DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Center for Veterinary Medicine
Office of Research

Food and Drug Administration
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Memorandum

Date: June 13, 2013

From: Philip James Kijak, Ph.D., Pak-Sin Chu, Ph.D., Hemakanthi De Alwis, Ph.D. and Hiranthi Jayasuriya, Ph.D.

Subject: **Milk Sampling Assignment: Internal Audit-Report of Findings**

To: Dr. William T Flynn, Deputy Director for Science Policy, Center for Veterinary Medicine

Through: Dr. Timothy C. Schell, Acting Director, Office of Research

SUMMARY

The FDA Office of Regulatory Affairs (ORA) completed a Center for Veterinary Medicine (CVM) assignment to test approximately 1800 milk samples for residues of 31 veterinary drugs. The samples were analyzed in three ORA laboratories. The Division of Residue Chemistry in CVM audited the results for appropriateness and consistency. The audit concurred that the laboratories used appropriate methods. Additionally the audit confirmed that assignment of positive and negative findings were based on CVM guidance. The review identified several instances where additional data were required to support method validation. Subsequently, these data were provided by the laboratories and CVM found that the methods were appropriately validated. Additionally, several presumptive positive samples were determined to be below the limit of confirmation for the method and were not listed as violative. CVM concurred with the findings of violative residues in 10 samples tested at the Denver District Laboratory, three samples tested at the Arkansas Regional Laboratory, and three samples tested at the Southeast Regional Laboratory. Three samples which tested positive for violative residues at the Southeast Regional Laboratory were shipped to the Denver District Laboratory where they were retested. No residue was found at the Denver Lab. The cause of this discrepancy could not be resolved by the audit. In summary, the audit confirmed that the method validation and data interpretation were conducted in accordance with CVM guidance. The results of the assignment with the noted exceptions were acceptable.

1. PURPOSE OF THE AUDIT

a. FDA Office of Regulatory Affairs (ORA) completed a Center for Veterinary Medicine (CVM) assignment to test approximately 1800 milk samples for residues of 31 veterinary drugs. Samples were analyzed in three ORA Laboratories, the Southeast Regional Laboratory in Atlanta Georgia, the Denver District Laboratory in Denver Colorado, and the Arkansas Regional Laboratory in Jefferson Arkansas over approximately one year. Following the completion of the assignment, CVM Office of Surveillance and Compliance requested CVM Office of Research Division of Residue Chemistry to conduct an audit of the results to determine that the laboratories:
    i. Used methods that were appropriately validated
    ii. Followed CVM guidance in the determination of a positive finding
    iii. Were consistent in the level of validation and application of confirmation criteria across all three laboratories

2. SCOPE OF THE AUDIT

a. In conducting the review, CVM Division of Residue (DRC) Chemists audited the data generated from the analysis of all samples found to contain drug residues. The audit includes a partial review of the results from samples that tested negative. Typically, all data were audited for samples assayed in an analytical set containing positive results.
b. The following areas of the testing were subject to the audit
    i. Method standard operating procedures and laboratory specific method validation data
    ii. Analytical standard preparation data
    iii. Sample preparation information
    iv. Mass spectral data
    v. Calculations
    vi. Determination if assignment of a positive or negative finding was done in accordance to the requirements of the method, assignment, and CVM guidance.
c. For the data from Southeast Regional Laboratory, the vendor specific software was available at DRC, and the electronic raw data files were examined. For the other two laboratories, pdf printouts were used to review the data.

3. BACKGROUND

The Milk Sampling Assignment did not list the method to be used for the analysis of the samples. However, all laboratories were instructed to use LIB 4443 as adapted for the specific equipment available for screening purposes except for the drugs tested by ELISA.

a. The Southeast Regional Laboratory of FDA in Atlanta Georgia analyzed 519 milk samples for the survey. The Milk Sampling Assignment is described in a memorandum dated Dec 22nd 2011 from the Drug Residue Compliance Team, HFV-233, Division of compliance, CVM. The Milk Assignment contains 31 drugs. Twenty-six of them were analyzed according to LIB 4443- “Optimization and Validation of multi-class, Multi-residue LC/MS/MS Screening and Confirmation Methods for Drug residues in Milk”. Three others [Chloramphenicol (CAP), Florfenicol (FF) and Tulathromycin (TUL)] that are not in this LIB, were analyzed according to a separate memorandum. TUL was analyzed using the same instrumentation as in LIB 4443

(in positive ESI mode). CAP and FF are analyzed in negative ESI mode with a different LC conditions than described in the LIB.
The quantitation of the compounds was carried out by a single point calibration with the 1X fortified sample. Therefore the concentrations indicated by SRL for the drugs detected are only estimates of the true concentrations.
Elisa immunoassay screening for neomycin and gentamicin was performed using EuroProxima Elisa kit, EuroProxima B.V. Arnhem, The Netherlands.

b. Denver District Laboratory (DDL) is the original method developer for LIB 4443 entitled, "Optimization and Validation of multi-class, Multi-residue LC/MS/MS Screening and Confirmation Methods for Drug residues in Milk". In addition to the drugs listed in LIB 4443, three additional drugs (CAP, FF, and TUL) were added to the analysis. TUL was analyzed in accordance with the procedure described in LIB 4443 in positive ESI mode, while CAP and FF were analyzed in negative ESI mode with a different LC condition than that described in the LIB. Neomycin and Gentamicin were screened by EuroProxima ELISA kits, while the presumptive positive samples were analyzed by a quantitative method entitled, "The Confirmation and Quantitation of 7 Aminoglycosides in Raw Milk by HILIC Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry with Solid-Phase Extraction". Sulfonamides, including sulfamethazine (SMZ), were screened by the LIB 4443 method, while the presumptive positive samples were analyzed by a quantitative method entitled, "Quantification and Confirmation of Nine Sulfonamides and Trimethoprim Residues in Whole and Raw Milk by LC-MS/MS". DDL analyzed approximately 1070 milk samples on a Thermo Quantum triple quadrupole Access mass spectrometer or on a Thermo Quantum triple quadrupole Classic mass spectrometer.

c. Arkansas Regional Laboratory (ARL) analyzed 332 milk samples under the Milk Assignment. We received data packages for all the batches that contained samples tested positive for one or more drugs listed in the Milk Assignment. These packages also contained data for all the samples in these batches that tested negative for the analytes. The methods used for the analysis of the drugs stated in the Milk Assignment were:
    i. LIB 4507 "High Throughput LC/MS/MS Screening and Confirmation of Drug Residues in Milk using Automated Solid Phase Extraction.
    This method is based on the LIB 4443, which was used in the Milk Assignment work at SRL and Denver laboratories, but utilized advanced technologies to increase sample throughput. These technologies included an automated solid phase extraction system, an AB Sciex QTRAP 5500 mass spectrometer in positive and negative ion mode coupled to an Agilent 1200 Rapid Resolution liquid chromatograph with alternating column regeneration. A comparison of method performance between the two LIBs is given by ARL in the document "Validation Statistical Evaluation". Briefly, in the new method (LIB 4507), standard deviation for most compounds are lower, and additional analytes, CAP, FF and TUL, have been incorporated and validated as reported in the relevant Memorandum of Analysis.
    ii. Elisa immunoassay screening for neomycin and gentamicin was performed using EuroProxima Elisa kit, EuroProxima B.V. Arnhem, The Netherlands.

4. REFERENCE

a. LIB 4443 "Optimization and Validation of Multi-class, Multi-residue LC/MS/MS Screening and Confirmation Methods for Drug Residues in Milk"

b. LIB 4507 “High Throughput LC/MS/MS Screening and Confirmation of Drug Residues in Milk using Automated Solid Phase Extraction”
c. “The Confirmation and Quantitation of 7 Aminoglycosides in Raw Milk by HILIC Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry with Solid-Phase Extraction”
d. “Quantification and Confirmation of Nine Sulfonamides and Trimethoprim Residues in Whole and Raw Milk by LC-MS/MS”
e. EuroProxima Elisa Kit Manual, EuroProxima B.V. Arnhem, The Netherlands.
f. CVM Guidance for Industry 118 Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues
g. Milk Sampling Assignment, CVM Assignment Reference # - VA 11- 02, December 22, 2011

5. DATA AUDIT FINDINGS FOR SOUTHEAST REGIONAL LABORATORY

a. Method verification:
    i. We only had access to the hard copies of the SRL document “Method Verification for the Multi-class, Multi-residue LC-MS/MS Screening and Confirmation Method for Drug Residues in Milk” for review.
    ii. The standard solutions of the drugs were prepared accurately. The method verification of the drugs were done using an Agilent triple quad 6460, which is different from Thermo TSQ quantum triple quad mass spectrometer used in the LIB. Therefore some transitions are different from the ones listed in LIB (Table 3-MS acquisition parameters for Agilent 6460). The control milk samples are fortified at 0.5X, 1X and 2X concentrations of drugs with ~20 replicates for each concentration. The concentration X is the established level, which had been set at the tolerance, safe level, or target level for the drugs. The lab provided us with tables of data for % recovery and % confirmed for these samples (Table 1). They also compared their data to data from LIB (Table 2) for milk samples fortified at 1X.
    iii. Limit of detection (LOD) is determined using signal to noise (S/N) value from milk fortified at 0.05X of all compounds. Five times S/N was used to calculate the LOD (Table 4 - Limit of detection - method verification document SRL)
    iv. The results of the validation experiment carried out at SRL indicate that their method can be used for screening of most of the drugs in the Milk Assignment except for PEN G. The confirmation rate for PEN G in SRL is 15%. This method cannot be used as an efficient screening method for PEN G in this lab, since it can generate false negatives. However, the low confirmation rate for PEN G will not cause false positives.
    v. One has to also keep in mind the inherent instability of PEN G in interpreting the data.
b. Positive samples
    i. The investigational milk samples were analyzed on a Sciex 4000 QTrap mass spectrometer.
    ii. We looked at all positives (26 positives in 24 samples) in ver. 2 of SRL spreadsheet. We looked at the results files of their analysis and found evidence to certify that these drugs are indeed present in these samples as indicated by the lab. We detected a few minor errors but they did not impact our interpretations.
    iii. Out of the 26 positives, 18 positives are for tetracyclines (OTC, TC, CTC), 1 for Tylosin (TYL) and 1 for Sulfadimethoxine (SDM) which have official tolerances. The amounts

detected are well below 50% of the established level. They should be reported as "not detected at or above X" according to the milk sampling assignment memo.

iv. PEN G:SRL 487 and PEN G and AMP:SRL 474:
    1. The data support the detection and confirmation criteria for these compounds. The method verification at SRL, showed low overall % recovery for PEN G and AMP (34.75 and 29.21 – Table 1 method verification document SRL - see attachment). Therefore, the amounts of these compounds present in SRL 487 (PEN G 5.6ppb) and 474 (PEN G 8.64 ppb and AMP 5.39 ppb) could be higher than the reported values.
    2. The positive milk samples 474 and 487 were sent to the Denver Lab for confirmation. Denver Lab could not detect the positives in these samples. There are several possible explanations for the inability to confirm these samples as positive in the Denver Lab. Both penicillin G and ampicillin have been found to be unstable in milk.

c. Violative samples:
    i. The rest of the positive findings were for CAP and FF in SRL samples 477, 115 and 313 and should be considered violative according to the assignment. The quantitation of the compounds was carried out by a single point calibration with the 1X fortified sample. Therefore the concentrations indicated by SRL for these compounds are only estimates of the true concentrations.
    ii. CAP: SRL 477
        1. Detected at SRL at 16 ppb and meets confirmation of identity. However Denver lab could not detect CAP in this sample. FDA does not have sufficient information on the stability of chloramphenicol in milk.
    iii. FF: SRL 115 and 313
        1. Detected at 3.01 ppb and 0.261ppb in samples 115 and 313 respectively, and meets criteria for confirmation of identity.

d. Controls:
    i. We also inspected the controls (method blank, matrix blank, spiked, spiked-duplicate) in all batches containing these violative samples. All QCs satisfied batch acceptance requirement with minor inconsistencies that did not affect the interpretation.

e. Conclusion:
    i. SRL followed the Milk Assignment and LIB 4443 (with reasonable modification for applicable drugs). The 3 violative cases of FF and CAP in SRL 313, 115 and 477 above are supported by analytical data, both detection and confirmatory. Lastly, because of the low confirmation rate of PEN G in SRL, there might be potentially more violative samples containing this drug that had not been found by this lab.

6. DATA AUDIT FINDINGS FOR DENVER DISTRICT LABORATORY

a. Method Verification:
    i. We reviewed the method validation data, which are included in the LIB 4443 and in data subsequently provided to us. The method validation data generated at DDL indicate that the method is suitable for confirming and screening all drugs specified in the Milk Assignment, with the exception of ampicillin. The LIB 4443 mentioned that the ion ratios for ampicillin varied more than the allowed ± 20% during method

validation. For this reason, the DDL method is suitable for screening but not for confirming the presence of ampicillin.

ii. We reviewed the preparation and calculation of standard solutions used for the analysis and verified that they were prepared correctly.

iii. A sample is confirmed positive for an analyte if the mass spectral and chromatography data meet the confirmation criteria specified in the method SOP and that the level found is above the limit of confirmation (LOC). The LOCs were determined and provided to us by DDL.

b. Negative Samples:

i. We examined data from all negative samples in the batches containing one or more violative samples, with approximately 200 negative samples audited. We also reviewed the quality control samples (blanks, control, fortified control) in each batch to make sure that the acceptance criteria were met.

ii. We have a comment on the analysis of sample set 457-480 (Jun 14 2012). In this sample set, the formic acid and control exhibited elevated levels of ciprofloxacin (CIP). As such, the quality control criteria for ciprofloxacin were not met that day. Such abnormality should have no impact on the analysis, because none of the samples in that set tested positive for ciprofloxacin. Moreover, DDL re-injected the sample set the next day. The CIP level in the control dropped to 0.7 ppb (below the limit of confirmation) and again all unknowns were found negative for CIP.

c. Positive samples:

i. We reviewed data for all the positive samples reported by DDL in the spreadsheet and verified that these drugs are indeed present in the samples as reported by the DDL, except for DEN 510 and DEN 817 noted below.

ii. The limit of confirmation for FF is 0.25 ppb. However, the FF level reported for DEN 510 and DEN 817 was 0.2 ppb, which is below the limit of confirmation. As such, these two samples should not have been reported as confirmed positive.

iii. The samples that were verified positive are summarized below

Sample Number	Drug	Screening/Confirmatory Assay Results	Quantitative Assay Result (ppb)	Safe/Tolerance Level (ppb)
DEN 096	FF	Positive		0
DEN 109	SMZ	Positive	175	10
DEN 116	FF	Positive		0
DEN 190	TUL	Positive		0
DEN 225	CIP	Positive		0
DEN 326	FF	Positive		0
DEN 463	TUL	Positive		0
DEN 525	FF	Positive		0
DEN 588	GEN	Positive	322	30
DEN 609	FF	Positive		0

d. Presumptive Positive Samples Reported by SRL:

i. The data obtained from the screening method support DDL's finding that penicillin G and ampicillin were not found in SRL 474 and that penicillin G was not found in SRL 487. However, we have a comment: In the analysis of the SRL 487 and 474

samples for penicillin G and ampicillin, Denver Lab received the presumptive positive samples from SRL. According to the Milk Assignment, a quantitative assay should be performed if a sample is screened positive. Upon receiving the SRL samples, DLL assayed the samples using the screening method. Because the results were negative for penicillin G and ampicillin, DDL did not perform further quantitative assays on these samples.

ii. SRL 477 was previously shown to contain 16.2 ppb of CAP by SRL. We reviewed the DDL's data and verified that chloramphenicol was not found in this sample.

e. Conclusion:
   i. Except for the FF levels reported for DEN 510 and 817, the data support Denver Lab's findings.

7. DATA AUDIT FINDINGS FOR ARKANSAS REGIONAL LABORATORY

a. Method verification: We reviewed the in-house validation packages of LIB 4507 and Elisa method, and found that the validation was satisfactory. The authentic standards used were within the expiry period. Preparations of standards, calibration standards, and quality controls were found to be in accordance with the method, except for two instances, which were reported in section 7.b.v. below. System suitability was satisfactory. The solvent standards, negative controls (blanks) and positive controls gave expected results.
   i. LIB 4507: Validation has been done for 33 analytes at 0.5X, 1X, and 2X, where X is the established level (tolerance, safe, or target level). The ions used for screening gave well defined peaks, their ion ratios were satisfactory, and the retention times were within expected ranges. ARL has summarized their data in the document "Validation Statistical Evaluation" where the method performance is also compared with that of LIB 4443. According to these data, ARL determined that the method as given in LIB 4507 is fit for the purpose of surveillance screening of milk samples for the 33 analytes at the 1X level. After reviewing the validation data, we agree with ARL that the method is suitable to be used for screening milk samples in the Milk Assignment.
   ii. Neomycin & gentamicin validation: ARL has documented the validation data analysis in the report "Validation Statistical Evaluation". The data shows linearity of calibration curve for the 1/4X to 1X range, and a R^2 value greater than 0.92. According to these data, ARL determined that the method is fit for the intended purpose of screening of milk samples. After reviewing the validation data, we agree with ARL's conclusion.

b. Milk assignment sample analysis:
   i. Violative samples:
      1. ARL 216 for FF: Reported 3.2 ppb >lowest level validated (2.5 ppb). FF has no tolerance/safe level in milk. Therefore, according to the Milk Assignment, residues at any detectable level that meet criteria for confirmation of identity are violative.
      2. ARL 171 for FF: Reported 1.2 ppb > lowest level validated (0.3 ppb)
      3. ARL 216 for TIL: Found 46 ppb > lowest level validated (45 ppb). Note: Although "ARL - Milk_Results Version 2" Excel spreadsheet gave the value as 92 ppb, the pdf document "Scanned Worksheet 090612-A" and Excel spreadsheet, "2012-09-06 A POS" gave a value of 46 ppb. After reviewing the data, we agreed with

46 ppb. ARL later confirmed that the value reported in the said Excel spreadsheet should be corrected as 46 ppb.

4. ARL 276 for FF: Reported 0.3 ppb = lowest level validated (0.3 ppb)

ii. Non-violative samples with established Safe/Tolerance levels:

1. ARL 129: OTC, Reported 44 ppb. We concur with ARL confirming that OTC is present in the sample. However, quantitative analyses by Denver gives a concentration (100 ppb) that is less than the tolerance level of 300 ppb. Therefore according to milk sampling assignment memo, this sample is considered non-violative.
2. ARL 133: SDM: Reported 4.7 ppb, Quantified (Den) 2.4 ppb < lowest level validated (5 ppb). We concur with ARL confirming that SDM is present in the sample. However, since the amount present is less than the tolerance level of 10ppb, sample is considered non-violative.
3. ARL 216: GEN (safe level 30 ppb), Reported 9 ppb, but not confirmed due to lack of sample. We concur with ARL's conclusion that presence of GEN cannot be confirmed with the available data.

iii. Analytes without established Safe/Tolerance levels:

1. ARL 076 for TUL: Reported 0.4 ppb. We do not concur with ARL that TUL is present in the sample as one of the chromatographic peaks (TUL3) is not well-defined. We contacted ARL on this. They agreed that their finding of TUL to be a trace, not meeting confirmation criteria.
2. ARL 216 for TUL: Found 1.3 ppb < lowest level validated (5 ppb). Note: Although "ARL - Milk_Results Version 2" Excel spreadsheet gives the value as 2.6 ppb, the pdf document "Scanned Worksheet 090612-A" and Excel spreadsheet, "2012-09-06 A POS" give a value of 1.3 ppb. After reviewing the data, we agreed with the value of 1.3 ppb. Attempts by ARL to confirm the presence of TUL at 1.3 ppb level were not successful because of an interfering peak with the third transition

iv. Samples tested negative for all analytes:

1. We reviewed data for 105 samples that tested negative for all analytes in the Milk Assignment. We found that the data were in agreement with the ARL conclusions. We, therefore, concur with ARL that these samples were negative for all analytes.
2. It was noted that ARL 132 and ARL 155 samples showed all three ions for FF at levels presumably lower than what was found in ARL 276 sample (0.3 ppb), judging from a comparison of chromatographic peak heights. However, we could not confirm if FF was present in these two samples since the peaks were not integrated

v. Noted inconsistencies and ARL Response.

1. On the extraction date 08/15/2012 C, spiking solution used was FDA-I-494, which had an expiry date of 07/18/12.

   ARL *response: ARL communicated to us that there had been an error in recording which standard was used. They had actually used the same spiking standard that was recorded with batch 08/15/12A, FAD-I-524. Corrections have been made to the extraction page and uploaded along with the other documents.*

   Conclusion: With this correction, we agree that data are in compliance.

2. In the document "MRM Extraction", there are two extraction dates (11/15/2012 and 11/28/12) for one batch. Which is the date that corresponds to the analytical data produced? The spiking solution used for this batch was given as FDA-I-548. This solution was to expire on 11/24/12, and therefore, was not suitable to be used on 11/28/12.

   ARL response: There is no significant impact of the inadvertent use of the slightly expired standard to the negative results on this batch of samples. The samples had been analyzed for 28 of the 29 residues before expiration of the standard and re-run after the expiration to pick up the final residue, sulfapyridine, one of more stable analytes.

   Conclusion: We agree with ARL reasoning that this discrepancy had no significant impact on the analysis.

c. Conclusion:
   i. The samples that are violative are ARL 171 and ARL 276 for FF.
   ii. The samples that are non-violative are ARL 129 for OTC, ARL 133 for SDM and ARL 076 for TUL.
   iii. ARL 216 was violative for FF and TIL and non-violative for TUL and gentamicin.

8. AUDIT CONCLUSION

The Division of Residue Chemistry scientists concurred that the ORA laboratories used appropriate methods. Additionally the audit confirmed that assignment of positive and negative findings were based on CVM guidance. All discrepancies in the interpretation of the assignment between the laboratories were addressed to ensure that a uniform standard was applied to the interpretation of the data from the three laboratories. The review identified several instances where additional data were required which the ORA laboratories provided. CVM concurred with the findings of violative residues in 16 samples. The table below summarizes the sample found to contain violative drug residues.

Sample Number	Drug	Screening/Confirmatory Assay Results	Quantitative Assay Result (ppb)	Safe/Tolerance Level (ppb)
DEN 096	FF	Positive		0
DEN 109	SMZ	Positive	175	10
DEN 116	FF	Positive		0
DEN 190	TUL	Positive		0
DEN 225	CIP	Positive		0
DEN 326	FF	Positive		0
DEN 463	TUL	Positive		0
DEN 525	FF	Positive		0
DEN 588	GEN	Positive	322	30
DEN 609	FF	Positive		0
SRL 477	CAP	Positive		0
SRL 115	FF	Positive		0
SRL 313	FF	Positive		0
ARL 216	FF, TIL	Positive		0
ARL 171	FF	Positive		0
ARL 276	FF	Positive		0

In summary, the audit confirmed that the method validation and data interpretation were conducted in accordance with CVM guidance. The results of the assignment with the noted exceptions were acceptable.

9. DEFINITIONS/GLOSSARY

a. Limit of Detection (LOD): The minimum mass or concentration of analyte that can be detected with acceptable certainty, though not quantifiable with acceptable precision. The term is usually restricted to the response of the detection system and is often referred to as the *Detection Limit*. When applied to the complete analytical method it is often referred to as the *Method Detection Limit* (MDL). (Guidelines for the Validation of Chemical Methods for the FDA Foods Program)
b. Limit of Quantitation (LOQ): The minimum mass or concentration of analyte that can be quantified with acceptable accuracy and precision. Limit of quantitation (or quantification) is variously defined but must be a value greater than the Method Detection Limit and should apply to the complete analytical method. (Guidelines for the Validation of Chemical Methods for the FDA Foods Program)
c. Limit of Confirmation (LOC): The concentration where the weakest diagnostic ion no longer appears at an acceptable signal-to-noise level or where the false negative rate becomes excessive. (CVM Guidance # 118)

10. ATTACHMENTS

a. Method Verification for the Multi-class, Multi-residue LC-MS/MS Screening and Confirmation Method for Drug Residues in Milk – SRL

11. SIGNATURES

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cc: Dr. Craig Lewis, HFV-1
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Milk Sampling Assignment: Internal Audit-Report of Findings: Addendum and Correction

An inconsistency was found in the description of the level of validation for florfenicol listed under section **7: DATA AUDIT FINDINGS FOR ARKANSAS REGIONAL LABORATORY,** b, I, 1-2. Part 1 lists the level of validation as 2.5 ppb whereas part 2 lists the value as 0.3 ppb. During the initial review of the results, the Arkansas Regional Laboratory provided data showing the method was validated at 2.5 ppb. Sample ARL 216 was listed as violative based on this data. ARL had claimed sample ARL 171 was violative, however, validation data was not presented to demonstrate that the method was acceptable to test below 2.5 ppb. The laboratory addressed this data gap, and provided the information to demonstrate that the method was validated to 0.3 ppb. The information was used to update the lowest level validated for sample ARL 171, but inadvertently not updated for sample ARL 216.

The correct value for the lowest level validated for florfenicol (FF) at the Arkansas Regional Laboratory is 0.3 ppb.