Screening Method for Flunixin and Ceftiofur in Bovine Kidney

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Abstract

Flunixin and ceftiofur are veterinary drugs detected in bovine tissue samples collected at slaughter. A 10 minute screening method was developed for detecting ceftiofur and flunixin and other beta-lactam drugs in kidney that involves a simple swab extraction followed by detection using a lateral flow test strip. The method was evaluated in blind studies using drug-spiked negative control kidney extract and incurred kidney samples. In spiked samples, penicillin G and flunixin were detected below a kidney tolerance of 50 ppb penicillin G and a kidney target-detection level of 100 ppb flunixin. Ceftiofur incurred samples were detected near the 400 ppb tolerance while flunixin incurred kidney samples were detected below 25 ppb, which is the tissue tolerance for flunixin. Results indicate the method fills a current detection gap in current screening methods used at slaughter and that it may be useful as a rapid screening method for reducing the incidence of these drug residues in animal tissue.

Introduction

Flunixin, a non-steroidal anti-inflammatory drug, and ceftiofur, a protein bound beta-lactam antibiotic, are detected frequently in bovine tissue as reported by the Food Safety Inspection Service (FSIS) (1). These residues are detected in inhibitory-screening-test-positive samples quarantined during slaughter (2). However, flunixin and ceftiofur-bound-metabolite are not inhibitory at tolerances and may elude detection (3). A rapid method is needed to screen for these drugs in tissue.

Purpose

1) Develop a lateral flow (LF) method for detecting ceftiofur and flunixin in kidney and

2) Assess method sensitivity using spiked synthetic negative control kidney extract and drug treated incurred kidney.

Methods

Beta-lactam and flunixin lateral flow (LF) test kit with Kidney Extraction Swab (KES), Negative Control (KESNC), ROSA 56C 8 min incubator, and reader were supplied by Charm Sciences, Inc. The 10 min procedure for performing the method is shown in Figure 1. Reader interpretation, negative or positive, compares a control line (C) to a beta-lactam detection line (BL) and a flunixin line (FLU) on the same LF strip. When positive the reader indicates which line BL or FLU or both BLFLU are positive relative to C line.

Spiking experiment: USP meglumine flunixin and potassium penicillin G were spiked at five levels into KESNC, split into (n=30) replicates, and blind coded with (n=60) negative samples. One hundred µl were added to KES and screw-activated into the KES vial containing buffer. An aliquot (300 µl) of the buffer-extract was applied to LF, incubated, and the completed test strip interpreted with reader.

Incurred Experiment: Frozen incurred kidney samples containing 343 ppb flunixin, 21 ppb flunixin, and 1150 ppb ceftiofur, supplied by Food and Drug Administration- Center for Veterinary Medicine (FDA-CVM), were cut into (n=10) pieces and blind coded along with (n=30) negative kidney pieces and tested following kit instructions. Positive extracts were diluted 3-fold with KESNC extract and tested to mimic 115 ppb flunixin, 7 ppb flunixin and 383 ppb ceftiofur incurred samples.

Data

Table 1: Summary of spiked KESNC experiments

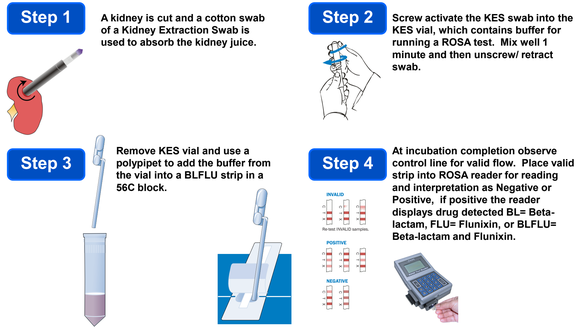
Penicillin G (ppb)	% positive (#positive/#tested)	Flunixin (ppb)	% positive (#positive/#tested)
0	5% (3/60)	0	8.3% (5/60)
10	20.0% (6/30)	20	43.3% (13/30)
20	73.3% (21/30)	40	73.3% (21/30)
30	96.7% (29/30)	60	93.3% (28/30)
40	100.0% (30/30)	80	100.0% (30/30)
50	96.7% (29/30)	100	100.0% (30/30)

Table 2: Summary of Incurred Kidney Experiments

Sample ID	% positive (#positive/#tested)	Reader Interpretation (# of interp/#samples)
Kidney #1- ceftiofur 1150 ppb	100% (10/10)	BLFLU[1] (10/10)
Kidney #5 – 21 ppb flunixin	100% (10/10)	FLU[2] (10/10)
Kidney #6 - 343 ppb flunixin	100% (10/10)	FLU (10/10)
Kidney #1 * Ceftiofur 383 ppb	100% (10/10)	BL[3] (6/10) BLFLU (4/10)
Kidney #5 * 7 ppb flunixin	70% (7/10)	FLU (7/10)
Kidney #6 * 114 ppb flunixin	100% (10/10)	FLU (9/10) BLFLU (1/10)
0 ppb Kidney (n=5 of 6 kidneys)	0% (0/30)	Negative (30/30)

1 BLFLU = both the BL line and FLU line are positive, lighter than C line
2 FLU = The FLU line is positive, lighter than C line and the BL line is negative, darker than C line.
3 BL = The BL line is positive, lighter than C line, and FLU line is negative, darker than C line.
*The samples are from retest of initial positive kidney sample using 1 part original extract diluted into 2 parts KESNC

Figure 1. KES Procedure



Results

The results of spiking experiments are shown in Table 1. Penicillin G was detected below 50 ppb tolerance at 20-40 ppb. Flunixin was detected below the 100 ppb kidney target level at 20-80 ppb. The penicillin G spiking experiments resulted in 3 false BL positive responses out of 60 samples and the flunixin experiment resulted in 4 false FLU positives and 1 false BL positive out of 60 samples. In these experiments seven of the eight total false positives were specific to the drug spiked into the samples, suggesting possible contamination of the negative control samples during the spiking experiment. No false positives were observed in the incurred study containing 30 negative kidney samples, shown in Table 2. All incurred samples (10/10) tested positive for total-ceftiofur-metabolite at 1150 ppb and 383 ppb levels (400 ppb kidney tolerance). All (10/10) samples containing flunixin at 21 ppb, 115 ppb and 343 ppb levels tested positive, while 7/10 containing 7 ppb were positive (25 ppb tissue tolerance). Reader interpretation of FLU contamination in the incurred tissue was accurate for 36 out of 37 positive flunixin samples. The reader positive interpretation of the 1150 ppb ceftiofur incurred sample, was a combined BLFLU positive interpretation. The 383 ceftiofur sample positive results were interpreted BL 60% of the time and BLFLU 40% of the time.

Significance

The 10 minute procedure detects ceftiofur and flunixin below or near tissue tolerances. This addresses a residue detection gap in slaughterhouse-inhibition-tests providing additional consumer protection. Additional kidney sample testing and beta site validation is planned.

References

1) USDA/FSIS. (2011). US National Residue Program 2009 Residue Sample Results. p. 110-111 http://www.fsis.usda.gov/PDF/2009_Red_Book.pdf

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3) Salter, R.S., M. Rossi, C. Lee, S. Son, S. Saul. (2004) Kidney Inhibition Swab (KIS) Test. Broad Spectrum Microbial Inhibition Test Meets Tolerances within 3 Hours. Poster presented to AOAC annual conference 2004 St. Louis, MO