



Lansing, Michigan USA

Reveal[®] for CAP/STREP: A Rapid, One-Step Lateral Flow Assay for Detection of Chloramphenicol and Streptomycin in Raw Commingled Bovine Milk

David Ankrapp, Joshua Kuipers, Coilin Walsh, Paul Lewis, Tim Goldy, Frank Klein and Jennifer Rice



INTRODUCTION

Dairy regulations in the Russian Federation specify stringent limits for the presence of chloramphenicol and streptomycin/dihydrostreptomycin drug residues in raw, commingled bovine milk (Russian Federal Law 163-2010). These regulations require that all milk imported into the Federation must be tested for antibiotic residues, and if a positive milk sample is detected, importation is not allowed from the supplier until testing confirms that drug residues are below the Russian limits. With the rapid and easy-to-use one-step Reveal for CAP/STREP assay, dairy suppliers to the Russian Federation can confidently test raw tanker milk to ensure it meets the more stringent antibiotic drug residue screening criteria of the Russian Federation.

PURPOSE

Demonstrate the robustness and reliability of the Reveal for CAP/STREP assay for detecting target drug levels. Validation testing was performed in a multi-operator, multi-day, multi-reader, multi-kit lot randomized blind experiment.

MATERIAL AND METHODS

MILK COLLECTION

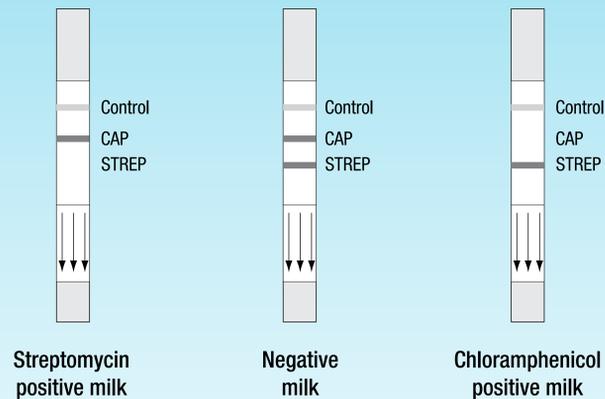
Raw, commingled bovine milk was obtained from a local dairy processing facility. The milk was kept at 2–8°C and used to prepare drug milk within 24 hours of collection.

PREPARATION OF DRUGGED MILK

Drugs were obtained from the U.S. Pharmacopeia Convention. Drug stock solutions were prepared at 1 g/L in deionized water; for streptomycin/dihydrostreptomycin, the stocks were adjusted for the anion portion to provide the correct concentration of active drug. Drug stocks were serially diluted into raw milk to obtain the final drug concentrations used for testing. Non-drug and drugged milk were then aliquoted, snap frozen in liquid nitrogen, and stored at -80°C. This stock of frozen non-drug and drug milk was used throughout the validation testing. On each day of testing, aliquots of the frozen non-drug and drugged milk were rapidly thawed in warm water and placed on ice prior to use.

REVEAL FOR CAP/STREP ASSAY KIT DESCRIPTION

The Reveal for CAP/STREP kit contains 25 lateral flow devices. Each device has all of the chemistries necessary for the specific detection of chloramphenicol and streptomycin drug residues. Specifically, the device has three lines: two antibiotic detection-specific test lines and a control line. One test line is for the detection of chloramphenicol (CAP) and one test line is for the detection of streptomycin/dihydrostreptomycin (STREP). A schematic of the test is shown below.



ROBUSTNESS PROTOCOL

- Multiple testing days
- Multiple AccuScan[®] Pro readers
- Multiple kit lots (N=4)
- Each operator tested multiple lots of Reveal for CAP/STREP kits in a randomized, blind fashion
- Multiple operators
- Four different operators were used over the course of the multi-day testing period. Each operator was unfamiliar with running the test.



ONE-STEP ASSAY PROCEDURE

1. Add 0.2 mL of drugged milk or negative milk (0.2 mL) to a glass vial and place vial into a heating block (47.5±10°C).
2. Immediately insert a Reveal for CAP/STREP device into the vial.
3. Incubate 5 minutes.
4. Remove device from vial and place device into an AccuScan Pro device holder.
5. Insert holder into AccuScan Pro reader
6. Results obtained in ~20 seconds.

Test results are scored either positive or negative based on the following criteria: The AccuScan Pro reader measures the signal intensity of each antibiotic test line and the signal intensity of the control line. A test line/control line ratio is then calculated.

- **POSTIVE RESULT:** Test line ratio < Test line-specific threshold ratio
- **NEGATIVE RESULT:** Test line ratio ≥ Test line-specific threshold ratio

RESULTS

Drug sensitivity and reliability of Reveal for CAP/STREP device from robustness study (Table 1)

- Chloramphenicol detection at 0.3 ppb ≥ 95%
- Streptomycin detection at 200 ppb ≥ 95%

CHLORAMPHENICOL

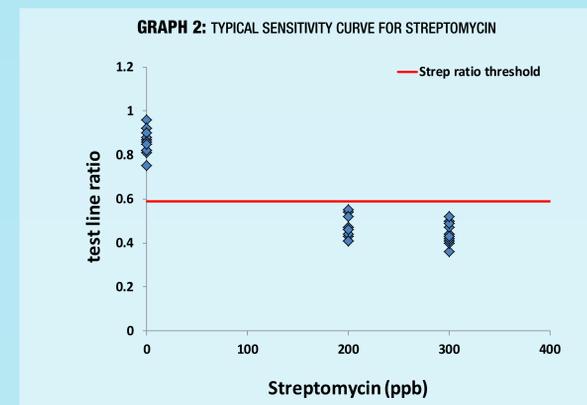
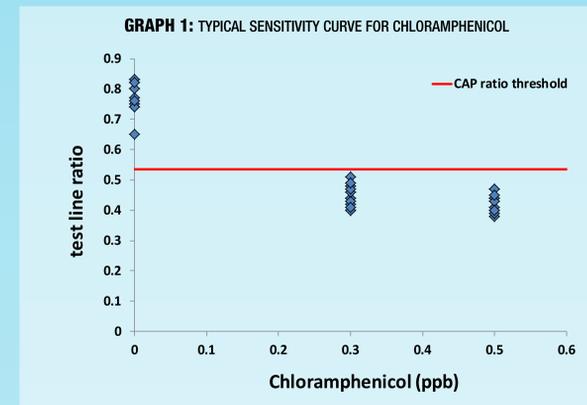
ppb	Positive	N	% Positive
0	0	332	0%
0.3	312	328	95%
0.5	331	333	99%

STREPTOMYCIN

ppb	Positive	N	% Positive
0	0	332	0%
200	312	328	95%
300	320	333	96%

CONCLUSION

Reveal for CAP/STREP is a simple, rapid, one-step test for detection of chloramphenicol and streptomycin/dihydrostreptomycin in raw, commingled bovine milk. With greater than 90% reliability for the detection of both chloramphenicol and streptomycin, the Reveal for CAP/STREP assay meets the strict standards of residue tolerance detection limits set forth by the Russian Federation.



Graphs 1–3 show typical drug sensitivity curves for chloramphenicol, streptomycin, and dihydrostreptomycin.

