

Single Vial and Multiplate Inhibition Assays with MRL Detection of Tetracyclines, Beta-lactams, Sulfa Drugs, Macrolides and Two Aminoglycosides

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Introduction:

Broad spectrum inhibition assays are essential in integrated monitoring plans to control antibiotics on dairy farms and prevent residues in milk. Charm Sciences' single-vial CowSide II and multiplate Blue Yellow II Tests are improved Geobacillus stearothermophilus based inhibition assays that detect a broader range of antibiotics at MRLs, including ceftiofur-total-metabolites and tetracyclines that have eluded earlier single-culture-inhibition assays used on-farm and in milk control stations.

Objective:

This study presents compiled internal QC and independent single laboratory validation data consistent with Community Reference Laboratories Guidelines for Validation of Screening Methods (1).

Methods:

The Blue Yellow II Test was evaluated with a reflectometric color scanning program (GEVIS, Fidenza, IT). Values ≥ 6.0 on a 0.0 to 10.0 scale, indicating some blue color, are positive. The test was evaluated internally and at ILVO-T&V (Melle, Belgium) with spiked MRL and >500 negative samples (2). The CowSide II Test was evaluated visually both internally and at Istituto Zooprofilattico Sperimentale (IZS)(Brescia, Italy) using negative milk and spiked at MRL (3).

Results and Discussion:

Data listing the compiled results of internal and external evaluations are presented in Tables 1 and 2. The BY II sensitivity, as presented by Reybroeck, are shown in figures 1-3 (2). All beta-lactam drugs evaluated, Figure 1, are detected at MRL including 100 $\mu\text{g}/\text{kg}$ total-ceftiofur-metabolite in internal study; desferoyl ceftiofur

was detected at 125 $\mu\text{g}/\text{kg}$ at ILVO-T&V and metabolite detected 200 $\mu\text{g}/\text{kg}$ by IZS. Tetracyclines are detected by BY II at 100 $\mu\text{g}/\text{kg}$ MRL, Figure 2, and between 100 and 200 $\mu\text{g}/\text{kg}$ with CowSide II. Detection of ceftiofur and tetracyclines at MRL and 2x MRL by these inhibition tests demonstrates enhanced sensitivity in comparison to other commercial inhibition tests. Tylosin and tilmicosin are detected at MRL, while erythromycin is detected at 2x-MRL. Lincomycin and pirimycin are detected at $\frac{1}{2}$ -MRL. Gentamicin and neomycin are the only aminoglycosides detected at $\frac{1}{2}$ -MRL. Sulfa drugs are detected at MRL to 2x-MRL depending on the specific drug, see Figure 3 and Table 1.

Over 2000 farm tank replicates with <500,000 somatic cells/ml, were evaluated for method selectivity and only 10 positives were observed, Table 2. Some of the positives may in fact contain inhibitors or natural inhibitors as the samples were positive by other screening tests. High somatic cell >500,000 SCC/ml, mastitic milk, may produce more false positives than milk from healthy cows. The method was evaluated with raw and UHT cow milk, but not other species milk.

Significance:

The two inhibitor methods provide enhanced MRL level detection of tetracyclines and ceftiofur compounds that were previously not detected by other inhibitor methods. The methods have a low false positive rate and meets CRL specifications for use a screening method for cows milk.

Table 1. Sensitivity Data Summary in Raw Commingled Cow Milk

Drug	EU MRL ($\mu\text{g}/\text{kg}$)	CowSide II Test				Blue Yellow II Test							
		Manual Claim 90% Positive with 95% Confidence	CC6 Concentration ($\mu\text{g}/\text{kg}$)	Instituto # of Samples / n / # of Positives	Internal # of Samples / n / # of Positives	Percent Positive	CRL α	Manual Claim 90% Positive with 95% Confidence	CC6 Concentration ($\mu\text{g}/\text{kg}$)	ILVO-T&V # of Samples / n / # of Positives	Internal # of Samples / n / # of Positives	Percent Positive	CRL α
Amoxicillin	4	3-4	4	6 / 30 / 30	5 / 18 / 18	100%	0%	2-3	3	2 / 20 / 20	4 / 40 / 40	100%	0%
Ampicillin	4	3-4	4	6 / 30 / 30	5 / 18 / 18	100%	0%	2-3	3	2 / 20 / 20	4 / 40 / 40	100%	0%
Cefalexin	100	75-100	100	6 / 30 / 0*	5 / 18 / 18	38%	63%	60-100	100	2 / 20 / 20	4 / 60 / 60	100%	0%
Cefalonium	20	15-20	20	6 / 30 / 30	5 / 18 / 18	100%	0%	10-15	15	2 / 20 / 20	4 / 40 / 40	100%	0%
Ceftiofur Metabolite (Parent)	100	60-100	(25)	6 / 30 / 0*	5 / 18 / 18	39%	63%	50-100	(20)	2 / 20 / 20*	4 / 60 / 60	100%	0%
Cephapirin	60	8-10	10	ND	5 / 18 / 18	100%	0%	4-6	6	2 / 20 / 20	4 / 20 / 20	100%	0%
Cloxacillin	30	10-25	25	6 / 30 / 30	5 / 18 / 18	100%	0%	10-20	20	2 / 20 / 20	4 / 40 / 40	100%	0%
Penicillin G	4	2-3	3	6 / 30 / 30	52 / 262 / 262	100%	0%	1-2	2	2 / 20 / 20	4 / 40 / 40	100%	0%
Oxytetracycline	100	75-100	100	6 / 30 / 8*	12 / 36 / 36	67%	33%	75-100	100	10 / 100 / 99	12 / 102 / 102	99.5%	0.5%
Chlortetracycline	100	NC	100	6 / 30 / 14*	ND	ND	ND	150-200	200	2 / 20 / 20	1 / 60 / 60	100%	0%
Tetracycline	100	50-100	100	E	4 / 14 / 14	100%	0%	50-100	100	2 / 20 / 20	6 / 60 / 60	100%	0%
Gentamicin	100	75-150	150	ND	4 / 14 / 14	100%	0%	75-150	100	2 / 20 / 20	4 / 60 / 60	100%	0%
Sulfadiazine	100	40-60	60	6 / 30 / 30	5 / 18 / 18	100%	0%	80-100	100	10 / 100 / 96	4 / 60 / 59	97%	3%
Sulfadimethoxine	100	25-50	50	6 / 30 / 16*	5 / 18 / 18	71%	29%	50-75	75	ND	4 / 40 / 40	100%	0%
Sulfadimidine (Sulfamethazine)	100	75-125	100	6 / 30 / 20*	48 / 234 / 234	96%	4%	75-125	100	2 / 20 / 14	14 / 242 / 231	94%	6%
Erythromycin	40	75-100	100	ND	5 / 18 / 18	100%	0%	100-150	100	2 / 20 / 19	6 / 94 / 94	99%	1%
Pirlimycin	100	25-50	50	6 / 30 / 30	5 / 18 / 18	100%	0%	50-100	100	2 / 20 / 20	4 / 20 / 20	100%	0%
Tilmicosin	50	25-35	25	ND	1 / 4 / 4	100%	0%	25-35	35	2 / 20 / 20	2 / 40 / 40	100%	0%
Tylosin	50	20-30	30	6 / 30 / 21*	5 / 18 / 18	81%	19%	20-30	30	2 / 20 / 20	4 / 40 / 40	100%	0%

E= excluded from analysis for technical reason

ND= Not evaluated

* next higher studied dilution 100% positive, additional replicates required to meet CRL Guidelines

Table 2. Selectivity Data Summary

Negative Control (Blank)	CowSide II Test				Blue Yellow II Test			
	Instituto # of Samples / n / # of Positives	Internal # of Samples / n / # of Positives	Percent Positive	CRL α	ILVO-T&V # of Samples / n / # of Positives	Internal # of Samples / n / # of Positives	Percent Positive	CRL α
Negative Raw Cow Milks	18 / 720 / 6	106 / 660 / 0	0.4%	0.4%	807 / 1717 / 4	45 / 272 / 0	0.2%	0.2%

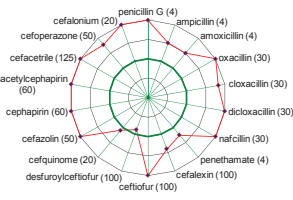


Figure 1. Test capability of Charm Blue Yellow II for β -lactams related to their respective MRL (Commission Regulation (EU) N° 37/2010 (situation on 15/06/2011)). Inner circle = 2 MRL; circle 2 = MRL; circle 3 = 0.5 MRL; circle 4 = 0.25 MRL.

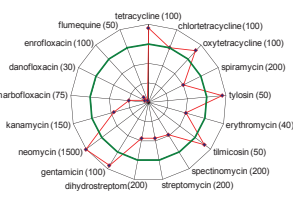


Figure 2. Test capability of Charm Blue Yellow II for tetracyclines, macrolides, aminoglycosides and quinolones related to their respective MRL (Commission Regulation (EU) N° 37/2010 (situation on 15/06/2011)). Inner circle = 10 MRL; circle 2 = 2 MRL; circle 3 = MRL; circle 4 = 0.5 MRL.

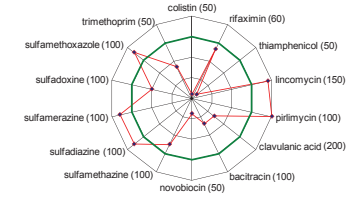


Figure 3. Test capability of Charm Blue Yellow II for colistin, rifaximin, thiamphenicol, lincosamides, clavulanic acid, bacitracin, novobiocin, sulfonamides and trimethoprim related to their respective MRL (Commission Regulation (EU) N° 37/2010 (situation on 15/06/2011)). Inner circle = 10 MRL; circle 2 = 2 MRL; circle 3 = MRL; circle 4 = 0.5 MRL.

References:

- Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines (Initial Validation and Transfer)- Community Reference Laboratories (CRLs) 20/1/2010
- Reybroeck, W., Ooghe, S., Aug 2011. Evaluation of the Charm Blue Yellow II, ILVO-T&V, Belgium
- Istituto Zooprofilattico Sperimentale Della Lombardia e Dell'emilia Romagna "Bruno Ubertini" (Ente Sanitario Di Diritto Pubblico), 2011, Brescia, IT

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