

**2019 NCIMS APPENDIX N  
MODIFICATION STUDY COMMITTEE PROGRESS REPORT**

**Testing for Drugs Other Than Beta-lactams  
Tetracycline Pilot Program**

**September 30, 2019**

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**2019 NCIMS APPENDIX N  
MODIFICATION STUDY COMMITTEE PROGRESS REPORT**

**Testing for Drugs Other Than Beta-lactams  
Tetracycline Pilot Program**

**DETERMINATION OF DRUG FAMILY, REGULATORY CONCERNS, AND COMMUNICATIONS**

The Pilot Program was based on Proposal 211 passed at the 2015 National Conference on Interstate Milk Shipments (NCIMS). The Proposal stipulated that veterinary drugs other than beta-lactams be considered for testing. The drugs chosen for testing were to be based on the Food and Drug Administration's (FDA's) output of the risk ranking model and were to be selected from the top 20 drugs of the 54 drugs analyzed. The top 20 drugs were from the following 8 families of drugs: beta-lactams, amphenicols (e.g. florfenicol), NSAIDs (specifically flunixin), sulfonamides, macrolides, tetracyclines, aminoglycosides, and avermectins. The frequency of sampling raw milk was based on a statistical analysis, to be no less than 1 in 15 bulk milk tankers and/or all raw milk supplies that have not been transported in bulk milk pickup tankers, except for sulfonamides, which shall be at no less than 1 in 7; however, the Appendix N Modification Study Committee (Committee) is only charged with "considering" these criteria, and the pilot may result in different required frequencies being established. The Committee was charged to develop a Pilot Program, establishing a regulatory framework by which testing raw milk for veterinary drugs other than beta-lactams would be required.

The Multicriteria-Based Ranking Model for Risk Management of Animal Drug Residues in Milk and Milk Products (risk ranking model) based the ranking of animal drug residues on five (5) criteria: the likelihood and magnitude of use of the drug in dairy cattle; the likelihood of the drug's presence in raw milk bulk pickup tanker; the impact of processing on drug residues present in raw milk; the magnitude of consumption of dairy products; and the health effects from human exposure.

In June of 2015, the Committee began deliberations to select a drug family to be tested in the Pilot Program. The Committee based the decision on the following criteria: existence of tolerance or target testing levels; availability of commercial test kits; relative test kit cost; relative time to perform the testing; and whether test kits were already in existence, and if not, the estimated timeline for development. These priority factors were given a score of 1 to 4, with 1 being low priority and 4 being high priority.

Avermectins and other anti-parasitic drug classes were considered challenging to develop a rapid test with current available technology. Concerns were also raised that testing methods to detect this drug class would need laboratory equipment and supplies that were not already present in most milk processing labs.

Relative to some of the other drugs classes, tolerances or target testing levels for macrolides and amphenicols would first need to be established, hence the anticipated timeline until a rapid test was commercially available would be longer. It was noted that currently there were not any approved test kits available for aminoglycosides.

Sulfonamides, tetracyclines, and Nonsteroidal Anti-inflammatory Drugs (NSAIDs) (specifically flunixin) were the three drug classes identified for additional consideration. From this, the tetracycline class (consisting of tetracycline, oxytetracycline, and chlortetracycline) was proposed as the first drug class to consider for implementation of expanded testing through the Pilot Program. This was largely based on perceived use on the dairy farm, an established tolerance, availability of test methods, and the potential for timely development and approval of additional test methods to speed implementation of the program.

The Committee was charged to develop a regulatory framework for a required testing program. The pilot's goal was to establish a regulatory compliance program, including regulatory consequences. Initially, the Committee decided that regulatory action for the Pilot Program would be the same as the beta-lactams program (Pasteurized Milk Ordinance (PMO) Appendix N). The start date for the Pilot Program for the testing of the tetracycline class of drugs was set at July 1, 2017 and to continue over an 18 months period ending on December 31, 2018.

The Committee also discussed whether Proposal 213 passed at the 2015 NCIMS should apply to the Pilot Program. Specifically, Proposal 213 requires that a regulatory program have at least two (2) NCIMS-accepted test kit methods available prior to initiating a testing program for any specific drug. The discussion centered around whether this requirement would apply to Proposal 211 with the requirement of two (2) tetracycline test methods that were FDA-evaluated and NCIMS-approved for the Pilot Program to move forward. It was decided that the Proposal 211 pilot would require two (2) or more FDA-evaluated and NCIMS-accepted test kits before tetracycline class testing would be implemented.

Flowcharts were developed to document the procedure to be used for tetracycline class testing and the enforcement action in the event of a positive load. The enforcement action included suspension of the permit, or equally effective measures, to prevent the sale of the raw milk and further pickup or use of the raw milk. The enforcement framework began to evolve upon further discussions of the topic. The end result was to assure that the raw milk testing positive for tetracyclines would not enter the human or animal food chain. The costs associated with the disposal of a violative load of milk was to be handled between the producer and supplier, with documentation of the action sent to the Regulatory Agency. It was also determined, since this is a Pilot Program and currently not in the PMO, a violative load of milk would not count against a producer's permit in regard to counting as a violation toward permit revocation if repeated three times in a twelve-month period, unless an individual Regulatory Agency's statutory authority dictates otherwise.

Communication was critical to the eventual implementation of the Pilot Program. The Committee had three subcommittees that addressed different aspects of the Pilot Program. The Communication Subcommittee worked hand and hand with the Question and Answer Subcommittee. It was important that all affected stakeholders were informed in a timely manner. One of the first major decisions in this regard was to post the [Appendix N Pilot Program Question and Answer document on the NCIMS website](#), so it would be available to any interested party. With the initial implementation of the Pilot Program, the Communication Subcommittee in conjunction with the Question and Answer Subcommittee, made it a point to anticipate any problems that may be forthcoming. To that end, it was communicated to the industry and States that if industry was experiencing hurdles with implementing the Pilot Program, they needed to contact their State and the State would work with them to resolve any issues. If this didn't work, the issue would be brought to the attention of the Committee and if no resolution was forthcoming, it would be communicated to the NCIMS Executive Board. Communication of the Pilot Program was performed through many different avenues including: FDA Regional Milk Seminars; National Association of Dairy Regulatory Officials (NADRO) meetings; the International Association for Food Protection (IAFP), which included state affiliate meetings; the 2017 NCIMS Conference; and industry groups such as the National Milk Producers Federation (NMPF). The States held various meetings for the industry to inform them about the Pilot Program and answer any questions. The NCIMS Laboratory Committee and FDA Laboratory Proficiency Evaluation Team (LPET) were instrumental in this process through contacting Laboratory Evaluation Officers (LEOs), giving them instructions and information concerning the test kits and testing methodologies. The LEOs in turn sent out information to the State and commercial laboratories. This led to a successful launch of the Pilot Program.

### **AVAILABILITY AND ACCEPTANCE of ANALYTICAL METHODS, AND DEVELOPMENT of the ANALYTICAL PROCESS**

The goal of the program was to have test kits developed that were rapid, easy to implement, cost effective, and met the criteria set forth by FDA Center for Veterinary Medicine (CVM). This involved a review of available tests that were suitable for the pilot, test kit manufacturers' commitment of resources, and the development and review of the analytical methods.

Prior to the Pilot Program, there was one NCIMS-approved tetracycline method: The Charm® II Tetracycline Drug Test (Competitive Assay). As the Committee deliberated on the analytical methods and regulatory program, it became apparent that for the States to act on results, the test methods used needed formal acceptance. In addition, test kit evaluation forms had to be developed. This resulted in the following methods that have been vetted and accepted through the NCIMS Appendix N Modification Study Committee, NCIMS Laboratory Committee, FDA LPET and FDA CVM reviews:

- Charm® II Tetracycline Drug Test (Competitive Assay) ^
- Charm® ROSA® Tetracycline-SL\*
- IDEXX SNAP® Tetracycline\*

- NEOGEN BetaStar<sup>®</sup> Advanced for Tetracycline
- Charm<sup>®</sup> ROSA<sup>®</sup> TRIO Beta-lactam, Sulfonamide, and Tetracycline\*\*

^Sample is diluted before the initial assay

\*Dilution step required after an initial positive result to determine presumptive positive or negative result.

\*\*The Committee voted in the majority to accept the Charm<sup>®</sup> TRIO FDA/NCIMS 2400 Form for the Pilot Program for screening tetracycline negatives. An initial positive test will have to be screened through the NCIMS accepted Charm<sup>®</sup> ROSA<sup>®</sup> Tetracycline-SL Tetracycline test.

In development of test methods, the Committee and FDA engaged suppliers to resolve the methodology, how the tests were to be used in a regulatory framework, confirmation procedures, and actions taken including reinstatement.

Multiple steps were vetted through meetings in developing the test methods.

1. Tolerances or Target Testing Levels had to be available prior to the development of a test method. Tetracycline levels were available.
2. Analytical methods were developed to meet NCIMS sensitivity criteria defined in FDA CVM protocol.
3. When a dilution step was needed, additional validation steps were required:
  - a. Acceptance from FDA and Appendix N Modification Study Committee for the use of a dilution confirmation was required.
    - i. Manufacturer developed a test, and a diluent buffer if needed, that met the performance criteria.
  - b. The Committee discussed and developed a flowchart for the dilution protocol to meet a regulatory framework.
4. Suppliers submitted sensitivity, selectivity, incurred residue, cross reactivity, and interference data to FDA. The suppliers were required to have an external laboratory validate performance of the test method.
  - a. Suppliers submitted three manufactured lots to a third-party laboratory for validation of dose response and 90% positive sensitivity with 95% confidence.
    - i. Suppliers used private labs to perform somatic cell and bacterial interference studies.
  - b. Suppliers conducted internal validation of method: robustness, freeze thaw stability, and chemical interferences.
  - c. FDA or supplier conducted incurred studies for chlortetracycline, tetracycline and oxytetracycline. Collected samples were analyzed with High Pressure Liquid Chromatography (HPLC) technology to determine quantitative data and then adjustments were made to the test kits to meet NCIMS study levels and demonstrate that incurred residues were detected as sensitively as parent drug studies.
    - i. In at least one case, a manufacturer had to re-design the dilution protocol to adapt to differences to the incurred results and then repeat the dilution sensitivity studies with the parent drugs.

- d. Suppliers submitted data to FDA CVM and when questions arose, conducted further work before the test kit gained acceptance to move forward to presentation to the NCIMS Laboratory Committee.
  - i. For example, one manufacturer conducted a proficiency study with ten laboratories to demonstrate inter-laboratory robustness and ability to correctly dilute for confirmation to reach desired sensitivity levels.
- 5. Suppliers and FDA, CVM submitted data for NCIMS Laboratory Committee review.
- 6. Suppliers initiated test kit evaluation forms for NCIMS Laboratory Committee consideration and acceptance.
- 7. NCIMS Laboratory Committee submitted test kit evaluation forms to Appendix N Modification Study Committee for acceptance into the pilot.
- 8. Appendix N Modification Study Committee developed Q&A and a regulatory program with flow charts outlining confirmatory procedures.
- 9. Appendix N Modification Study Committee submitted the analytical method and accompanying test kit evaluation form for each test kit to the NCIMS Executive Board for acceptance.

### **HURDLES AND QUESTIONS ENCOUNTERED**

Hurdles and questions were encountered through the vetting process of the analytical test method and development of the regulatory framework. These included:

- 1. Regulatory Framework
  - a. Interpretation between testing that was to be voluntary versus testing to be within the Pilot Program.
  - b. Was this a monitoring program for residues in milk or a regulatory enforcement program?
  - c. Development of a three-way agreement for testing outside of the pilot after the pilot ends.
- 2. Analytical Test Kit Development - Time and Cost
  - a. Time required and cost to suppliers to develop an NCIMS accepted test.
  - b. Time needed for suppliers to build test kit inventory.
  - c. Time needed for industry to prepare and “gear up” for testing.
  - d. State Commissioners concerns about cost burden to the industry.
  - e. Available manpower and time cost to the industry.
  - f. Cost of the test.
- 3. Test Protocols
  - a. Establishment tests for drug family to be piloted.
  - b. Drug tolerance/target testing levels and available method detection levels as they relate to the tolerance/target testing levels and the NCIMS requirement that tests not be sensitive to less than 50% of the target.
  - c. Decisions on how the test was to be used such as requiring a dilution step first or as a confirmation step for the correct test detection levels.
  - d. Clarification of the protocols for submission to FDA and eventually the NCIMS Laboratory Committee.

- e. Reinstatement/clearing sample testing protocol (Single positive versus repeating two times with positive and negative controls protocol).
  - f. Using the wrong “channel” or procedures for the test kit.
  - g. Initial establishment of negative control.
  - h. Transition of prior unapproved method procedures to approved method procedures.
4. Reporting
    - a. Drug Residue Database Reporting was not clear regarding coding of tests.
    - b. Database reporting manual dated 2003 needed to be updated.
  5. Funding
    - a. Lack of federal funding to assist in program implementation.
  6. Multidrug, Multianalyte Platforms
    - a. How should multidrug testing protocol be managed when confirmation has to be performed using a different test?
    - b. Under which test code should results be reported? The multidrug test or the confirmation test.
    - c. Reinstatement protocol when a multidrug test is used.
  7. Limited Raw Bulk Milk Pickup
    - a. State with minimal producer(s) (<2).
    - b. Small processors receiving minimal amount of raw milk, such as one bulk milk pickup tanker per week.
  8. Testing could not commence until:
    - a. Test methods were available.
    - b. Test protocols were established.
    - c. Tests were NCIMS accepted.
    - d. 2400 forms were developed.
    - e. All of the above were communicated.

### **REGULATORY TESTING AND LABORATORY REQUIREMENTS SECTION**

During the initial Pilot Program discussions, the Committee primarily relied on the FDA Risk Assessment in choosing the class of drug to start testing bulk milk pickup tankers. One key consideration factored into the Committee’s choice was the availability of validated test kits. Manufacturers of beta-lactam test kits that were currently NCIMS approved for Appendix N testing were asked if they had test kits available for the various drugs and which would be the easiest and quickest to implement into the Pilot Program.

Charm® Sciences, Inc. and IDEXX Laboratories, Inc. both indicated that they had test kits for several of the drugs and felt that their test kits for the tetracycline family would provide the easiest and quickest acceptance for use in the Pilot Program. However, both Charm® and IDEXX indicated that their test kits were significantly more sensitive than the current U.S. tolerance of 300 ppb for oxytetracycline. Charm® put forward that with a dilution process, the detection sensitivity could be brought to the U.S. tolerance. The use of a dilution protocol had previously been accepted when the U.S. tolerance for the tetracycline family was increased to 300 ppb in 1998. IDEXX indicated that their



test kit could also meet the U.S. tolerance with dilution. Neogen Corporation indicated that they did not currently have a tetracycline family test kit ready but were in the process of developing one and felt that they could have the test kit ready for the implementation of testing. Neogen indicated that their test kit would not require dilution to meet U.S. tolerance.

Based on this information, the Committee agreed to proceed with the tetracycline family and to have the manufacturers submit their test kits to FDA, CVM for evaluation. The Committee decided to use an evaluation process that would be a modified version of the protocol used to evaluate beta-lactam test kits. Initially these tetracycline family test kits would be FDA evaluated and Appendix N Modification Study Committee accepted for use in the 211 Pilot Program but could not be listed as an NCIMS approved test method in M-a-85. However, the pilot drove technology to provide for NCIMS approved methods.

The tetracycline family test kit evaluations took longer than anticipated, which delayed initiation of pilot testing from January 1, 2017 until July 1, 2017. Acceptance by the Committee of all three test kits under the Pilot Program evaluation protocol was completed prior to July 1, 2017. Both the Charm® ROSA® SL Tetracycline Test (with Dilution Protocol) and Neogen BetaStar® Advanced Test for Tetracyclines were NCIMS-approved and met the criteria to claim detection of chlortetracycline, oxytetracycline and tetracycline at U.S. tolerance. The IDEXX Snap® Tetracycline Test (with Dilution Protocol) was NCIMS-approved and met the criteria to claim detection of oxytetracycline and tetracycline at U.S. tolerance. The IDEXX test was not able to meet the detection level for chlortetracycline but was able to detect that drug at less than the upper limit of 600 ppb. During this period, Charm® and Neogen both made decisions to expand the evaluation of their kits to meet M-a-85 criteria.

The NCIMS Laboratory Committee was approached to develop Pilot Program test kit evaluation forms to allow use of the tetracycline family test kits to test raw cow milk prior to processing. The NCIMS Laboratory Committee completed this task prior to the July 1, 2017 initiation date. Relative to use of these accepted test kits, several questions were raised regarding approval/certification of analysts and LEO responsibilities under the Pilot. For example, to expedite an analyst's ability to conduct testing with these newly accepted test kits, LPET proposed an evaluation process, with NCIMS Laboratory Committee input and agreement, to allow analysts that were currently approved for beta-lactam test kits be able to use the tetracycline family test kit for the same testing platform without the need for LEOs to conduct on-site evaluations. These questions were captured and resulted in a document titled "Appendix N Modification Study Committee LEO Responsibilities For New Tetracycline Test Kits" that was posted on the NCIMS website. The Committee accepted the test kit evaluation forms and "Appendix N Modification LEO Responsibilities" protocol. With the Pilot Program test kit evaluation forms and the LEO responsibility's protocol in place, testing for the tetracycline family under the Pilot Program was initiated on July 1, 2017.

To expedite the testing process, it was agreed that the initial screening test for receipt of raw cow milk would be done without dilution for the Charm® and IDEXX test kits. If an initial positive result was determined, the analyst performing the initial test would need to retest controls and retest the same sample in duplicate using the dilution protocol for the kit used. Any subsequent testing would also require use of the dilution protocol when using the Charm® and IDEXX test kits. The dilution protocol did not apply to the Neogen test kit. It was agreed that the testing and follow-up process for any initial positive testing would follow the current process Appendix N has in place (completion of presumptive testing, tanker confirmation, producer traceback, and producer re-instatement) as used for beta-lactams and clarified in Q&A's found in M-I-03-13 and M-I-12-9. Discussion with the NCIMS Laboratory Committee also precipitated issuance of M-I-17-6 "Clarification Of The Testing Requirement And Procedure For The Clearance Sample For Reinstatement Due To A Confirmed Positive Using An Approved Test Method To Allow The Sale Of Milk For Human Food, When A Representative Sample From The Dairy Producer's Milk, Prior To Commingling With Any Other Milk, Is No Longer Positive For Drug Residues." The FDA, CVM was also tasked with developing a table showing which tetracycline family test methods may be used to establish test kit equivalence for the purposes of confirmation (presumptive positive), producer trace back, and producer reinstatement. This table was published in the "[Appendix N Pilot Program Question and Answer](#)" document and posted on the NCIMS website.

After the initiation of tetracycline family testing under the Pilot Program, both the Charm® ROSA® SL Tetracycline Test (with Dilution Protocol) and Neogen BetaStar® Advanced Test for Tetracyclines completed M-a-85 testing evaluation by the FDA, CVM and the NCIMS Laboratory Committee, and they were approved by the NCIMS Executive Board in October of 2017. The NCIMS Laboratory Committee revised the Pilot Program test kit evaluation forms for these kits, to become official FDA/NCIMS 2400 forms. At that time, IDEXX also chose to submit their kit for M-a-85 evaluation. To date, the IDEXX data for their IDEXX Snap® Tetracycline Test has been evaluated by FDA, CVM, and reviewed by the NCIMS Laboratory Committee, and approved by the NCIMS Executive Board.

The "Appendix N Modification LEO Responsibilities For New Tetracycline Test Kits" document required LEOs to include tetracycline family drugs in their drug split sample programs. Concentrates of chlortetracycline, oxytetracycline and tetracycline were prepared to allow preparation of these drug residues at 300 ppb in raw cow milk and then provided to LEOs by LPET. No issues have been reported with regards to the detection of tetracycline family drugs by the Pilot Program approved test kits through split sample testing.

A second set of split samples was provided to State Central Laboratories by LPET. The 2018 LPET proficiency test contained samples with 300 ppb of chlortetracycline, oxytetracycline and tetracycline as well as samples without tetracyclines added. Data was submitted for the Charm® ROSA® Tetracycline SL Test and IDEXX Snap® Tetracycline Test. No sample data was available for the Neogen BetaStar® Advanced Test for Tetracyclines. The Charm® test was able to detect samples containing chlortetracycline, oxytetracycline, and tetracycline when undiluted samples were screened as well as

for completion of presumptive testing (duplicate testing with controls) with diluted samples. The IDEXX test was able to detect samples containing chlortetracycline, oxytetracycline, and tetracycline when undiluted samples were screened and able to detect samples containing oxytetracycline and tetracycline when completion of presumptive testing was performed with diluted samples. About 84% of participating analysts using the IDEXX test were able to detect samples containing chlortetracycline when completion of presumptive testing of samples with this drug was performed. No false positive results for either test was submitted for samples not containing tetracyclines. These results substantiate the kit validation results submitted to the FDA and NCIMS in support of the approval of both the Charm and IDEXX test kits.

### **DRUG RESIDUE REPORTING AND MANAGEMENT OF DATA**

The data derived from the eighteen-month Pilot Program must be coded, submitted, and ultimately analyzed in an accurate manner in the National Milk Drug Residue Database (NMDRD). It became clear through the institution of the Pilot Program that continued training and updating would serve all state partners in submitting accurate and timely data. Diligence on the States' part must come into play. With the advent of new processors, new analysts, new reporters of data, etc., an ongoing effort to continue to report timely and accurate data was vital.

The Committee determined early in the process that the drug kit technology would have to evolve. This was key in being able to commence the Pilot Program. According to the protocol of the Pilot Program for incoming loads of milk, the initial sample would be tested undiluted using test kits IDEXX Snap® Tetracycline Test (test code 51) and the newly created Charm® ROSA® SL Tetracycline Test (with Dilution Protocol) Orange (test code 95v). If found to be positive, then the diluted protocol would be used on the same tests. There was much discussion relative to having separate test kit codes for diluted and undiluted samples. Ultimately, for simplistic reasons the Committee decided to use the same codes (51v and 95v).

The reality that multi-drug test kits were already NCIMS approved posed a concern for the Committee on how to report the data. The Charm® TRIO, for example, can test for beta-lactams, sulfonamides, and tetracyclines. After a much discussion by the Committee, FDA, and the third-party database group, it was resolved by having each drug family detected by a test reported as a different test in the database. For example, a TRIO detection of three drug families is reported as a TRIO-Beta-lactam, a TRIO Sulfonamide, and a TRIO Tetracycline test.

### **REDUCTION OF REQUIRED BETA-LACTAM TESTING**

A reduction of required Beta-lactam testing was proposed as an option to manage the burden to industry when considering an expansion of Appendix N to include animal drugs other than beta-lactams. In review, required Beta-lactam testing is covered under "APPENDIX N, DRUG RESIDUE TESTING AND FARM SURVEILLANCE" and "Section 6, The Examination of Milk and/or Milk Products" of the PMO. As stated in the PMO:

1. "APPENDIX N. DRUG RESIDUE TESTING AND FARM SURVEILLANCE, I. INDUSTRY RESPONSIBILITIES, MONITORING AND SURVEILLANCE"
  - a. Industry shall screen all bulk milk pickup tankers and/or all raw milk supplies that have not been transported in bulk milk pickup tankers, regardless of final use, for Beta-lactam drug residues.
2. "Section 6. The Examination of Milk and/or Milk Products"
  - a. Bacterial counts, somatic cell counts, and cooling temperature checks shall be performed on raw milk for pasteurization, ultra-pasteurized, aseptic processing and packaging, or retort processed after packaging.
  - b. Drug tests for Beta-lactams on each producer's milk shall be conducted at least four (4) times during any consecutive six (6) months.
  - c. All pasteurized and ultra-pasteurized milk and/or milk products required sampling and testing to be done only when there are test methods available that are validated by FDA and accepted by the NCIMS, otherwise there would not be a requirement for sampling. "Required bacterial counts, coliform counts, drug tests for beta-lactams, phosphatase and cooling temperature determinations shall be performed on Grade "A" pasteurized and ultra-pasteurized milk and/or milk products defined in this *Ordinance* only when there are validated and accepted test methodology." (PMO Section 6)

There were several issues identified with a reduction of required beta-lactam testing:

1. Pasteurized milk is required to be tested for beta-lactams and could be at risk if there is a reduction of testing of bulk milk pickup tanker.
2. There is the potential of processed and packaged milk resulting from a bulk milk pickup tanker that was not tested but resulted in a Section 6 producer test positive. The Committee reviewed concerns with this scenario as it would require not just the reduction of required beta-lactam testing but also the coordinated prohibition of testing under Section 6 of producers from that same raw milk from the bulk milk pickup tanker for beta-lactams. This is needed to prevent a situation where an allowed reduction in testing would result in a producer positive and potential regulatory action.

Validation of microbiological testing results required for inhibitors could result in positives of packaged product processed from raw milk bulk pickup tankers not tested due to the reduction of the required beta-lactam testing. The requirement that all bacteriological plate counts were accompanied by inhibitor testing could result in a positive inhibitor such as beta-lactam that was not tested on the raw milk bulk pickup tanker could occur from FORM FDA/NCIMS 2400 Cultural Procedures - General Requirements, "34. Sample Bench Sheet Requirements", b, d, "Results of inhibitor tests accompany all plate counts. Inhibitor controls performed, and results recorded for each group of samples." The cause of the highest incidence of animal drug residue is beta-lactams and reduction of testing could be counterproductive to the program that had resulted in the significant reduction of beta-lactam violative residues. In the most recent 2018 data on Beta-lactam animal drug residue 328 loads tested

positive out of a total of 3,179,848 industry loads tested (Table 7, NATIONAL MILK DRUG RESIDUE DATA BASE FISCAL YEAR 2018 ANNUAL REPORT October 1, 2017 - September 30, 2018). In a reduction program those positives could have been missed.

In conclusion it was determined by the Committee that reduction of testing for beta-lactams, and where covered under microbiological testing for inhibitors, was not prudent.

**TETRACYCLINE PILOT PROGRAM STUDY RESULTS - Data Review and Summary**

**BACKGROUND**

A Pilot Project for the testing of tetracycline residues in milk was developed by the Appendix N Modification Study Committee of the National Conference on Interstate Milk Shipments (NCIMS) in response to 2015 Proposal 211. The Pilot Program began on July 1, 2017 and concluded on December 31, 2018 to test a percentage of Bulk Milk Pickup Tanker Samples and/or Raw Milk Supplies That Have Not Been Transported In A Bulk Milk Pickup Tanker (BMP) for tetracycline drug residues. Further details regarding the pilot can be found at: <http://ncims.org/programs/appendix-n-pilot-program/>.

Results of the testing were reported to the National Milk Drug Residue Database (NMDRD). The NMDRD was authorized at the 1991 meeting of the NCIMS, when the voting delegates approved a national program to compile the results of milk drug residue testing by industry and State Regulatory Agencies. Subsequently, FDA awarded a contract to develop the NMDRD which is operated by an independent third party, under contract to the FDA. The Pilot Program for testing of tetracyclines spanned three Fiscal Year Annual Reports of the NMDRD; FY 2017, FY 2018 and FY 2019.

Under the terms of the NMDRD, industry reporting of drug residue testing is voluntary; therefore, the samples and tests reported to the database do not necessarily represent one hundred percent (100%) of the milk supply from every regulatory agency.

**TETRACYCLINE PILOT PROGRAM PARTICIPATION**

**State Participation**

The tetracycline data was reported by the fifty (50) States, Puerto Rico, and two (2) Third Party Certifiers (TPCs) authorized under the International Certification Program (ICP) and is termed “Regulatory Data Reporters.”

<b>STATE PARTICIPATION</b>				
<b>Regulatory Data Reporters</b>	<b>FY 2017 July 2017 – Sept. 2017</b>	<b>FY 2018 Oct. 2017 – Sept. 2018</b>	<b>FY 2019 Oct. 2018 – Dec. 2018</b>	<b>Average for FY 2017, FY</b>

				<b>2018, and FY 2019</b>
Number of Regulatory Data Reporters*	40 out of 53	43 out of 53**	33 out of 53***	39 out of 53
Percentage of Regulatory Data Reporters Participated	75%	81%	62%	73%
<p>*Fifty (50) States, Puerto Rico and two (2) Third Party Certifiers. Puerto Rico submitted partial data. Third Party Certifiers did not submit Tetracycline data.  **FY 2018 is a full 12 months report  ***FY 2019 reported as of March 2019</p>				

**Industry Participation**

Industry participation accomplished (Table 7 NATIONAL MILK DRUG RESIDUE DATA BASE FISCAL YEAR 2018 ANNUAL REPORT, October 1, 2017 - September 30, 2018)

<b>INDUSTRY PARTICIPATION</b>		
<b>Number of Industry Test for Beta-lactams</b>	<b>Number of Industry Test for Tetracyclines over the same period</b>	<b>Sampling Rate of Participation from Industry</b>
3,179,848	293,692	9.24% (1/11)
<p>Notes:</p> <ol style="list-style-type: none"> <li>Using a denominator of 3,179,848 from Table 7-1 Bulk Milk Pickup Tankers tested for beta-lactams, tetracycline testing provided for a 9.24% of Bulk Milk Pickup Tankers that were tested (293,692 industry tests for tetracycline/3,179,848 industry test for beta-lactam bulk milk pickup tanker). A 1/15 or 6.67% sampling rate was recommended for tetracycline and the pilot yielded a ~1/11 or 9.24% rate for October 1, 2017 - September 30, 2018</li> <li>This could be equated to a 2.57% higher test rate or 81,722 more tests (2.57% of 3,179,848) beyond the 1/15 rate.</li> <li>Participation did not include State sponsored testing for small manufacturers or other extra testing performed.</li> </ol>		

**COST TO INDUSTRY TO PARTICIPATE IN THE PILOT STUDY**

Industry was surveyed regarding the cost of tetracycline tests. The survey revealed that the cost was between \$4.00 - \$5.00 per test, without considering the cost of controls and other additional testing. An average cost of \$4.50 per test was used.

<b>COST TO INDUSTRY TO PARTICIPATE IN THE PILOT STUDY</b>			
<b>July 1, 2017 to December 31, 2018</b>			
<b>Family/Drug</b>	<b>Number of Industry Test</b>	<b>Average Cost per Tetracycline Test</b>	<b>Total Cost to Industry</b>

Tetracyclines	399,900	\$4.50	\$1,799,550
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**TETRACYCLINE TEST RESULTS REPORTED TO THE NMDRD**

(July 1, 2017 – December 31, 2018 Submitted by: GLH, Incorporated)

Tables 5, 6, 7-1 and 8-a show the number of Tetracycline Pilot Program testing results extracted from the FY 2017, FY 2018 and pending FY 2019 NMDRD Annual Reports (July 1, 2017 to December 31, 2018).

<b>TABLE 5 -- Number of BMP Tests Conducted by Family/Drug July 1, 2017 to December 31, 2018*</b>		
<b>Family/Drug</b>	<b>Total BMP Tests</b>	<b>Total Positive</b>
TETRACYCLINES	406,415	7

\* Includes Industry and Regulatory testing: Table 5 presents the total number of both industry and regulatory Bulk Milk Pickup Tanker Tetracycline testing and the number of positive tested reported to NMDRD.

Table 6 shows the number of tests and number of positives for the specific Tetracycline tests used. The testing methods with the largest use were: Charm® SL – Tetracyclines (green strip) and Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip. Although the Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip was available and validated with a report code of 95, for the entire Pilot Study timeframe, it was found that some plants and states continued to submit incorrectly Code 89 to the NMDRD when the correct code should have been 95. Therefore, the number of tests conducted on the Charm SL-Tetracyclines (green strip) and the Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip may not be accurate, but the combined total of the two (2) tests and positives submitted to the NMDRD are accurate.

In January 2017, the NCIMS conference approved the Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip. It could be estimated that nine (9) months out of the twelve (12) months or three fourths of the tetracycline tests conducted during the timeframe of this report were the Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip for fiscal year 2018. Regardless of whether the Charm® Tetracycline SL- green strip or the Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip were used, for all intended purposes the two tests are equivalent. FDA has confirmed that all the tetracycline positives reported with tests codes 89 (Charm® Tetra SL- green strip) and 95 (Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip) were confirmed positive on 95 (Charm® ROSA® Tetracycline-SL (Dilution Confirmation) orange strip. Therefore, all the

tetracycline reported positives were confirmed at the safe tolerance level of 300 ppb (parts per billion).

**TABLE 6 -- Number of Tests Conducted by Method by Family/Drug  
July 1, 2017 to December 31, 2018**

Tests Used By Family/Drug	Number of Tests*	Number Positive	Percent Positive
TETRACYCLINES			
Charm II Tablet Competitive	2,560	0	0.000%
Charm ROSA Tetracycline-SL (Dilution Confirmation) Orange Strip (Code 95v)	162,829	2	0.00123%
Charm SL – Tetracyclines (Green Strip) (Code 89)**	236,489	5***	0.00211%
Charm Trio Test - Tetra	3,608	0	0.000%
IDEXX SNAP - Tetracycline**	8,855	0	0.000%
Neogen BetaStar Advanced For Tetracycline	1,190	0	0.000%

\*Includes Tetracycline Tests conducted for Grade “A” and Non-Grade “A” BMP, Grade “A” and Non-Grade “A” Pasteurized Fluid Milk and Milk Products, Grade “A” and Non-Grade “A” Producer, and Grade “A” and Non-Grade “A” Other Testing (Milk from Milk plant tanks/silos, milk transport tankers, etc.)

\*\* Non-Validated Test Method

\*\*\*Verified to be 5 positives rather than 6. The NMDRD will be amended to reflect this change.

**Table 7 presents the number of industry and regulatory BMP Tetracycline testing, the number of positive tests and the percent of positive samples reported to the NMDRD.**

**TABLE 7-1 -- Grade A Bulk Milk Pick-Up Tanker Testing  
July 1, 2017 to December 31, 2018**

Family/Drug	Number of Industry Test	Number of Positive Industry Test	Number of Regulatory Test	Number of Positive Regulatory Test	Total Test	Total Positive Test	Total Percent Positive
Tetracyclines	399,900	7	6,516	0	406,415	7	0.00172%



Tetracyclines	3,119 more tetra test submitted since March 13 <sup>th</sup> cut off when the Intermittent Data Report was submitted.	409,534	7	0.00171%
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**SUMMARY OF ACTIONS OF THE APPENDIX N MODIFICATION STUDY COMMITTEE REGARDING THE TETRACYCLINE PILOT PROGRAM**

- Discussed hurdles on reduction of beta-lactam testing. It was determined by the Appendix N Modification Study Committee that reduction of testing for beta-lactams, and where covered under microbiological testing for inhibitors, was not prudent.
- Drug to be tested:
  - Discussed and determined which class of drug would be the best to start the Pilot Program.
  - Tetracycline data from the Pilot Program will be used to further refine statistical sampling.
- Availability of Test methods:
  - Discussed availability and testing issues with test kit manufacturers (Charm, DSM, IDEXX and Neogen).
  - Selected three tetracycline test kits (Charm® ROSA®-SL (Dilution Confirmation), IDEXX SNAP® and Neogen BetaStar® Advanced) to implement Pilot Program testing as kits were readily available.
  - Worked out initial testing (undiluted for Charm and IDEXX) and follow up confirmation testing (when to start dilutions, after initial positives for Charm and IDEXX) practicalities and reinstatement protocols. Neogen BetaStar® Advanced does not require dilution for testing. All follow up testing must be done at the U.S. regulatory level.
  - Through FDA, CVM the program agreed on an evaluation process and approved the test kits to be used.
  - All three kits have subsequently been submitted for recognition as M-a-85 methods. Evaluation forms have now been updated to FDA/NCIMS 2400 status.
- Regulatory framework:
  - The testing of different drugs other than beta-lactams – determination of drug or drugs, and their significance.
  - Requiring approved NCIMS test methods, and protocols for approval.
  - Determination of sampling rates: During the Pilot Program the sampling frequency rate for tetracycline was 1 out 11. This gave a 90 percent precision rate. Based on FDA statisticians this is an acceptable and an effective sampling rate given the low prevalence of tetracycline.
  - Determination of funding for the program as several States provided testing service to small processors. No funding was provided from FDA on this project.
  - Developing Database reporting.
  - Providing for communications of reporting, duration of program.
  - Determining regulatory action(s) direction for States.

- **Laboratory framework:**
  - NCIMS Laboratory Committee developed the test kit evaluation forms to be used in consultation with the test kit manufacturers and Appendix N Modification Study Committee.
  - Developed LEO responsibilities and a process to recognize testing analysts (based on platforms used to test for beta-lactams).
- **Database development:**
  - Discussed how results of testing were to be incorporated into the National Milk Drug Residue Data Base Annual Report.
  - Cleaned up out dated testing codes and added new codes for new tests to be used in the National Milk Drug Residue Data Base Annual Report.
  - Updated NMDRDB manual.
- **Determination of drug to be tested:** Initiated discussions on next class of drug for the Pilot to test the regulatory framework. Gentamicin was determined as the next drug to test the regulatory framework.

### **CONCLUSIONS OF THE APPENDIX N MODIFICATION STUDY COMMITTEE REGARDING THE TETRACYCLINE PILOT STUDY**

The purpose of the Pilot Program is to develop and test a regulatory framework where by drugs other than beta-lactams could be investigated. This regulatory framework has resulted in several main elements that involves the following:

1. A foundation of understanding the use and potential presence of an animal drug residue to be found in milk.
2. Determination of a frequency of testing.
3. Setting of regulatory tolerance and/or testing limits.
4. Designing a testing path that involves method and procedures to carry out the method(s) development through the NCIMS validation program, determining equivalent methods that could be used, and the reporting into the National Milk Drug Residue Database (NMDRD).
5. Defining regulatory actions as needed.
6. Communication, implementation, and documentation of the testing program.

The Tetracycline pilot resolved many of these issues and drove technology to provide for NCIMS accepted methods. The participation from States and Industry showed an 81% participation (43/53). Industry ran a total of 399,900 tests with a potential spend of \$1,799,550 over the 18-month period. The positive rate was at 0.0017% compared to 0.01% for beta-lactam over the same period (2018 ANNUAL REPORT, October 1, 2017 - September 30, 2018).

The Appendix N Modification Study Committee would like to thank all those that participated in the initial phase of this pilot program created with the purpose of establishing the regulatory framework for the testing of veterinary drugs other than beta-lactams in the nation's raw milk supply, if deemed necessary. This initial phase of testing, specific to the tetracycline family class of animal drugs, began on July 1, 2017 and ended on December 31, 2018. At least 409,534 tests were conducted by state regulatory and industry laboratories during this 18-month pilot phase. Seven positive samples were

detected indicating an overall estimated incidence of 0.0017%. When compared to a 0.01% (Fiscal Year 2018 annual report revised, TABLE 7-1, Grade "A" Bulk Milk Pickup Tanker Testing, October 1, 2017 to September 30, 2018) incidence rate for beta-lactams during the 12-month period the incidence rate for tetracycline family class of animal drugs in bulk milk pickup tankers observed during this pilot project was statistically smaller. Based on the results, bulk milk pickup tanker testing is more likely to find beta-lactam residues than tetracycline residues. The data collected in this pilot on tetracyclines provide adequate information to estimate the incidence rate in bulk milk pickup tankers. The results of the pilot study, and the 2015 "Multicriteria-Based Ranking Model for Risk Management of Animal Drug Residues in Milk and Milk Products" do not indicate a need for mandatory routine testing. The committee recommends that testing for tetracyclines continue to be conducted on a voluntary basis.

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