

Drug Residue Testing – NCIMS Approved Drug Residue Tests

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WISCONSIN DEPARTMENT OF AGRICULTURE, TRADE AND CONSUMER PROTECTION
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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





HOT OFF THE PRESS!!!!!



BIG NEWS!

- ALL 2400 forms have been updated with new footers.
- Removed "FDA" in footer as the NCIMS Lab Committee now revises all the forms.
- All labs need to replace their old forms with these new forms.

Not so big news:

 Only one form has had more changes than just the revision date. This is the 2400 form for the Charm SL, Charm 3 SL-3, and Charm BL30SEC tests.

HOWEVER - (BIG NEWS!!):

- This 2400 form has several MAJOR updates (more on this later)
- These changes only apply to the tests in this form.

FORM NCIMS 2400n Appendix N - General Requirements Rev. 03/2024



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Just learnt that people around the world consume the milk of goats, water buffalo, llamas, reindeer, horses, sheep, camels, 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





NCIMS APPROVED TESTS FOR SHEEP MILK



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 FLUSLBL FLUNIXIN AND BETA-LACTAM ASSAY HAS BEEN DISCONTINUED)

Commonly Used Tests

- Charm SL
- IDEXX SNAP
- · Charm 3 SL-3 Delvo 5 Pack
- Charm BL30SEC Delvotest P
- · Charm SULF
- Charm Tetracycline-SL
- Charm TRIO



Uncommonly Used Tests

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

- Quantitative



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- · 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
- Cephapirin
- 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 $_{\circ}$ However, if used, results must be reported
 - •Required for Appendix N confirmation
- Refer to 2400 form for reporting details



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
- 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
- •Idexx New SNAP®
- Charm BL30SEC
- Charm TRIO



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ALL CHARM TEST STRIP ASSAYS SHARE THESE SAME REQUIREMENTS:

- · Charm SL
- Incubator temperature 56±1°C
- Charm 3 SL-3
- Charm BL30SEC
- Negative control -600 or more negative
- Charm SULF
- Positive control +400 or more positive
- · Charm Tetracycline-SL
- Charm TRIO



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BASIC PROCEDURE FOR CHARM TEST STRIP ASSAYS

- 1. Take out test strips
 - ı. 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



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).
 - 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 INCUBATE AND READ PROCEDURE

Charm EZ Reader automatically sets 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 Reader automatically prompts for further testing when positive



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CHARM EZ READER

• Charm is replacing old ROSA (and Pearl) readers with the EZ reader if the ROSA stops working.



- Is 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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CHANGES TO THE CHARM SL/3 SL-3/BL30SEC 2400 FORM

- New EZ-Protect Reader is included on form (but not available yet)
- 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





CHANGES TO THE CHARM SL/3 SL-3/BL30SEC 2400 FORM NEGATIVE CONTROL QUALIFIER HAS BEEN ADDED

- ONLY approved for Charm SL, Charm 3 SL-3, and Charm BL30SEC tests.
- · No other tests can use this option
- Other Charm SL-type tests to be approved to use the NCQ soon
- 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):
- Rehydrate Negative Control Qualifier
- 2 Test NCQ and raw milk twice (all results ≤ -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 VI.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:
 - ${\tt m\,Close}$ 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:
- \bullet 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 Reader in 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 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 drugspecific tests – not Charm TRIO).
- ${\tt m}$ Tetracycline $\underline{{\sf only}}$ positive: Verification and confirmation tests are diluted.
- ${\tt n}\,{\sf Beta}\text{-lactam}$ test picks up ${\sf all}\,\,{\sf six}\,\,{\sf BL}$ 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 Reader 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±5°C
 - Testing area must be well lit
- Kits stored at 0-7°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.



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) μL 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



CHARM SULF SULFONAMIDE TEST

- Uses ROSA Reader VI.03 or higher set to SULF channel
 - or use EZ 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 VI.03 or higher set to TETRA channel
 - or use EZ 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 Reader 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)



CHARM ROSA TETRACYCLINE SL

- DILUTION STEP REQUIRED FOR CONFIRMATION
- Initial screen, producer traceback, and negative clearing samples are run UNDILUTED
 - \bullet 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
 - \bullet Dilute the sample using 1 mL Tetracycline Dilution Buffer and ImL sample/control and mix before testing (ImL = 1000μ L)



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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.





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
- $\bullet\,$ Radioactive material needs proper handling and disposal





CHARM II TESTS

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

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

 ${\it Carefully \ review \ forms-procedures \ are \ intermixed}$



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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
 - \bullet 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
 - \bullet Dilute the sample using one part sample to nine parts tetracycline negative milk (fresh or frozen)
 - Controls are not diluted



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