EVALUATION OF COMMERCIAL RAPID TESTS FOR ß-LACTAM ANTIBIOTICS IN RAW MILK

Silke Kroll, Ewald Usleber, Klaus-Jürgen Zaadhof, Elisabeth Schneider, and Erwin Märtlbauer

Institute for Hygiene and Technology of Food of Animal Origin, University of Munich, Veterinaerstrasse 13, 80539 Munich, Germany

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Abstract

To comply with drug residue regulations set by the EU (maximum residue limits, MRL), and to minimize economic losses, milk testing for ß-lactam antibiotics at the truck site, before pumping the contents into the silo, is increasingly applied in dairy industry. Because of the time requirements for microbiological inhibition tests, several companies offer more rapid commercial ß-lactam tests. The aim of this study was to evaluate five commercial test systems ("Beta Star", "Charm MRL", "Delvo-X-Press ßL-II", "Penzyme", "SNAP beta-lactam"). Three microbiological inhibition tests ("BRT MRL-Screening Test", "BRT Inhibitor Test", "Delvotest SP") were used in parallel as "reference" methods. Major parameters checked in the study were (i) the capability to detect ß-lactams at the MRL-level (spiked milk samples), (ii) the percentage of false-negative and false-positive results (incurred and blank milk samples), (iii) the agreement with microbiological inhibition tests used as "reference" methods (incurred samples), and (iv) test ruggedness (somatic cell count, total germ count, pH; spiked samples). The results showed that, although no test was optimal in view of all aspects, three tests (Beta Star, Charm MRL, SNAP beta-lactam) were found to be acceptable, while two others (Delvo-X-Press, Penzyme) have to be considered as less suitable, in particular considering low sensitivity for MRL substances.

Introduction

The ß-lactam antibiotics are still the most frequent cause of inhibitor-positive results in milk in Germany (1-3). To comply with EU regulations, the control of every milk tank for residues of ß-lactams, before unloading into the silo, is now widely accepted as good dairy practice. Microbiological inhibition tests require more than 2 hours and are therefore not suitable for this purpose. A range of commercial "rapid tests" is available for ß-lactams, enabling qualitative (yes/no) detection in milk within 10-20 min (4-7). Since several of these tests are relatively new products, little independent information is available about these tests. The aim of this study was therefore to compare the performance of five commercial tests in aspects of test sensitivity (MRL levels), ruggedness (milk quality), and reliability.

Materials und Methods

The test systems as listed in Table 1 were included in the study. For use as "reference" methods, three microbiological inhibition tests were used in parallel throughout the study. Inhibitor-free raw cows‘ milk was from the herd of the Veterinary Faculty of Munich (about 100 lactating cows). Inhibitor-positive and -negative milk samples were obtained from the Bavarian milk testing organisation (Milchprüfring Bayern e.V.). Samples were stored at 4-6 °C and analysed within two days. All samples were checked for inhibitors using the microbiological methods before analysis with the rapid tests. During analyses, milk samples were kept at 10 °C in a water bath. The rapid tests were performed according to manufacturers‘ instructions. The evaluation of the results was performed visually (except Delvo-X-Press) and by instrumental reading (except Penzyme). The study design followed the IDF "guidance for the standardized description of microbial inhibitor tests" as closely as possible, with the necessary modifications for multiple test evaluation. The test sensitivity with regard to ß-lactams for which MRLs have been set was determined using blank milk samples individually spiked with these substances at levels corresponding to 1/2 x MRL, 1 x MRL, and 2 x MRL. Ten replicates per level (plus unspiked "zero" samples) per test were analysed, each five on two different days. To check test ruggedness, the parameters "high somatic cell count", "high total germ count", and "altered pH values" were used. For each parameter, ten replicate analyses of "blank" milk and a blank milk spiked with penicillin G at 4 ng/ml were performed. Finally, the rapid tests were compared with the "reference" methods using inhibitor-positive samples (n=54). These samples had been verified to be positive in our lab before inclusion in the study.

Table 1: Rapid ß-lactam tests under study and microbiological inhibition tests used as "reference" methods

Test system	**Visual evaluation**	**Instrumental reading**	**Manufacturer**
	Rapid tests		
Beta Star	yes	yes	UCB Bioproducts, Belgium
Charm MRL	yes	yes	Charm Sciences, USA
Delvo-X-Press ßL-II	no	yes	DSM, The Netherlands
Penzyme 50	yes	no	UCB Bioproducts, Belgium
SNAP	yes	yes	IDEXX, USA
Microbiological inhibition tests (B. stearothermophilus)			
BRT MRL-Screening Test	yes	yes	AIM, Germany
BRT Inhibitor Test	yes	yes	AIM, Germany
Delvotest SP	yes	(yes)*	DSM, The Netherlands

*, instrumental absorbance reading is possible only after manipulation of the microtiter plate.

Results and Discussion

The levels at which individual ß-lactam antibiotics gave at least 90% positive results in the rapid tests are listed in Table 2. No test detected all 12 substances at the MRL niveau. The Charm MRL (11/12) gave best results in this part of the study, the Beta Star (9/12) and the SNAP (8/12) were estimated as still acceptable. In contrast, the Delvo-X-Press (6/12) and the Penzyme (4/12) showed poor sensitivity for a range of compounds. Looking at the frequency of false-positive results for inhibitor-free milk samples, a different ranking was obtained (Table 3). Even with instrumental reading, the Charm MRL gave a high percentage of false-positive results. The other tests were found to be acceptable (<3% false positives). All tests except the Delvo-X-Press are designed for visual evaluation. Therefore the frequency of doubtful results, i.e. tests which could not be clearly scored as negative or positive, was of interest. With the Penzyme, the result "doubtful" (which has to be interpreted as positive) is part of the method description, while the Beta Star, the Charm MRL, and the SNAP should give only yes/no results according to the manufacturer. However, about 6-10% of these three test systems could not be visually evaluated without doubt, even by a trained user (Table 4). All doubtful visual results were recorded as positive throughout this study. Instrumental reading did improve the evaluation in the case of the SNAP, while the instrumental reading devices of the Beta Star and the Charm MRL frequently gave erratic results. The results of the ruggedness study are compiled in Table 5. High somatic cell counts and changes in pH had a strong influence on some tests, while the total germ count had little or no effect at all. To study the test performance for “real” sample material, a total of 54 inhibitor-positive samples from routine control were reanalysed and the results compared with the three microbiological "reference" methods. Since the “reference” methods have different test sensitivity, the agreement was dependent of which microbiological method was used as the “reference”, although the ranking of the rapid tests was the same (Table 6). The SNAP and the Charm MRL detected the highest percentage of positives, the other tests missed a significant number of samples. However, if a less sensitive "reference" method (BRT inhibitor test) was used, the Charm MRL and the SNAP would give a high percentage of false-positive results, probably caused by sub-MRL levels of penicillin G (1-2 ng/ml) in these milk samples. In conclusion, the results of the study show that no individual rapid test was optimal under all aspects studied, and that the suitability of a certain test also depends on the requirements. However, while the Beta Star, the Charm MRL, and the SNAP seem to be acceptable, the Delvo-X-Press and the Penzyme cannot be recommended, mainly because of poor compliance with MRLs.

Table 2: Detectability* of ß-lactam antibiotics by rapid tests for ß-lactam antibiotics in relationship to European Union MRLs (Council Regulation No. 2377/90).

Substance	MRL (ng/ml)	Concentration (ng/ml) resulting in ≥90% positive test results				
		Beta Star[1]	Charm[1]	Delvo-X-Press[1]	Penzyme[2]	SNAP[1]
Penicillin G	**4**	4	2	4	**8**	2
Amoxicillin	**4**	**8**	4	**> 8**	**8**	**8**
Ampicillin	**4**	4	2	**> 8**	**8**	**8**
Oxacillin	**30**	15	30	**60**	**60**	**60**
Cloxacillin	**30**	15	15	**60**	**60**	30
Dicloxacillin	**30**	15	30	30	**> 60**	30
Nafcillin	**30**	15	**60**	**> 60**	**60**	**> 60**
Cefacetril	**125**	62.5	62.5	62.5	62.5	62.5
Cefazolin	**50**	**100**	25	25	25	25
Cefquinom	**20**	10	20	**40**	**40**	20
Ceftiofur	**100**	100	50	50	100	50
Cephapirin	**10**	**20**	5	5	10	5

* All substances were tested at levels of 1/2 x MRL, 1 x MRL, and 2 x MRL. The concentration level at which ≥90% positive results were obtained (n=10) was regarded as "detected". Please note that the actual test sensitivity could be lower than the lowest level tested. 1, instrumental reading; 2, visual evaluation.

Table 3: False-positive results obtained for raw milk samples free of ß-lactam antibiotics (n=110)

Test system	% false-positive results	
	visual evaluation	instrumental reading
Beta Star	0	2.73
Charm MRL	7.27	7.27
Delvo-X-Press	not applicable	1.82
Penzyme	0	not applicable
SNAP	2.73	2.73

Table 4: Percentage of tests with doubtful visual results* (n=440)

Test system	% of tests
Beta Star	8.18
Charm MRL	9.77
SNAP	5.91

*Doubtful results were scored as "positive" throughout the study for all calculations.

Table 5: Percentage of false-positive (FP) and false-negative (FN, at 4 ng/ml penicillin G) results obtained for raw milk samples differing from "normal" quality (n=10)

Parameter	Beta Star[1]		Charm MRL[1]		Delvo-X-Press[1]		Penzyme[2,3]		SNAP[1]	
	FP	FN	FP	FN	FP	FN	FP	FN	FP	FN
High somatic cell count (10^6 per ml)	**10**	0	0	**40**	0	**80**	0	**10**	0	0
High total germ count (6.5 x 10^5 per ml)	0	0	**10**	0	0	0	0	**80**	0	0
Low pH (6.0)	0	0	0	0	0	0	0	**30**	**20**	0
High pH (7.5)	0	0	0	0	0	**100**	0	**90**	0	0

1, instrumental reading, 2, visual evaluation; 3, false-negative results by the Penzyme are not necessarily due to changes in milk quality, since the detection limit for penicillin G is > 4 ng/ml in "normal" milk.

Table 6: Agreement of the rapid tests with three microbiological inhibition tests for inhibitor-positive raw milk samples (n=54). Samples were obtained during May/June 1999 from Bavarian Milchprüfring e.V.

Test system (n pos./n total)	Microbiological "reference" method (n pos./n total)				
	BRT MRL-Screening Test (54/54)	BRT Inhibitor Test (46/54)		Delvo SP (53/54)	
	% TP	% TP	Number of "FP"[1] results	% TP	Number of "FP"[1] results
	54=100%	46=100%	n=8	53=100%	n=1
Beta Star (34/54)	63	74	0	64	0
Charm MRL (53/54)	98	98	8	98	1
Delvo-X-Press (26/54)	48	57	0	49	0
Penzyme (24/54)	44	52	0	45	0
SNAP (54/54)	100	100	8	100	1

TP, true positives; FP, false-positives; 1, False-positive if this microbiological inhibition test was used as the "reference" method (although the sample was positive in the BRT MRL-Screening Test).

References

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