Milk Sample Collection Strategy

Background:

FDA is responsible for ensuring that food derived from animals is safe to eat. FDA works with USDA and the states to monitor meat and poultry for unapproved or unsafe drug and chemical residues. USDA’s Food Safety and Inspection Service (FSIS) tests meat and poultry for a variety of drug and chemical residues and reports violative findings to FDA for follow-up and potential enforcement action.

Although only 7.7% of the cattle slaughtered in the United States are adult dairy cattle, they represent 67% of the tissue residue violations reported by USDA’s FSIS. Drug residue violations in dairy cattle tissues often result from poor practices on the farm. These practices may include: failure to maintain treatment records, failure to identify treated animals, failure to follow labeled withdrawal times, increasing the labeled dosage, increasing the length of treatment, or giving the drug by an unapproved route of administration, i.e., intravenous vs. intramuscular.

FDA is concerned that the same poor practices on the dairy farm that cause positive tissue residues may also result in drug residues in milk, especially from non-beta-lactam drugs.

FDA and the states participate in the National Conference on Interstate Milk Shipments (NCIMS), a voluntary coalition of regulators established to ensure the safety and wholesomeness of milk in the United States. FDA publishes the Grade “A” Pasteurized Milk Ordinance (PMO) as a model ordinance for states to adopt. The PMO requires bulk milk pickup tankers to be tested for the presence of at least four of six specific beta-lactam drugs (penicillin, ampicillin, amoxicillin, cloxacillin, cephapirin, and ceftiofur). Beta-lactams are the most widely-used group of antibiotics used on dairy farms. However, there are many other classes of drugs that are approved and may be used on dairy farms that are not routinely tested for in milk.

FDA is initiating a milk sampling survey that will provide information to determine whether there are drug residues in milk and if so, the extent of the problem associated with these dairy farms whose practices result in positive tissue residues.
**Revised Sampling Plan:**

In November 2010, FDA announced a milk sampling assignment to determine whether dairy farms with histories of drug residue violations in meat from dairy cattle may also have violative drug residues in milk due to poor on-farm drug use practices. FDA received feedback from state regulators, dairy industry associations, and other affected stakeholders about the impact of the plan, and has sought input on revising the plan in an effort to avoid disruption to the milk supply.

In response to the feedback it has received, the Agency has moved from a sampling/enforcement strategy where action would have been taken against an individual dairy producer with a violative residue to a survey sampling approach, utilizing a third-party, that thoroughly blinds the origin of the samples and, hence, enforcement action is not possible. FDA believes such a non-regulatory survey will provide the agency with the information it needs to determine whether dairy farms with histories of drug residue violations in meat from dairy cattle may also have violative drug residues in milk.

The plan calls for the sampling to be conducted utilizing the “Universal Sample” as defined under the PMO. Sampling would be conducted as follows (See Attachment 1 for Sampling Plan Flow Diagram):

1. FDA District Offices will be given a confidential list of dairy producers who were identified using a relative risk ranking process (Selected list) and a list of laboratories identified by State Milk Regulatory Agencies who receive and hold Universal Samples.

2. FDA Investigators will visit the laboratories identified by State Milk Regulatory Agencies and collect an equal number of random samples and samples from the Selected list. The goal is to collect a total of 900 Selected samples and 900 Random samples. The FDA investigator will give unmarked sterile sample containers to lab staff and observe the samples being poured into containers. Each container of Selected samples will be marked as ‘71V020’ and each Random sample container, as ‘71V021’.

3. Samples will then be sent to the Institute for Food Safety and Health (IFSH) at Illinois Institute of Technology where they will be stored at negative 20° F or colder. IFSH will receive samples from all Districts and will initially hold samples for 1 month to assure a sufficient pool of samples for randomization.
4. IFSH will then randomly choose from the pool of stored, frozen milk samples and ship the appropriate number of Random (71V021) and Selected (71V020) samples to the appropriate FDA laboratory.

5. FDA intends to complete sample collection and analysis within approximately 12 months. FDA will collect samples in the most efficient and effective manner, taking into consideration sample storage time and FDA laboratory analytical capability.

6. All samples will be analyzed for residues of 30 different animal drugs. (See Attachment 2 for additional information on analytical methods and list of drugs to be tested).

7. Once all samples have been analyzed, FDA will prepare and make public a report summarizing the results of the sampling survey. The report will provide a summary of the results of the analytical tests and will make clear which results represent non-violative drug residues (those within existing established tolerances for drug residues in milk) and which results represent “violative” residues of animal drugs.
Flow Diagram for Milk Sampling Plan

FDA Investigators to visit labs designated to receive/hold Universal samples
- Investigator will have list of dairies Selected for sampling
- Investigator instructed to collect equal number of Random samples (dairies not on the selected list)

FDA Investigator will review Selected list with lab director to determine which samples are available for collection

Lab representative is asked to transfer available samples to FDA-provided vials
- Selected samples placed in vials coded 71V020
- Random samples placed in vials coded 71V021

FDA investigator logs samples into FACTS using only the following information:
- Vial code (71V020 or 71V021)
- Farm name: “Blinded Milk Sampling Assignment”

FDA Investigator ships frozen sample vials to 3rd party (The Institute for Food Safety and Health)

3rd party receives samples from all investigators and stores frozen samples
Samples held until sufficient pool of samples is obtained to provide for randomization

3rd party randomly ships designated number of samples to designated FDA labs for analysis

All samples will be analyzed using methods described in Attachment 2

In cooperation with stakeholders FDA will prepare and make public a report summarizing the results of the sampling survey
Milk will be analyzed for the following drugs:

<table>
<thead>
<tr>
<th>Drug 1</th>
<th>Drug 2</th>
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<tbody>
<tr>
<td>Ampicillin (AMP)</td>
<td>Sulfadimethoxine (SDM)</td>
</tr>
<tr>
<td>Cephalin (CEPH)</td>
<td>Sulfamethazine (SMZ)</td>
</tr>
<tr>
<td>Cloxacillin (CLOX)</td>
<td>Sulfadiazine (SDZ)</td>
</tr>
<tr>
<td>Penicillin G (PEN G)</td>
<td>Sulfathiazole (STZ)</td>
</tr>
<tr>
<td>Erythromycin (ERY)</td>
<td>Trimethoprim (TRIP)</td>
</tr>
<tr>
<td>Tylosin (TYL)</td>
<td>Thiabendazole (THBZ)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>Flunixin (FLU) (5-hydroxyflunixin)</td>
</tr>
<tr>
<td>Sarafloxacin (SAR)</td>
<td>Bacitracin (BAC)</td>
</tr>
<tr>
<td>Chlortetracycline (CTC)</td>
<td>Virginiamycin (VIR)</td>
</tr>
<tr>
<td>Oxytetracycline (OTC)</td>
<td>Tilmicosin (TIL)</td>
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<tr>
<td>Tetracycline (TC)</td>
<td>Florfenicol (FF)</td>
</tr>
<tr>
<td>Doxycycline (DC)</td>
<td>Chloramphenicol (CAP)</td>
</tr>
<tr>
<td>Sulfachloropyridazine (SCP)</td>
<td>Tulathromycin (TUL)</td>
</tr>
<tr>
<td>Sulfadiazine (SDZ)</td>
<td>Gentamicin (GEN)</td>
</tr>
<tr>
<td>Sulfamerazine (SMR)</td>
<td>Neomycin (NEO)</td>
</tr>
</tbody>
</table>

Description of analytical methods

For Gentamicin and Neomycin:

- **Screening**: Enzyme-Linked Immunosorbent Assay (ELISA) with Solid Phase Extraction (SPE) cleanup (Europroxima ELISA kit, with sample extraction/cleanup) - (Based on “Determination of Gentamicin in Bovine Milk Using Liquid Chromatography with Post-column Derivatization and Fluorescence Detection” (Journ. Chrom B, 691 (1997), 377-382))

For all other drugs listed in table above:

- **Screening/Confirmation**: Multi-residue LC-MS/MS method as described in LIB# 4443: “Optimization and Validation of Multi-class, Multi-residue LC-MS/MS Screening and Confirmation Method for Drug Residues in Milk” with memorandum of analysis to include chloramphenicol, florfenicol, and Tulathromycin. A link to the laboratory information bulletin (LIB 4443) is listed below: [http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM239311.pdf](http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM239311.pdf)
• **Quantitative follow-up methods:** For those drugs for which tolerances have been established for residues in milk: presumptive positive results from the screening methods will be additionally analyzed by quantitative methods to determine the amount of drug residue(s) present.

Those drugs with no established tolerance in milk will not be quantitated because no amount is allowed in the milk and this method (LIB 4443) is confirmatory as to the identification of the drug.

• Florfenicol, chloramphenicol and tulathromycin were added to the above method (LIB#4443) and validated in the field labs per their local validation protocols. The details are listed in the following memorandum of analysis.

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**Memorandum of analysis for Chloramphenicol (CAP), Florfenicol (FF) and Tulathromycin (TUL) in milk multi-residue method (LIB# 4443)**

The above new analytes have been added (and validated in house per field lab’s protocol) to the procedure described in LIB 4443. To incorporate these new analytes the following steps have been added to the LIB:

1. Stock standards (at 100μg/mL) in methanol are prepared for the above and are good for one year when refrigerated at 4º C.

2. The following amounts of these stock solutions are additionally added to make IMS mixed standard two as shown on pg. 4 of LIB 4443: 50μL of CAP stock std., 50μL of FF stock std., and 100μL of TUL stock std. This corresponds to “safe levels” of 5, 5, and 10ng/mL for these analytes. The sample extraction, spiking and QA/QC protocol remain the same.

3. For MS detection, TUL is analyzed using the same instrument parameters as LIB 4443 (positive mode ESI). CAP and FF however, are analyzed in negative ion mode ESI with a different column and a different mobile phase than described in LIB 4443- a separate injection is required for these two analytes.

4. The following instrument parameters (using a Thermo TSQ quantum triple quadrupole MS) apply for CAP, FF analysis: column-Phenomenex Luna 5u C8(2) 100A 150 x 2.0 mm, spray voltage 2500V, sheath gas 50, aux gas 0 and capillary temperature of 320ºC, tube lens=-80V. The following mobile phase gradient was used (where A=water and B=ACN): initial mobile phase concentration @ 250μl/min. is 95:5 A:B, ramp to 35:65 A:B at 4 min. and hold for 2 min. then back to 95:5 A:B at 8 min and hold for two min. for a total run time of 10 minutes. Injection volume is 10μL. Oven temperature is 35ºC, Divert valve to MS at 3.6 min. The parent mass for CAP is 321 m/z with product ions monitored of 152, 194, and 257 m/z. The parent mass for FF is 356 m/z with product ions monitored of 185, 219, and 336 m/z.
5. The parent mass of Tulathromycin is the doubly charged m/z of 404 with product ions monitored of 158, 420 and 577 m/z. All product ions monitored were optimized for collision energy by infusion.