Adjusting Sensitivity of ROSA MRL Beta-lactam Tests to More Closely Detect Cefalonium at MRL

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Introduction:
Receptor based beta-lactam detection in rapid milk screening assays uses broad-spectrum screening of chemical functional-groups. The sensitivity of any one chemical structure is related to 3-D conformational fit into the ligand binding sites. The Charm ROSA (Rapid One Step Assay) formatted test uses a patented down-regulation with competing ligand-binding agents to adjust hyper-sensitivity to more closely match drug tolerance levels in milk, e.g. maximum residue limits (MRL) or Safe Level (SL). Cefalonium with MRL of 20 ppb is a beta-lactam that is hyper-sensitive at 3-6 ppb in receptor binding assays; and it is reported to be a cause of unnecessary milk rejection in countries such as Ireland and New Zealand where the drug is a popular dry-cow treatment. Recent development of a Kiwi Test with sensitivity cefalonium adjustment has helped NZ authorities confirm MRL violations without loss of milk from oversensitive initial truck screening tests. (1)

Purpose:
Adjust the sensitivity of the Charm MRL Beta-lactam Test, Charm MRL-3 Beta-lactam Test, and Charm MRL Beta-lactam and Tetracycline Tests to more closely detect cefalonium at the MRL of 20 ppb.

Methods:
Cefalonium ligand-binders were produced with beta-lactam-protein conjugate injected into animals. The Charm MRLBL, MRL3, and MRLBLTET tests were modified with application of affinity purified ligand-binders to the strips. The methods were evaluated n=60 negatives and n=30 positives at the detection claim levels.

Results:
The modified tests had 90% sensitivity with 95% confidence at 15 ppb cefalonium compared to the original tests 4 ppb sensitivity. Reader averages shown in Table 1 of negative results and of detected beta-lactam drugs and tetracycline drugs, were not shifted while reader averages of cefalonium 10 ppb were shifted from positive to negative. Concentration response ranges of the modified tests were 10-14 ppb cefalonium and the original originals tests were 3-5 ppb; see Figure 1 example of dose-response shift using Charm MRLBL test.

Significance:
It is possible to adjust sensitivity of broad antibiotic screening tests to be more compliant to specific drug tolerances.

Table 1. **Reader averages of negative and antibiotic spiked positive raw milk using different Charm ROSA tests with and without cefalonium ligand sensitivity adjustment**

Charm ROSA Test	Negative Raw Milk		10 ppb Cefalonium		4 ppb Ampicillin		100 ppb Oxytetracycline	
	Avg w'out Ligand	Avg with Ligand	Avg w'out Ligand	Avg with Ligand	Avg w'out Ligand	Avg with Ligand	Avg w'out Ligand	Avg with Ligand
Charm MRL 8 minute Beta-lactam	-1889	-1772	1669	-834	2645	2559	NA	NA
Charm MRL3 3 minute Beta-Lactam	-1250	-1299	2634	-534	990	995	NA	NA
Beta-lactam and Tetracycline Combo MRLBLTET	-1849	-1832	2850	-435	1299	1330	1273	1150

The higher the positive averages the more positive the results. The lower the negative averages the more negative the results. Differences in averages +/- 200 are not significant. Only the 10 ppb cefalonium samples show significant change from positive to negative result when tests are treated with cefalonium specific ligand.
NA= Not applicable

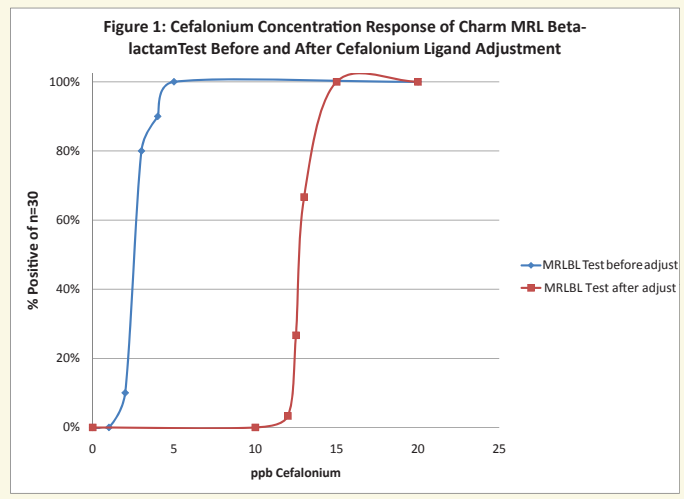

Figure 1.

References:

1) Douglas, D., S. Law, R. Salter, and R. Markovsky (2011) Adjusting Sensitivity of ROSA Beta-lactam Tests to more Closely Detect Cefalonium at MRL. Poster Presented at the European Conference of IAFP, Ede Netherlands May 2011.