bovine) milk, people around the world consume the milk of goats, sheep, water buffalo, camels, llamas, reindeer, horses and yaks



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NCIMS Tests Approved For Goat Milk

- CHARM SL
- CHARM II
 - Sequential Assay
- CHARM BsDA
- Delvotest P
- Delvo 5-Pack
- IDEXX New SNAP





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NCIMS Approved Tests for Sheep Milk

- CHARM SL



Sheep milk may be frozen prior to testing.

- Sample must be taken prior to freezing
- Sample and container frozen and maintained together for up to 60 days
- Sample thawed and tested within 24 hours
- Frozen sheep milk sample(s) and controls must be centrifuged per procedure before running the assay



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NCIMS Approved Tests



Water Buffalo milk:

- CHARM SL
- Delvotest® P



Camel milk:

- IDEXX New SNAP



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Test Kits Approved for Appendix N testing

(Note: The Charm FLUSBL Flunixin and Beta-lactam Assay has been discontinued)

Commonly Used Tests

- Charm SL
- Charm 3 SL-3
- Charm BL30SEC
- Charm SULF
- Charm Tetracycline-SL
- Charm TRIO



Uncommonly Used Tests

- Charm BsDA
- Charm II Beta-Lactam Assays:
 - Competitive
 - Sequential
 - Quantitative
- IDEXX Tetracycline
- Charm II Non-Beta-Lactam Assays:
 - Sulfonamide
 - Tetracycline



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Visual Read Tests

- Charm BsDA
- Delvotest® P Mini
- Delvotest® 5-Pack

All tests use *Geobacillus stearothermophilus* spores and take about 2.5 to 3 hours for the spores to germinate at 64°C. Presence of inhibitors will kill spores in seeded medium.



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Visual Read Tests

Visual Read tests are recognized to detect:

- Penicillin
- Amoxicillin
- Ampicillin
- Cephalosporin
- May also be able to detect other drugs as well, but not consistently



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Visual Read Tests

- Confirmation requires samples and controls heated in an 82±2°C water bath for two minutes (temperature control (TC) required), tested in duplicate
- Beta-lactamase:
 - Optional for verification of the initial positive in screening sites
 - However, if used, results must be reported
 - **Required for Appendix N confirmation**
- Refer to 2400 form for reporting details



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Delvotest®



Delvotest P Mini

- Individual test wells detect Beta-lactams

Delvotest 5 Pack

- 96-well blocks can be broken into strips of 16 wells. Usually used for Section 6 (milk quality) testing due to minimum volume of wells necessary per use



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Delvotest Requirements

- Room temperature range for testing 16-27°C (61-81°F)
- Heater block temperature:
 - 64±1°C for Delvo P Mini
 - 64±2°C for Delvo 5-Pack
- Nutrient tablets maintained in original opened bottle at room temperature with desiccant enclosed (mark with date opened)
 - Discard remaining tablets when last ampoule/well used.
- Kits stored at 0-15°C
- Beta-lactamase instructions now refer to manufacturer for directions on use, due to multiple manufacturers



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Delvotest Procedure

- Add one tablet to each labeled ampoule/well. Make sure nutrient tablets are touching the media.
- Add 100µL mixed sample.
- Seal Delvotest 5 Pack wells with provided sealing strips.
- Incubate at 64°C **for the time period specified by the manufacturer** (about 2.5 hours). Controls must show proper color reactions.
- Ampoules **yellow** or **yellow/purple** after incubation are negative (NF).
- Ampoules **purple** are presumptive positive and must be confirmed with heating step.
- After heating, any **yellow** in the ampoule/well is negative (Not Found).



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Common Tests using a reader/printer -
beta lactam assays:

•Charm Sciences

• Charm SL

• Charm 3 SL 3

• Charm BL30SEC

• Charm TRIO

•Idexx New SNAP®



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ALL CHARM TEST STRIP ASSAYS
SHARE THESE SAME REQUIREMENTS:

• Charm SL

• Charm 3 SL-3

• Charm BL30SEC

• Charm SULF

• Charm Tetracycline-SL

• Charm TRIO

• Incubator temperature 56±1°C

• Negative control -600 or more negative

• Positive control +400 or more positive



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Basic Procedure for Charm Test Strip Assays

1. Take out test strips

1. As needed

2. Or store extras in a dry, labeled container at room temperature

1. Dispose of unused test strips at the end of the day

2. Label each test strip, avoid crushing sample compartment

3. Mix sample, pipette within three minutes



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Basic Procedure for Charm Test Strip Assays (cont.)

4. **Place** first strip in incubator.
5. **Peel** strip cover back.
6. **Pipette** sample (avoid foam and bubbles).
 - i. With the pipettor/pipet vertical, slowly dispense into sample compartment.
7. **Seal** strip and repeat for next sample.



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Basic Procedure for Charm Test Strip Assays (cont.)

- All samples completed within test kit time frames after placing first strip in incubator
- Close and latch incubator cover, start timer
- Total time of incubation as per test kit
- Hold strips vertically if not reading immediately
- Read within time specified per test kit



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Charm EZ and EZ Protect + Incubate and Read Procedure

Charm EZ Readers automatically set channel and incubation temperature when strip is inserted

- Mix sample, pipette within three minutes
- **Place** strip in EZ Reader
- **Peel** strip cover back
- **Pipette** sample (avoid foam and bubbles)
 - With the pipettor/pipet vertical, slowly dispense into sample compartment
- **Seal** strip
- Close door to begin test
- EZ Readers automatically prompt for further testing when positive



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Charm EZ or EZ Protect + Readers

- Charm is replacing old ROSA (and Pearl) readers with EZ or EZ Protect + readers if the ROSA reader stops working.
- Are considered an **equivalent** (not the same) reader as the ROSA and Pearl readers.
 - Switching between readers is not allowed within a testing process (verification, load confirmation, etc.) when using both readers at a facility.



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Charm EZ Reader

- If you wish to switch modes between "Read Only" and "Incubate and Read", you will first need to send the reader to Charm Sciences so they can change the mode and recalibrate the reader.



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EZ Protect +

Primary Parts

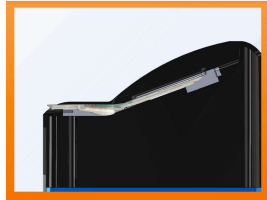
- Display or Base
 - Touch Screen – Serial Number
- Smart Optics
 - 2 Optic Modules
- Module Extender
 - 4 Additional Optic Modules
- Printer or Data Download



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EZ Protect +

Placing the Strip will feel different
Unit Detects the milk flow
May take longer than Charm EZ



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More changes to the Charm SL/3 SL-3/BL30SEC 2400 Form

- Removed "EZ Compatible Strips" statement since all strips are now EZ compatible
- SL-3 strips can now be shipped unrefrigerated if received within seven days
 - Over seven days must be refrigerated
- A few wording updates/clarifications



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Changes to the Charm SL/3 SL-3/BL30SEC 2400 Form

Negative Control Qualifier has been clarified

- Approved for Charm SL, Charm 3 SL-3, and Charm BL30SEC tests.
 - Also approved for Charm SULF, TETRA, and TRIO
- Charm Freeze Dried Negative Control Qualifier (NCQ)
 - Can be used to qualify an unknown raw milk sample to become negative control if no previously tested negative control milk is available for use
 - Basic procedure (see 2400 form for specifics):
 1. Rehydrate Negative Control Qualifier
 2. Test NCQ and raw milk twice (all results $\leq$ -600) and compare average to see if raw milk sample can be used as a Negative Control



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Charm SL

- Detects **five** of six Beta-lactams using ROSA Reader V1.03 or higher
- Centrifuge required if using frozen controls
 - After slowly thawing controls, centrifuge 3 minutes and cool
 - Test portion below fat layer without mixing
- Testing procedure specifications:
 - Close lid within 2 minutes of placing first strip in incubator
 - Total incubation time: 8 minutes, 9 minutes MAXIMUM
 - Read within 5 minutes



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Charm 3 SL-3

- Detects **all six** Beta-lactams
- ROSA Pearl Reader V3.00 or higher
- Due to tight time frames of test, use an extra timer when running multiple samples to maintain time limits
- Testing procedure specifications:
 - Close lid within 1 minute, 15 seconds of placing first strip in incubator
 - Total incubation time: 3 minutes
 - 3 minutes, 30 seconds MAXIMUM
 - Read within 3 minutes



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Charm BL30SEC

- Detects **all six** Beta-lactams
- Testing procedure specifications:
 - EZ and EZ Protect + Reader: incubate and read mode required for this test



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Charm TRIO

- Detects Beta-lactams, Sulfonamides, and Tetracyclines
 - **Confirmation must be performed with separate drug-specific tests**
- Must read with EZ or EZ Protect+ Reader
- EZ Reader must be in "Read Only" mode for Verification step



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Charm TRIO requirements

- Can initially screen samples and verify in duplicate with TRIO for BL and Sulfa. However, ANY positives in verification step must be confirmed with individual tests.
- Confirmation procedure must run each drug **separately** (using drug-specific tests – not Charm TRIO).
 - Tetracycline only positive: Verification and confirmation tests are diluted.
 - Beta-lactam test picks up **all six** BL drugs, so must be confirmed with an equivalent test.
- Positive control contains all three drugs.
- Negative control must be cleared on all three drugs.



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Charm TRIO procedure

- Testing procedure specifications:
 - Close lid within 90 seconds of placing first strip in incubator
 - Total incubation time: 3 minutes, 4 minutes MAXIMUM
 - Read within 3 minutes
- EZ Readers will indicate what drug line(s) is(are) causing a positive
 - Follow procedure in 2400 form for each drug's verification and confirmation step
 - If duplicate testing detects drugs other than the drugs detected on the initial test, redo verification step for new drug that was detected



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Idexx New SNAP®



- Individual test kits for use with reader
- Testing area must be well lit
- Testing complete in about 10 minutes



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Idexx New SNAP Requirements

- Heater Block $45 \pm 5^{\circ}\text{C}$
 - Testing area must be well lit
- Kits stored at $0-7^{\circ}\text{C}$
- Must use SNAPshot DSR Reader with printer
- Reader Performance Check Set must be within limits
- Can use frozen negative control. Positive control cannot be frozen, but can be made with frozen negative milk.



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Idexx New SNAP Procedure

- Set out SNAP devices, samples tubes, and pipettes for each sample
 - Discard unused devices at the end of day
- Label each device and sample tube
- Place SNAP device on incubator block
 - Make sure blue reagent pellet is in bottom of tube before removing cap
- Mix sample, pipette within three minutes
- Pipette $450 (\pm 50) \mu\text{L}$ sample into sample tube
 - Sample tube should not be placed in heater block until after sample is pipetted into tube.



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Idexx New SNAP Procedure

- Incubate tube in heater block for 5 minutes (use timer)
- Pour contents of tube into sample well of SNAP device
- Unit must be snapped when blue activation circle first **BEGINS** to disappear (unit remains in heater block)
- Incubate device for 4 minutes (use timer)
- Inspect control and test spots to determine if test is valid
- Read IMMEDIATELY after final incubation with IDEXX Reader
 - Read within **30 seconds maximum** – test no more than 2 samples at a time to stay within this time limit



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Common Tests Using a Reader/Printer Other Inhibitor Assays:

Testing for other than Beta-lactams:

- Currently, you are not required to test for other inhibitors, BUT
- If you test for it and find it, you must have it confirmed

- Charm Sciences (ROSA)
 - Charm SULF
 - Charm Tetracycline SL
 - Charm TRIO



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Charm SULF Sulfonamide test

- Uses ROSA Reader V1.03 or higher set to SULF channel
 - or use EZ or EZ Protect+ Reader
- Positive control made with 10ppb Sulfamethazine
- Test times are the same as SLBL (8-minute) test
- All testing is run on an UNDILUTED sample (screening, verification, and confirmation)
- EZ Reader must be in "Read Only" mode for Verification and Confirmation steps



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Charm ROSA Tetracycline SL Test



- Uses ROSA Reader V1.03 or higher set to TETRA channel
 - or use EZ or EZ Protect+ Reader
- Test times are the same as SLBL (8-minute) test
- Screening and producer traceback run with UNDILUTED sample
- Verification, load confirmation, and producer confirmation run with DILUTED sample
- EZ Readers must be in “Read Only” mode for Verification and Confirmation steps

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Charm ROSA Tetracycline SL

- Negative control
 - Previously tested tetracycline negative raw milk
- Positive control
 - Prepare with three tablets 100ppb Oxytetracycline in 5mL negative control (raw milk)
- ROSA Reader – TETRA slow blink
- Test procedure is the same as SL Beta-lactam (screen)

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Charm ROSA Tetracycline SL

- DILUTION STEP REQUIRED FOR CONFIRMATION
- Initial screen, producer traceback, and negative clearing samples are run UNDILUTED
 - Any positive load or producer result is retested in duplicate with the dilution step
- Load verification, load confirmation, and producer confirmation samples are run DILUTED
 - Dilute the sample using 1mL Tetracycline Dilution Buffer and 1mL sample/control and mix before testing (1mL = 1000µL)

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Uncommon Tests – Beta-lactam Assays:

- Charm BSDA
- Charm II Beta lactam Assays
 - Competitive Assay
 - Sequential Assay
 - Quantitative Assay



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Uncommon Tests Using a Reader/Printer

Other Inhibitor Assays:

- IDEXX Tetracycline
- Charm II Non-Beta-Lactam Assays:
 - Sulfonamide
 - Tetracycline

Testing for other than Beta-lactams

- Currently, you are not required to test for other inhibitors, BUT
- If you test for it and find it, you must have it confirmed.

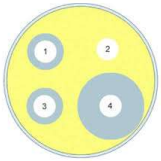


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Charm BsDA (Bacillus stearothermophilus Disk Assay)

Visual Read Test



- One of the oldest approved tests
- Uses media prepared in-house
- 2.5- to 3-hour incubation
- May detect other inhibitors as well as Beta-lactams

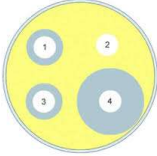


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Charm BsDA (Bacillus stearothermophilus Disk Assay)

- Paper discs placed 10mm apart on agar media (use template).
- Dispense 90 µL of positive control (5ppb Pen G standard), negative control (zero standard), and up to five samples per plate.
- Incubate at 64±2°C, 2.5 to 3 hours until well defined zones of inhibition are obtained on the positive control.
- Valid positive control zone is 16-20mm (use calipers readable to 0.1mm).
- Measurable zones >12.7mm must be promptly confirmed to report as positive for inhibitor or Beta-lactam residue.



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Charm II Tests

- Uses all equipment shown below to run test
- It takes about 20 minutes to complete the test
- Radioactive material needs proper handling and disposal



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Charm II Tests

All Charm II tests have been consolidated into two forms (Rev 4/16):

- Charm II Beta-Lactam Assays:** Competitive, Sequential, Quantitative, and Cloxacillin
 - Cloxacillin test has been withdrawn.
- Charm II Other Inhibitor Assays:** Sulfonamides, Tetracyclines, and Chloramphenicol (this test is not NCIMS approved)

Carefully review forms – procedures are intermixed



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Charm II Tests

- Incubation temperatures are different for each assay – check form for temperature requirements
- Vortex mixer and centrifuge required
- Scintillation fluid expires six months after opening (mark with open date); dispose of properly
- Must establish positive and negative control points (watch percentages)
- Sample results less than 50 greater than control point are recounted (Beta-lactam Competitive, Sequential, and Quantitative assays only)
- Tests use radioactive materials – handle properly



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Idexx SNAP® Tetracycline Test Requirements

- Same test requirements as Idexx SNAP Beta-lactam test
- Same test procedure as Beta-lactam test (screening only)

HOWEVER...



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Idexx New SNAP® Procedure

- DILUTION STEP **REQUIRED** FOR CONFIRMATION
- Initial screen, producer traceback, and negative clearing samples are run **UNDILUTED**
 - Any positive load or producer result is retested in duplicate with the dilution step
- Load verification, load confirmation, and producer confirmation samples are run **DILUTED**
 - Dilute the sample using one part sample to nine parts tetracycline negative milk (fresh or frozen)
 - Controls are not diluted



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