Comparison of Detection Sensitivity of Five Microbial Inhibition Tests for the Screening of Aminoglycoside Residues in Fortified Milk

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Abstract

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The assessment of detection sensitivity of five microbial inhibition tests (MITs), STAR (screening test for antibiotic residues) with the test strain *Bacillus subtilis* BGA, Delvotest® SP-NT, Total Antibiotics, Kalidos TB, and Kalidos MP with the test strain *Bacillus stearothermophilus* var. *calidolactis* to five aminoglycosides (AMGs), gentamicin, neomycin, streptomycin, kanamycin, and spectinomycin in fortified milk samples were studied. The sensitivity of MITs to AMGs was evaluated on the basis of experimental determination of detection limits (LODs) of MITs for AMGs. The LODs of these tests were compared with the maximum residue limits (MRLs) established for milk by the Commission Regulation (EU) No. 37/2010. LODs of STAR for AMGs in fortified milk samples were at the levels of MRL for neomycin (1.50 μg/g), gentamicin (0.10 μg/g), streptomycin (0.20 μg/g) and kanamycin (0.15 μg/g). Spectinomycin (0.20 μg/g) was not detected at the level of MRL. The LODs determined by Delvotest® SP-NT, Total Antibiotics and Kalidos MP were comparable, but only gentamicin and neomycin were reliably detected at the levels of MRL. Kalidos TB was more sensitive to AMGs than Delvotest® SP-NT, Total Antibiotics and Kalidos MP. Gentamicin, neomycin and streptomycin were detected at the levels of MRL.

Keywords: antibiotic residues; microbial screening; detection limits

Antimicrobial drugs have been used in the dairy industry for more than five decades. They are principally administered to treat udder infections, and they are also applied for the treatment of other diseases (Stead *et al*. 2008). Treatment of lactating animals with antimicrobials may lead to residues appearing in milk.

The presence of antibiotic residues in milk constitutes a potential hazard for the consumer because of allergic reactions, intestinal dysbiosis and resistant populations of bacteria in the general population (Linge *et al*. 2007). Great losses are also incurred in fermented products by inhibiting the bacterial processes involved in the elaboration of cheese and cultured milk products (Althaus *et al*. 2003).

Because the antibiotic residues in food of animal origin pose a potential risk for the health of consumers, the Commission Regulation (EU) No. 37/2010 established the MRLs for antimicrobials in foodstuffs of animal origin. The MRL is the maximum concentration of residues of a pharmacologically active substance which may be permitted in food

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of animal origin. The residues of pharmacologically active substances in products of animal origin should be carefully controlled and monitored in accordance with the Council Directive 96/23/EC of 29 April 1996, on measures for monitoring certain substances and residues thereof in live animals and animal products, regardless of the origin of the product.

Microbial inhibition tests (MITs) are used for the primary screening of antibiotic residues in milk. MITs have to comply with the basic legislative specifications and reliably identify the presence of antimicrobial residues in the examined matrices in concentrations equal to MRLs (Commission Decision 2002/657/EC).

Principally two types of microbial screening assays can be distinguished: (multi-)plate test systems and tube test. In (multi-)plate assays the sample is applied on top of the agar layer or to punch holes in an inoculated agar layer. The presence of antibiotics will show as a growth inhibition zone around the sample which will become visible after incubation (Four Plate Test, STAR, NAT test). All test plates are specific to one or two groups of antibiotics, allowing presumptive identification. Tube tests contain an agar medium seeded with a pH or redox indicator. The presence of an antimicrobial compound becomes apparent from a delayed colour reaction or absence of colour change due to impaired growth of the test bacterium. The most commonly used bacterial strain for this type of test is *Bacillus stearothermophilus* var. *calidolactis* (Delvotest® SP-NT, Eclipse 100®, Kalidos TB, and Kalidos MP) (Pikkemaat *et al*. 2009).

AMGs have been widely used in dairy cattle to treat mastitis and other bacterial infections. Monitoring of AMG residues in milk is, therefore, very important. An inhibition test is useful for the detection of an antibacterial substance if the detection limit (LOD) of this substance is at the level of MRL. The LOD is the lowest concentration of antimicrobial substance which completely inhibits the growth and multiplication of the test organism in the respective MIT test. Because the majority of studies on residues is focused on the microbial detection of β-lactam antibiotics in milk and since 2010 the Kalidos TB and Kalidos MP have replaced the Delvotest® SP-NT test for routine analysis of antibiotic residues in milk, the subject of our comparative study was to examine the sensitivity of five MITs: STAR (CH 12.19, 2006), Delvotest® SP-NT (CH 12.17, 2006), total antibiotics (Euroclone, Milano, Italy), Kalidos TB and Kalidos MP (CH 12.21, 2009) for the detection of aminoglycodside (AMG) residues in fortified milk samples. The LODs for five AMGs (streptomycin, gentamicin, neomycin, kanamycin, spectinomycin) were determined in fortified milk samples and compared with MRLs established for AMGs in milk by the Commission Regulation (EU) No. 37/2010.

MATERIAL AND METHODS

Chemicals and reagents. The antibiotic standards neomycin (Sigma N 6386), streptomycin (Sigma S 9137), gentamicin (Sigma G 46305), kanamycin (Sigma K 1876-1G) and spectinomycin (Sigma S 85555-5G) were purchased from Sigma-Aldrich (Lyon, France). Delvotest® SP-NT kits were supplied by DSM (DSM Food Specialities, Delft, The Netherlands). Kalidos TB, Kalidos MP and Total Antibiotics were obtained from Euroclone (Euroclone S.p.A., Pero, Italy). *Bacillus subtilis* BGA spores were obtained from Merck (Darmstadt, Germany). Antibiotic medium No. 11 was from Difco (New Jersey, USA).

Preparation of milk samples. Stock solutions of AMG antibiotics were prepared by dissolving 10 mg of standards in 10 ml of sterile distilled water to a concentration of 1000 μg/ml. Working solutions of AMGs were prepared by diluting the stock solutions of AMGs in milk to the required concentrations. The concentrations used are presented in Table 1. Milk samples were collected from a farm in Eastern Slovakia and were frozen and stored at –18°C until analysis. Antibiotic-free milk samples were used as a "negative" control. Frozen milk samples were thawed in a water bath at 45°C, shaken well, and analysed according to procedures set by the official methods or producer's instructions (STAR, Delvotest® SP-NT, Total Antibiotics, Kalidos TB, and Kalidos MP).

Table 1. The tested concentrations of AMG standards in milk used for the detection of LODs of MITs (STAR, Delvotest®SP-NT, Kalidos TB, MP and total antibiotics)

Antimicrobials	Concentrations (μg/l)
Gentamicin	1.00, 0.50, 0.20, 0.10, 0.05, 0.01
Neomycin	1.00, 0.50, 0.20, 0.10, 0.05, 0.01
Streptomycin	1.00, 0.50, 0.20, 0.10, 0.05, 0.01
Kanamycin	2.00, 1.50, 1.00, 0.50, 0.20, 0.10, 0.05
Spectinomycin	2.00, 1.50, 1.00, 0.20, 0.10, 0.05

Test systems and interpretation of results. The STAR method comprises five test plates, each of them is preferentially sensitive to one or two families of antibiotics. *Bacillus subtilis* BGA was used as a test strain for the detection of AMGs. Sterile paper disks (Whatman No. 1, diameter 9 mm) were soaked in fortified milk samples and placed onto the agar surface. The plates were incubated at 30°C for 18 hours. In the presence of AMGs a growth inhibition zone was produced around the paper disks. The inhibition zones were measured from the edge of the paper disks to the outer edge of the inhibition zones to the nearest 0.1 mm using a vernier calliper and the mean diameter of the inhibition zones was expressed as arithmetic mean ($\bar{x}$) ± standard deviation (SD). To check the sensitivity of the test strain *Bacillus subtilis* BGA of the STAR to AMGs, a control solution of streptomycin was used at the concentration of 2.00 μg/ml. The positive control paper disk should present an inhibition zone of 4.5 ± 1.5 mm, set by the method.

Delvotest® SP-NT, Kalidos TB, Kalidos MP and Total Antibiotics are the tube tests employing the test strain *Bacillus stearothermophilus* var. *calidolactis*. Fortified milk samples (100 μl/Delvotest® SP-NT, 50 μl/Kalidos MP and Total Antibiotics, 25 μl/Kalidos TB) were transferred to agar test ampoules. The ampoules were sealed using a plastic film supplied with the test kit and were incubated (at 64°C ± 0.5°C/Delvotest® SP-NT, at 65°C ± 0.5°C/Kalidos TB, MP and Total Antibiotics) in a thermostatic block for 2.5–3 hours. If no antimicrobial substances were present in the milk or their concentration was lower than the LOD of the method, the media colour in the tubes turned from purple to yellow. If antimicrobial substances were present in the milk samples or their concentration was at or above the LOD of the method, the media colour remained unchanged (purple). Antibiotic-free milk was used as a negative control. Each spiked sample was tested in eight replicates.

Statistical analysis. The means of LODs of AMG residues in fortified milk samples detected by MITs were analysed by GraphPad Prism version 4.00 (2003). Results are expressed as $\bar{x}$ ± SD and were calculated from eight measurements. The mean LODs of MITs for AMGs in fortified milk samples in each row were compared with each other by one-way ANOVA test. Tukey's multiple comparison test was used to determine significance of differences between the values at a level of P < 0.05 (GraphPadPrism 2003).

RESULTS AND DISCUSSION

STAR is the five plate test that was developed by the EU Community Reference Laboratory in Fougerés (France). The mean diameters of inhibition zones (mm ± SD) produced by fortified milk samples on the plates seeded with *Bacillus subtilis* BGA are presented in Table 2. The mean diameters of inhibition zones were dependent on the concentration of AMGs in the milk samples and sensitivity of the respective bacterial strain. The highest sensitivity of *Bacillus subtilis* BGA was detected for gentamicin (LOD 0.05 μg/ml). The mean of inhibition zone was 0.43 ± 0.10 mm. LODs for neomycin and kanamycin were 0.10 μg/ml and the mean inhibition zone was 1.10 ± 0.05 mm for neomycin and 1.53 ± 0.19 mm for kanamycin. LOD for streptomycin was 0.20 μg/ml and the mean inhibition zone was 1.35 ± 0.27 mm. The lowest sensitivity of *Bacillus subtilis* BGA was detected for spectinomycin (LOD 1.50 μg/ml with the mean inhibition zone of 0.45 ± 0.27 mm). Spectinomycin was not detected at the level of MRL. The inhibition zones produced with the positive control disks complied with the limit value set by the STAR.

The first version of Delvotest® SP-NT to be developed in 1970 was the Delvotest P, designed to detect β-lactams. Later on the Delvotest SP was capable of detecting a wider spectrum of substances, notably sulphonamides, but it also achieved increased sensitivity to tylosin, erythromycin, neomycin, gentamicin and other antimicrobials (Kantiani *et al.* 2009). The currently used version is Delvotest® SP-NT. All versions of Delvotest were produced by DSM (DSM Food Specialities, Delft, The Netherlands). The detectable concentrations of AMG residues in fortified milk samples by using Delvotest® SP-NT are shown in Table 3. Delvotest® SP-NT had a different sensitivity to selected AMG antibiotics. LOD for gentamicin was 0.10 μg/ml, 0.20 μg/ml for neomycin, 0.50 μg/ml for streptomycin, 2.00 μg/ml for kanamycin, and 1.00 μg/ml for spectinomycin. Only gentamicin and neomycin were reliably detected at the level of MRL.

Total antibiotics is produced by Euroclone (Pero, Italy) for the detection of antimicrobials in meat and milk. The detectable concentrations of AMG residues in fortified milk samples by using Total Antibiotics are shown in Table 3. The sensitivity of this test is comparable with Delvotest® SP-NT and Kalidos MP. The LODs for gentamicin and neomycin were 0.10 μg/ml, the lower sensitivity

Table 2. STAR – the mean diameters (mm ± SD) of inhibition zones, range of inhibition zones (min–max) produced by AMGs in fortified milk samples (n = 8)

AMGs	Concentrations (μg/ml)	$\overline{x}$ (mm) ± SD	Range of the zone (mm) min–max
Neomycin	1.00	3.75 ± 0.05	3.67–3.80
	0.50	3.26 ± 0.18	3.00–3.59
	0.20	2.25 ± 0.12	2.13–2.44
	0.10	1.10 ± 0.05	1.03–1.20
	0.05	–	–
Gentamicin	1.00	4.36 ± 0.44	3.79–4.94
	0.50	3.09 ± 0.40	2.68–3.80
	0.20	2.56 ± 0.11	2.34–2.70
	0.10	1.29 ± 0.12	1.20–1.55
	0.05	0.43 ± 0.10	0.32–0.57
	0.01	–	–
Streptomycin	1.00	3.77 ± 0.66	2.60–4.33
	0.50	2.75 ± 0.27	2.42–3.10
	0.20	1.35 ± 0.27	0.99–1.96
	0.10	–	–
Kanamycin	1.00	4.10 ± 0.40	3.76–4.60
	0.50	2.96 ± 0.19	2.85–2.96
	0.20	2.31 ± 0.18	2.12–2.66
	0.10	1.53 ± 0.19	1.18–1.72
	0.05	–	–
Spectinomycin	2.00	1.26 ± 0.20	1.00–1.65
	1.50	0.45 ± 0.27	0.16–0.99
	1.00	–	–

was for streptomycin (LOD 0.50 μg/ml), spectinomycin (LOD 1.50 μg/ml), and kanamycin (LOD 2.00 μg/ml). Only gentamicin and neomycin were detected at the levels of MRLs.

Kalidos TB and Kalidos MP are also produced by Euroclone S.pA. (Pero, Italy). Both these tests are designed for the detection of antimicrobials in milk. The detectable concentrations of AMG residues in fortified milk samples by using Kalidos TB and Kalidos MP are shown in Table 3, resp. Kalidos TB was the most sensitive for gentamicin (LOD 0.01 μg/ml), neomycin (LOD 0.05 μg/ml), and streptomycin (LOD 0.20 μg/ml). Kanamycin (LOD 0.50 μg/ml) and spectinomycin (LOD 1.00 μg/ml) were not detected at the levels of MRL.

Lower sensitivity for AMG residues in fortified milk samples was detected by using Kalidos MP. Detection sensitivity of this test is at the level of Delvotest®SP-NT and Total Antibiotics. LOD for gentamicin was 0.10 μg/ml, 0.20 μg/ml for neomycin, 1.00 μg/ml for streptomycin, 1.50 μg/ml for spectinomycin and 2.00 μg/ml for kanamycin.

A comparison of the mean LODs of the MITs (STAR, Delvotest® SP-NT, Total Antibiotics, Kalidos TB, and Kalidos MP) for AMG residues in fortified milk is presented in Table 4. The sensitivity of MITs to gentamicin in fortified milk samples was different. LODs determined by STAR and Kalidos TB were comparable ($P > 0.05$) and these tests were the most sensitive for the detection of gentamicin. Detection sensitivity ($P > 0.05$) of Delvotest® SP-NT, Total Antibiotics, and Kalidos MP was the same but in comparison with STAR and Kalidos TB, the difference was significant ($P < 0.05$).

LODs for neomycin obtained by MITs were comparable at about the same level. STAR and Kalidos TB were the most sensitive again. Higher LODs were detected by Delvotest® SP-NT and total antibiotics and sensitivity of these tests was lower in comparison with STAR, Kalidos TB, Delvotest®

Table 3. Delvotest® SP-NT, Total Antibiotics, Kalidos TB, and Kalidos MP – detectable concentrations (μg/ml) of AMG antibiotics in fortified milk samples

AMG	0.01	0.05	0.10	0.20	0.50	1.00	1.50	2.00
Delvotest ®SP-NT								
Gentamicin	–	–	±	+	+	+	+	+
Streptomycin	–	–	–	–	±	+	+	+
Neomycin	–	–	–	±	+	+	+	+
Kanamycin	–	–	–	–	–	–	–	±
Spectinomycin	–	–	–	–	–	±	+	+
Total Antibiotic								
Gentamicin	–	–	±	+	+	+	+	+
Streptomycin	–	–	–	–	±	+	+	+
Neomycin	–	–	±	+	+	+	+	+
Kanamycin	–	–	–	–	–	–	–	±
Spectinomycin	–	–	–	–	–	–	±	+
Kalidos TB								
Gentamicin	±	+	+	+	+	+	+	+
Streptomycin	–	–	–	±	+	+	+	+
Neomycin	–	±	+	+	+	+	+	+
Kanamycin	–	–	–	–	±	+	+	+
Spectinomycin	–	–	–	–	–	±	±	+
Kalidos MP								
Gentamicin	–	–	±	+	+	+	+	+
Streptomycin	–	–	–	–	–	±	+	+
Neomycin	–	–	–	±	+	+	+	+
Kanamycin	–	–	–	–	–	–	–	±
Spectinomycin	–	–	–	–	–	–	±	+

+ positive milk samples, – negative milk samples, ± dubious milk samples

SP-NT, and Total Antibiotics but the difference was insignificant ($P > 0.05$). The lowest sensitivity for neomycin detection was determined by Kalidos MP and it differed significantly ($P < 0.05$) from that of STAR and Kalidos TB.

The highest sensitivity to streptomycin in fortified milk samples was found by STAR and Kalidos TB ($P > 0.05$) and it was significantly lower in Delvotest® SP-NT, Total Antibiotics, and Kalidos MP ($P < 0.05$). The highest LOD of streptomycin was detected by Kalidos MP and it differed significantly ($P < 0.05$) from Total Antibiotics.

Kanamycin was reliably detected at the level of MRL only by STAR while the sensitivity of Delvotest® SP-NT, Total Antibiotics, Kalidos TB, and Kalidos MP was lower ($P < 0.05$). Kalidos MP was the least sensitive to kanamycin similarly like Delvotest® SP-NT, but they were significantly different from the other tested MITs ($P < 0.05$).

Spectinomycin was not detected at the levels of MRL in any tested MITs. The most sensitive were Kalidos TB and Delvotest® SP-NT ($P > 0.05$). The LODs of STAR, Total Antibiotics, and Kalidos MP were higher and these tests had the same sensitivity ($P > 0.05$) to AMGs. STAR had the lowest sensitivity, which differed significantly from that obtained by Kalidos TB and Delvotest® SP-NT ($P < 0.05$).

MITs play an important role in the integrated system for the detection of antimicrobial residues in food of animal origin. They have a wide detection spectrum, are simple to carry out, are not costly and can be used for the screening of a large number of samples (Mitchel *et al.* 1998). However, these methods have their drawbacks that limit their use: they do not enable specific antibiotic identification, have limited levels for series of antibiotics and are only qualitative. Many studies also proved that natural antimicrobial agents, if present in milk in

Table 4. Comparisons of the mean LODs (μg/ml) determined by MITs (STAR, Delvotest® SP-NT, Total Antibiotics, Kalidos TB, and Kalidos MP) for AMG residues in fortified milk (One-way ANOVA, Tukey's comparison test) (n = 8)

Aminoglycosides	STAR	Delvotest® SP-NT	Total Antibiotics	Kalidos TB	Kalidos MP
Gentamicin	0.06 ± 0.02^{b}	0.12 ± 0.04^{a}	0.12 ± 0.04^{a}	0.04 ± 0.01^{b}	0.12 ± 0.04^{a}
Neomycin	0.11 ± 0.04^{b}	0.16 ± 0.05^{ab}	0.15 ± 0.05^{ab}	0.06 ± 0.02^{b}	0.25 ± 0.12^{a}
Streptomycin	0.20 ± 0.00^{c}	0.75 ± 0.27^{ab}	0.66 ± 0.25^{b}	0.30 ± 0.15^{c}	1.08 ± 0.20^{a}
Kanamycin	0.11 ± 0.04^{d}	1.83 ± 0.25^{ab}	1.50 ± 0.31^{a}	0.66 ± 0.25^{c}	1.91 ± 0.20^{b}
Spectinomycin	1.66 ± 0.25^{b}	1.08 ± 0.20^{a}	1.33 ± 0.25^{ab}	1.00 ± 0.00^{a}	1.33 ± 0.25^{ab}

Values with different superscripts in a row differ significantly (P < 0.05)

higher concentrations, can bring false positive results (Carlsson *et al.* 1989; Navrátilová 2008). Several authors have related false positive results with the presence of natural inhibitors, somatic cell counts, microorganisms or free fatty acids (Andrew *et al.* 1997; Kang & Kondo 2001). Some naturally occurring compounds, such as lactoperoxidase system, lactoferrin or lysozyme, could have a significant negative effect on the growth of bacterial indicators used in microbiological assays reflected in false positive results, mainly due to their antimicrobial activity (Yamaki *et al.* 2006).

The STAR was validated for antibiotics in milk by Gaudin *et al.* (2004). They found out that *Bacillus subtilis* BGA showed sensitivity to AMGs regarding the MRL, but not to all substances from the AMG group. Althaus *et al.* (2009) determined the antimicrobial substances including AMGs in milk by using the test strain of the six-plate test. The obtained results demonstrated the sensitivity of *Bacillus subtilis* BGA to AMGs around the MRL, while Pikkemaat *et al.* (2009) compared the sensitivity of the test strain *Bacillus subtilis* BGA of the STAR method and the Nouws antibiotic test (NAT-screening) to AMGs. The use of Plate Count Agar adjusted to different pH values and changes in sample preparation and sample procedure increased the sensitivity of the test strain of NAT-screening to AMGs in comparison with the STAR and showed a higher number of suspect samples.

Althaus *et al.* (2003) also reported lower coefficient values for AMGs, macrolides and tetracyclines compared to those obtained for β-lactam antibiotics. This demonstrates the low sensitivity of *Bacillus stearothermophilus* var. *calidolactis* in detecting residues of these antimicrobial agents compared with the beta-lactam antibiotics. Le Breton *et al.* (2007) detected ten different antibiotics by Delvotest® SP-NT and Copan Milk Test. Both tests were found to detect β-lactam and sulphonamides at or below the MRLs. Other groups of antibiotics (tetracyclines and aminoglycosides) were detected with low sensitivity. The authors reported better sensitivity for Delvotest® SP-NT when working at the control time (the time when the blank sample turns from purple to yellow).

In spite of the fact that the MITs are designed for a group-specific identification of antibiotics, our results allowed us to conclude that the STAR and Kalidos TB should be used in combination for the wide-spectrum detection of AMGs in milk to ensure the health safety of milk. Of all MITs tested in the present study the STAR method with the test strain *Bacillus subtilis* BGA was the most sensitive to AMGs. The LODs of STAR for AMGs were reliably detected at or below the MRL, with the exception of spectinomycin, which was detected above the MRL. The sensitivity of Delvotest® SP-NT, Total Antibiotics, and Kalidos MP was comparable. Only gentamicin and neomycin were reliably detected at the level of MRL. Kalidos TB was more sensitive than Delvotest® SP-NT, Total Antibiotics, and Kalidos MP. The LODs of Kalidos TB were at the levels of MRL for gentamicin, neomycin and streptomycin. The LODs of commercially produced MITs for AMGs obtained in our study were comparable with LODs determined by the producers.

To obtain relevant results the positive samples should be confirmed and quantified by a relevant confirmatory method.

References

Althaus R.L., Torres A., Montero A., Balash S., Molina M.P. (2003): Detection limits of antimicrobials in ewe milk by Delvotest-photometric measurements. Journal of Diary Science, **86**: 457–463.

Althaus R.L., Berruga M.I., Montero A., Roca M., Molina M.P. (2009): Evaluation of microbiological multi-residues system on the detection of antibacterial substances in ewe milk. Analytica Chimica Acta, **623**: 156–162.

Andrew S.M., Frobish M.J., Paape M.J., Maturin L.J. (1997): Evaluation of selected antibiotic residue screening tests for milk from individual cows and examination of factor that affect the probability of false-positive outcomes. Journal of Dairy Science, **80**: 3050–3057.

Carlsson A., Bjorck L., Persson K. (1989): Lactoferrin and lysozyme in milk during acute mastitis and their inhibitory effect in Delvotest P. Journal of Dairy Science, **72**: 3166–3175.

CH 12.17 (2006): Detection of residues of inhibitory substances in milk and milk products by DELVOTEST®SP-NT. List of official methods for laboratory diagnostic of food and feed. Bulletin of Ministry of Agriculture of SR, **38**: 60–63.

CH 12.19 (2006): Screening test for determination of antibiotic residues using bacterial strain (STAR). List of official methods for laboratory diagnostic of food and feed. Bulletin of Ministry of Agriculture of SR, **38**: 68–81.

CH 12.21 (2009): Detection of residues of inhibitory substances in milk by Kalidos TB, MP. List of official methods for laboratory diagnostic of food and feed. Bulletin of Ministry of Agriculture of SR, **41**: 6–10.

Commission Decision 2002/657/ES of 12 august 2002 implementing Council Directive 96/23/ES concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities, **L 221**: 8–36.

Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of the European Union, **L 15**: 1–72.

Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. Official Journal of the European Union, **L 125**, 10–36.

Gaudin V., Maris P., Fuselier R., Ribouchon J.L., Rault A. (2004): Validation of microbiological method: the STAR protocol, a five-plate test for the screening of antibiotic residues in milk. Food Additives and Contaminants, **21**: 422–433.

GraphPad Prism version 4.00 for Windows (2003): GraphPad Software, San Diego, USA.

Kang J.H., Kondo F. (2001): Occurrence of false-positive results of inhibitor on milk samples using the Delvotest SP assay. Journal of Food Protection, **64**: 1211–1215.

Kantiani L., Farré M., Barceló D. (2009): Analytical methodologies for the detection of beta-lactam antibiotics in milk and feed samples. Trends in Analytical Chemistry, **28**: 729–744.

Le Breton M.H., Savoy-Perroud M.C., Diserens J.M. (2007): Validation and comparison of the Copan Milk Test and Delvotest SP-NT for the detection of antimicrobials in milk. Analytica Chimica Acta, **586**: 280–283.

Linge B., Gonzalo C., Carriedo J.A., Asensio J.A., Blanco M.A., De La Fuente L.F., San Primitivo F. (2007): Performance of blue-yellow screening test for antimicrobial detection in ovine milk. American Dairy Science Association, **90**: 5374–5379.

Mitchel J.M., Griffiths M.W., McEwen S.A., McNab W.B., Yee A.J. (1998): Antimicrobial drug residues in milk and meat: cause, concerns, prevalence, regulation, tests, and test performance. Journal of Food Protection, **61**: 742–756.

Navrátilová P. (2008): Screening methods use for the detection of veterinary drug residues in cow milk – a review. Czech Journal of Food Sciences, **26**: 393–401.

Nouws J., Van Egmond H., Smilders I., Loeffen G., Schouten J., Stegeman H. (1999): A microbiological assay system for assessment of raw milk exceeding EU maximum residue levels. International Dairy Journal, **9**: 85–90.

Pikkemaat M.G., Rapallini M.L.B.A., Oostra-van Dijk S., Elfering J.W.A. (2009): Comparison of three microbial screening methods for antibiotics using routine monitoring samples. Analytica Chimica Acta, **637**: 298–304.

Sierra D., Contreras A., Sánchez A., Luengo C., Corrales J.C., Morales C.T., de la Fe C., Guirao I. (2009): Short communication: Detection limit of non β-lactam antibiotic in goat's milk by microbiological residues screening tests. Journal of Dairy Science, **92**: 4200–4206.

Stead S.L., Ashwin H., Richmond S.F., Sharman M., Langeveld P.C., Barendse J.P., Stark J., Keely B.J. (2008): Evaluation and validation according to international standards of the Delvotest®SP-NT screening assay for antimicrobial drugs in milk. International Dairy Journal, **18**: 3–11.

Yamaki M., Berruga M.I., Althaus R.L., Molina M.P., Molina A. (2006): Screening of antibiotic residues in ewe milk destined to cheese by a commercial microbiological inhibition assay. Food Additives and Contaminants, **23**: 660–667.

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