

VALIDATION OF THE BETASTAR S COMBO FOR THE RAPID SCREENING OF MILK FOR RESIDUES OF β -LACTAMS AND TETRACYCLINES

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Introduction

The BetaStar S Combo (Neogen Corporation, Lansing, MI) is a qualitative lateral flow assay for a rapid screening for residues of β -lactams (separate channel for (desfuroyl)ceftiofur) and tetracyclines in raw milk. The time to result for the assay is 5 minutes.

Aim

Evaluation study of the BetaStar S Combo according to Commission Decision 2002/657/EC and the CRLs' Guidelines for the validation of screening methods for residues of veterinary medicines (Anon., 2010).

Evaluation parameters

Following analytical parameters were evaluated: detection capability, test repeatability, test selectivity and test robustness (impact of changes in the test protocol, evaluation for influences from compositional components or milk quality, applicability to test milk of different animal species, rate of false positive results and participation in a national ring trial).

Results

1. Detection capability

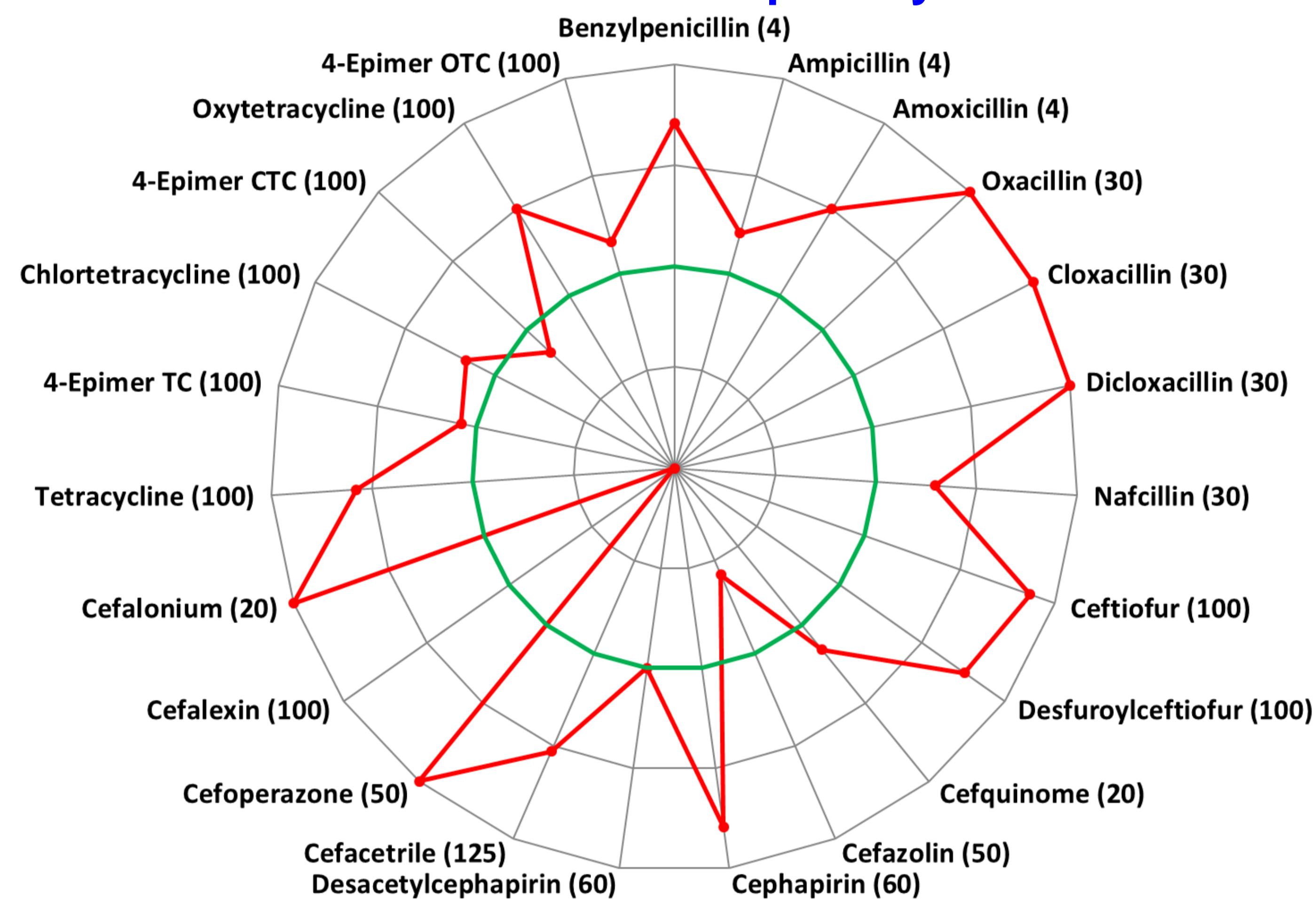


Figure 1. Detection capability of BetaStar S Combo for β -lactams and tetracyclines related to their respective MRL (Commission Regulation (EU) N° 37/2010 (situation on 01/01/2016)). Inner circle = 2 × MRL; circle 2 = MRL; circle 3 = 0.5 × MRL; circle 4 = 0.25 × MRL. MRL ($\mu\text{g}/\text{kg}$) in cows' milk in between brackets after the name of each substance. Results obtained with Accuscan Pro reader and cut-off = 1.00.

Notes: TC: tetracycline; CTC: chlortetracycline; OTC: oxytetracycline.

2. Test repeatability

Table 1. Repeatability of the BetaStar S Combo on 3 different result levels.

	Desfuroylceftiofur			β -lactam			Tetracyclines		
	Mean ratio	s_r	CV	Mean ratio	s_r	CV	Mean ratio	s_r	CV
Blank milk	2.23	0.148	6.63	2.06	0.160	7.76	2.15	0.178	8.28
Positive milk	0.84	0.021	2.51	0.80	0.032	4.00	0.82	0.131	15.92
	0.48	0.065	13.63	0.37	0.038	10.26	0.33	0.072	21.79

Notes: s_r , standard deviation of repeatability; CV, coefficient of variation

3. Test selectivity

The test is very selective for the detection of β -lactams and tetracyclines. Interference was just noticed for clavulanic acid, a β -lactamase inhibitor, on the β -lactam channel and for cefquinome on the desfuroylceftiofur channel from 30 $\mu\text{g}/\text{kg}$ on.

4. Test robustness

Table 2. Robustness of the BetaStar S Combo.

Parameter	Impact on test result (ratio)			
	Blank milk	Doped milk		
		Pen G 2 $\mu\text{g}/\text{kg}$	DCF 35 $\mu\text{g}/\text{kg}$	TC 50 $\mu\text{g}/\text{kg}$
Milk volume	no	no	no	no
Milk temperature	no	no	no	no
Shorter or longer incubation	no	no	no	no
Delay in reading	↓	no	↓	no
High somatic cell count (>10 ⁶ per ml)	↑	no	no	↑ *
High bacterial count (>5×10 ⁵ CFU/ml)	no	no	no	no
Low fat (<2 g per 100 ml)	no	no	no	no
High fat (>6 g per 100 ml)	↑	no	↑ *	↑ *
Low protein (<2.5 g per 100 ml)	no	no	no	no
High protein (>4 g per 100 ml)	no	no	↑ *	↑ *
Low pH (6.0)	no	no	no	no
High pH (7.5)	no	↓	no	↑ *
UHT milk	no	↑	no	↑ *
Sterilized milk	no	↑	no	no
Reconstituted milk powder	no	no	no	↑ *
Thawed milk	no	no	no	no
Goats' milk	no	no	no	↑ *
Ewes' milk	no	↓	no	↑ ↑ *
Mares' milk	↓	no	no	no
Reagents batch differences	no	no	no	no
False positive results	no	---	---	---

Notes: ↑: increased ratio, lower detection capability;

↓: decreased ratio, better detection capability;

*: false negative results;

Pen G: benzylpenicillin; DFC: desfuroylceftiofur; TC: tetracycline.

Conclusions

The BetaStar S Combo with its easy one-step test protocol could be used for the screening of tanker milk on the presence of β -lactam and tetracycline antibiotics at the entrance of the dairy plant. The test is giving reliable test results for different milk types and for milk of different animal species (cow, goat, ewe, mare).



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