



2011

## Microbial system for identification of antibiotic residues in milk

Follow this and additional works at: <https://www.jfda-online.com/journal>

---

### Recommended Citation

Nagel, O.; Molina, P.; and Althaus, R. (2011) "Microbial system for identification of antibiotic residues in milk," *Journal of Food and Drug Analysis*: Vol. 19 : Iss. 3 , Article 6.  
Available at: <https://doi.org/10.38212/2224-6614.2185>

This Original Article is brought to you for free and open access by Journal of Food and Drug Analysis. It has been accepted for inclusion in Journal of Food and Drug Analysis by an authorized editor of Journal of Food and Drug Analysis.

# Microbial System for Identification of Antibiotic Residues in Milk

ORLANDO NAGEL<sup>1</sup>, PILAR MOLINA<sup>2</sup> AND RAFAEL ALTHAUS<sup>1\*</sup>

<sup>1</sup>. Cátedra de Biofísica, Departamento de Ciencias Básicas, Universidad Nacional del Litoral,  
R.P.L. Kreder 2805. 3080 Esperanza, República Argentina.

<sup>2</sup>. Instituto de Ciencia y Tecnología, Universidad Politécnica de Valencia, Camino de Vera 14, 46071 Valencia, España.

(Received: August 18, 2010; Accepted: June 2, 2011)

## ABSTRACT

The aim of this study was to evaluate the ResScreen<sup>®</sup> microbiological system for the identification of antibiotic residues in milk. This microbiological system consists of two methods, the BT (betalactams and tetracyclines) and BS (betalactams and sulfamides) bioassays, containing spores of *G. stearothermophilus* subsp. *calidolactis*, culture media and indicators (acid-base and redox). The detection limits of 29 antimicrobial agents were calculated using a logistic regression model.

Both methods detect residues of penicillin-G, ampicillin, amoxicillin, cloxacillin, oxacillin, cephalexin, cefoperazone and ceftiofur<sup>®</sup> at levels close to their Maximum Residue Limits (MRL). The BT bioassay also presents good sensitivity to tetracycline and oxytetracycline residues, whereas the BS bioassay detects sulfadiazine, sulfamethoxazole and sulfathiazole residues in milk.

The simultaneous use of both bioassays identifies betalactam, tetracycline and sulfamide residues in milk. Neomycin, tylosin and lincomycin residues can also be detected, but these molecules are positive with the BT and BS bioassays, e.g., betalactams, given the microorganisms' sensitivity to these molecules.

Key words: screening test, microbiological inhibition system, betalactams, tetracyclines, sulfamides, milk

## INTRODUCTION

The presence of certain antibiotic residues in milk is a potential risk for consumers because they may be toxic and dangerous for human health, and may potentially cause antimicrobial resistance<sup>(1,2)</sup> and technological problems during dairy product manufacturing<sup>(3-5)</sup>.

For this purpose, several commercially available tests have been developed for the swift, precise detection of the presence of antibiotic residues in milk<sup>(6,7)</sup>. Many screening tests are based on the inhibition of microorganism growth by the presence of drug residues. Among the most widely used microorganisms, we find *Geobacillus stearothermophilus* subsp. *calidolactis* in the following tests: Delvotest<sup>®</sup><sup>(8)</sup>, BRT<sup>®</sup> AiM<sup>(9)</sup>, Eclipse<sup>®</sup><sup>(10)</sup> and Charm<sup>®</sup> AIM-96<sup>(11)</sup>.

These methods can nonspecifically detect the presence or absence of antibiotic residues in milk. To identify  $\beta$ -lactam or sulfonamide compounds however, positive and doubtful samples are tested using penicillinase and p-aminobenzoic acid (PABA) solutions. Thus, antibiotic residues can be classified into betalactam antibiotics or sulfamides<sup>(12)</sup>.

However, the penicillinase and PABA methods do

not suffice to identify other antimicrobial agents such as tetracyclines. So, when Yamaki *et al.*<sup>(13)</sup> investigated 2686 samples of ewe's milk, 47 samples were found to be positive with the Delvotest SP test. When using penicillinase and PABA methods, only 29.8% of the samples were identified as containing betalactam residues, while the remaining milk samples (70.2%) remained unidentified. These authors suggested that this methodology is insufficient for a complete identification of milk antibiotic residues.

In order to identify a higher number of antibiotic groups, Althaus and Nagel<sup>(14)</sup> proposed to use a microbiological system which not only complies with the International Standardization Organization guidelines<sup>(15)</sup>, but also identifies betalactam, tetracycline and sulfonamide residues.

This microbiological system consists of two methods, the BT (betalactams and tetracyclines) and BS (betalactams and sulfamides) bioassays, containing spores of *G. stearothermophilus* subsp. *calidolactis*, culture media and indicators (acid-base and redox). Moreover, this system includes synergistic components that improve the sensitivity of tetracycline<sup>(16)</sup> and sulfamide<sup>(17)</sup> residues in milk.

Thus, the objective of this research was to evaluate the ResScreen<sup>®</sup> system for the identification of antimicrobial agent residues in milk by means of studying detection limits.

\* Author for correspondence. Tel: + 00-54-3493420639 ext. 131;  
E-mail: ralthaus@fcv.unl.edu.ar

## MATERIALS AND METHODS

### I. Animals and Milk Samples

The animals came from cattle herds of Las Colonias (Santa Fe, Argentina). For this study, milk samples corresponding to the morning machine milking session (6 am) of 16 cows were collected in the 60 - 90 day postpartum period. The animals received no pharmacological treatment throughout the sampling period<sup>(18)</sup>.

The chemical composition and pH values of the selected samples were normal for bovine milk, with low somatic cell counts (SCC < 400,000 cells/mL) and an acceptable bacterial count for cow's milk (CFU < 100,000 cfu/mL).

### II. Antimicrobial Solutions and Spiked Samples

The drugs used for the preparation of antimicrobial solutions were stored and handled according to the manufacturers' instructions before use. All the dilutions were prepared in 100 mL volumetric flasks at the time the analyses were carried out in order to avoid the possibility of unstable solutions.

Antimicrobial solutions were prepared using antimicrobial-free milk<sup>(18)</sup>, as determined by the Delvotest<sup>®</sup>. The final drug concentrations in milk ( $\mu\text{g/L}$ ) were achieved after serial dilutions so that the volume of the antimicrobial agent solution did not exceed 1% of the volume of the final solution to be analyzed<sup>(18)</sup>.

### III. ResScreen<sup>®</sup> Test

The system consists of two microbial bioassays using *Geobacillus stearothermophilus* subsp. *calidolactis* C-953 spores. The microbiological method is based on growth inhibition of bacteria-test when milk containing residues of antibiotics.

The BT bioassay (Betalactams and Tetracyclines) is composed of a culture medium containing spores of thermophilic microorganism, chloramphenicol and bromocresol purple indicator<sup>(16)</sup>. If the milk sample is free of antibiotics and allows bacteria-test growth and changes in color of the acid base indicator (purple to yellow). Otherwise the test will remain the same color.

Moreover, the BS bioassay (Beta-lactams and Sulfonamides) use a medium inoculated with a microorganism spore suspension, brilliant black indicator, toluidine blue and trimethoprim<sup>(17)</sup>. So, the absence of antibiotic residues in milk causes bacteria-test growth, producing a color change of indicators from black to amber.

The ResScreen<sup>®</sup> system was carried out according to the manufacturer's instructions. Thus, 50  $\mu\text{L}$  milk sample was added to individual plates of the BT and BS ResScreen<sup>®</sup> methods. Plates were incubated in a water bath at  $64 \pm 1^\circ\text{C}$  for 3 (BT ResScreen<sup>®</sup>) and 4 h (BS ResScreen<sup>®</sup>) until the color change of the negative samples had taken place.

Visual interpretation was performed independently by 3 trained persons, and was assessed visually as negative and positive; doubtful qualifications were interpreted as positive<sup>(19)</sup>.

### IV. Detection Limits and Cross Specificity

#### (I) Detection limits

The following substances (Sigma Chemical Co, St. Louis, MO) were used to determine the ResScreen<sup>®</sup> system detection limits:

1. Ten betalactams: amoxicillin, ampicillin, cloxacillin, oxacillin, penicillin-G, cefadroxil, cephalexin, cefoperazone, cefuroxime and ceftiofur<sup>®</sup>.
2. Four sulfonamides: sulfadiazine, sulfadimethoxine, sulfamethoxazole and sulfathiazole.
3. Three tetracyclines: chlortetracycline, oxytetracycline and tetracycline.

The detection limits of the antimicrobial agents were established according to the Codex Alimentarius guidelines<sup>(18)</sup>. For this purpose, 12 concentrations were prepared with different levels of each drug. For each concentration, 16 replicates were prepared using antibiotic-free milk samples.

#### (II) Cross specificity

The Codex Alimentarius guidelines<sup>(18)</sup> were used to calculate the detection limits of the following antibiotics (Sigma Chemical Co, St. Louis, MO):

1. Four aminoglycosides: gentamycin, kanamycin, neomycin and streptomycin.
2. Four macrolides: erythromycin, lincomycin, tylosin and spiramycin.
3. Four quinolones: ciprofloxacin, norfloxacin, enrofloxacin and marbofloxacin.

### V. Statistical Analysis

The results were obtained by following the SAS<sup>®</sup> Logistic procedure<sup>(20)</sup>. A logistic regression model was also done to calculate the detection limits, as follows:

$$L_{ij} = \text{logit} [P_{ij}] = \beta_0 + \beta_1 [A]_i + \varepsilon_{ij}$$

where:  $L_{ij}$  = lineal logistic model;  $[P_{ij}]$  =  $\text{logit} [P_p / (1-P_p)]$ : the probability of positive response / probability of negative response);  $\beta_0$ ,  $\beta_1$  = coefficients estimated for the logistic regression models;  $[A]_i$  = antimicrobial concentration.  $\varepsilon_{ij}$  = residual error. The concordance coefficient<sup>(20)</sup> was applied as the rank correlation between the observed responses and the predicted probabilities.

The detection limit of the visual interpretation of the ResScreen<sup>®</sup> system was estimated as the concentrations at which 95% of the results were assessed as positive or doubtful<sup>(19,21)</sup>.

## RESULTS AND DISCUSSION

### I. Detection Limits

The results of applying the logistic regression model to the positive relative frequency of the BT and BS ResScreen<sup>®</sup> system for the different antimicrobial agents assayed are shown in Table 1.

The concordance coefficients obtained by applying the logistic model were high, between 89.2% for oxytetracycline (BT ResScreen<sup>®</sup>) and 99.4% for tetracycline (BS ResScreen<sup>®</sup>), demonstrating the correct adjustment achieved by the logistic model.

The  $\beta_1$  coefficient represents the sensitivity of *G. stearothermophilus* to the antibiotics studied. This parameter reached higher values for penicillin antibiotics (amoxicillin, ampicillin, cloxacillin, oxacillin and penicillin-G) than for the rest of the antimicrobial agents assayed, demonstrating the sensitivity of *G. stearothermophilus* to detect the residues of these antimicrobials.

The  $\beta_1$  coefficients values of cephalosporins (cefadroxil, cephalixin, cefoperazone, ceftiofur<sup>®</sup> and cefuroxime) were similar to those calculated for tetracyclines (BT ResScreen<sup>®</sup>) and sulfamides (BS ResScreen<sup>®</sup>). In contrast, the  $\beta_1$  parameter of tetracyclines (BS ResScreen<sup>®</sup>) and sulfonamides (BT ResScreen<sup>®</sup>) were very low, showing low sensitivity for detection purposes.

Figures 1 and 2 show the effect of penicillin and cephalosporin concentrations on the visual interpretations of the ResScreen<sup>®</sup> system, as well as the curves constructed by the logistic model ( $\beta_0$  and  $\beta_1$  coefficients, Table 1). The concentrations of ampicillin, amoxicillin, oxacillin and penicillin-G (high  $\beta_1$  coefficient values) underwent a slight increase to produce 100 % positive results, whereas the concentrations of cephalosporins (Figure 2) had to undergo greater increments to obtain positive results in both methods (lower  $\beta_1$  coefficient values).

The dose-response curves for tetracyclines (BT ResScreen<sup>®</sup>, Figure 3) and sulfonamides (BS ResScreen<sup>®</sup>, Figure 4) showed adequate sensitivity to detect the residues belonging to both antibiotic groups. Conversely, high concentrations of tetracyclines (BS ResScreen<sup>®</sup>, Figure 3) and sulfonamides (BT ResScreen<sup>®</sup>, Figure 4) were needed given the low  $\beta_1$  coefficients values (Table 1).

The detection limits of the ResScreen<sup>®</sup> system calculated by means of logistic regression models for betalactams, tetracyclines and sulfonamides are shown in Table 2.

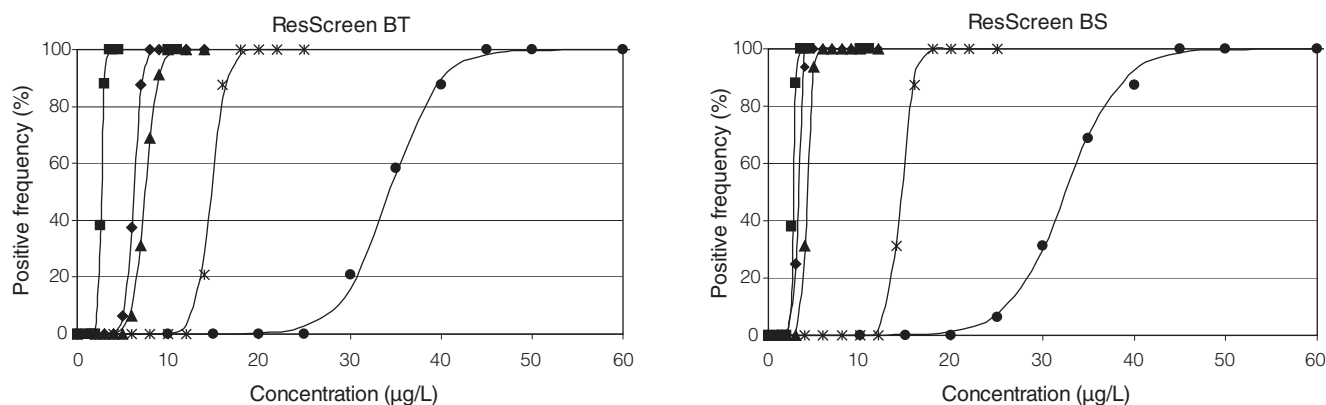
Amoxicillin, ampicillin, cloxacillin, oxacillin, penicillin-G, cephalixin, cefoperazone and ceftiofur<sup>®</sup> showed similar detection limits (Table 2) for the ResScreen<sup>®</sup> system to their respective Maximum Residue Limits (MRLs).

For betalactam antibiotics, other microbiological methods such as BRT<sup>®</sup> AiM<sup>(22,23)</sup>, Charm<sup>®(24)</sup>, Delvotest<sup>®</sup> SP<sup>(22,25)</sup>, Eclipse<sup>®</sup> 100ov<sup>(26)</sup> have similar detection limits to

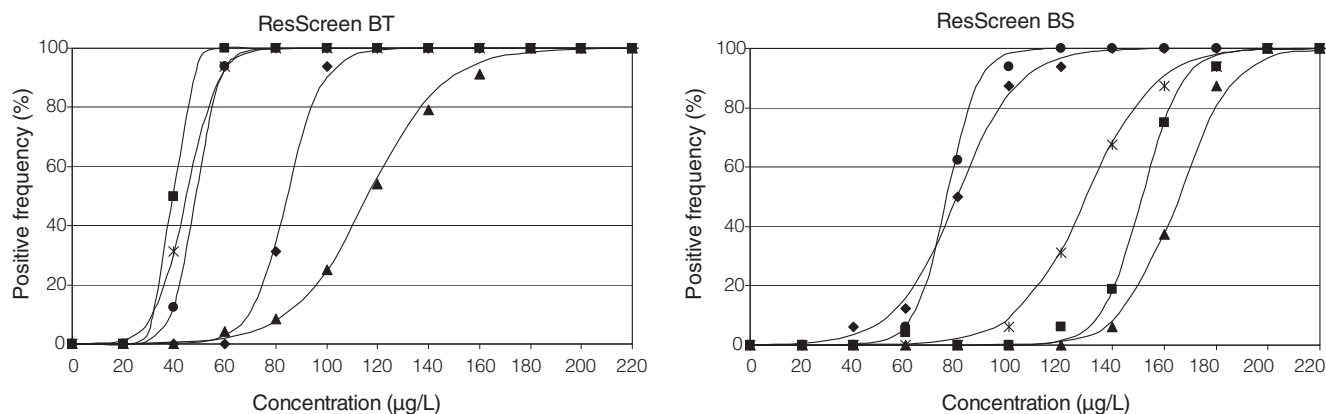
**Table 1.** Summary of the logistic regression model parameters of antibiotics in milk for the ResScreen<sup>®</sup> system

Antibiotics	ResScreen <sup>®</sup> BT	C	ResScreen <sup>®</sup> BS	C
	Logit = $\beta_0 + \beta_1*[A]$		Logit = $\beta_0 + \beta_1*[A]$	
<i>Betalactams</i>				
Amoxicillin	Logit = -11.3966 + 1.5185*[A]	96.7	Logit = -15.8159 + 3.7160*[A]	97.7
Ampicillin	Logit = -14.7862 + 2.3659*[A]	98.5	Logit = -21.6358 + 6.8009*[A]	99.1
Cloxacillin	Logit = -13.1755 + 0.3835*[A]	97.9	Logit = -10.9673 + 0.3371*[A]	97.5
Oxacillin	Logit = -18.4151 + 1.2483*[A]	98.6	Logit = -22.3155 + 1.5409*[A]	98.9
Penicillin G	Logit = -16.1514 + 6.1636*[A]	98.9	Logit = -22.5024 + 8.1827*[A]	99.4
Cefadroxil	Logit = -7.9435 + 0.0683*[A]	95.7	Logit = -16.0260 + 0.0970*[A]	97.5
Cephalixin	Logit = -10.0512 + 0.1313*[A]	97.4	Logit = -9.9664 + 0.0767*[A]	97.0
Cefoperazone	Logit = -11.0985 + 0.2277*[A]	98.3	Logit = -12.6755 + 0.1669*[A]	97.9
Ceftiofur <sup>®</sup>	Logit = -12.1451 + 0.1438*[A]	98.8	Logit = -6.7069 + 0.0841*[A]	94.3
Cefuroxime	Logit = -13.07 + 0.3282*[A]	98.9	Logit = -20.0044 + 0.1321*[A]	99.6
<i>Tetracyclines</i>				
Clortetracycline	Logit = -9.4066 + 0.0556*[A]	90.7	Logit = -10.1408 + 0.0036*[A]	97.7
Oxytetracycline	Logit = -10.8242 + 0.0933*[A]	89.2	Logit = -9.9616 + 0.0153*[A]	97.0
Tetracycline	Logit = -9.0156 + 0.0627*[A]	89.8	Logit = -26.5938 + 0.0309*[A]	99.4
<i>Sulfonamides</i>				
Sulfadiazine	Logit = -8.2241 + 0.0002*[A]	95.0	Logit = -22.089 + 0.1525*[A]	91.0
Sulfadimethoxine	Logit = -18.8281 + 0.0018*[A]	98.7	Logit = -11.9029 + 0.0577*[A]	90.0
Sulfamethoxazole	Logit = -16.7196 + 0.0015*[A]	97.9	Logit = -11.0868 + 0.1167*[A]	89.3
Sulfathiazole	Logit = -20.2747 + 0.0017*[A]	98.6	Logit = -9.0399 + 0.1246*[A]	89.3

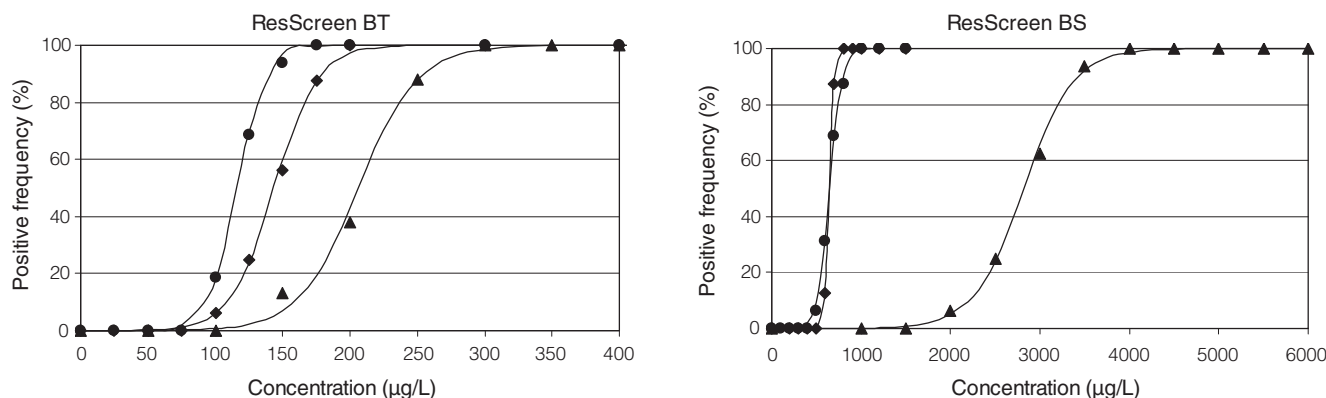
$\beta_0$ ,  $\beta_1$  = coefficients estimated for the logistic regression models; [A]: antimicrobial concentrations; C: concordance coefficients.



**Figure 1.** Dose-response curves for different penicillin concentrations in milk analyzed by the Rescreen<sup>®</sup> system (▲: amoxicillin, ◆: ampicillin, ●: cloxacillin, X: oxacilina, ■: penicillin-G).



**Figure 2.** Dose-response curves for different cephalosporin concentrations in milk analyzed by the Rescreen<sup>®</sup> system (▲: cefadroxil, X: cephalixin, ●: cefoperazone, ◆: ceftiofur<sup>®</sup>, ■: cefuroxime).



**Figure 3.** Dose-response curves for different tetracycline concentrations in milk analyzed by the Rescreen<sup>®</sup> system (▲: chlortetracycline, ●: oxytetracycline, ◆: tetracycline).

the ResScreen<sup>®</sup> system.

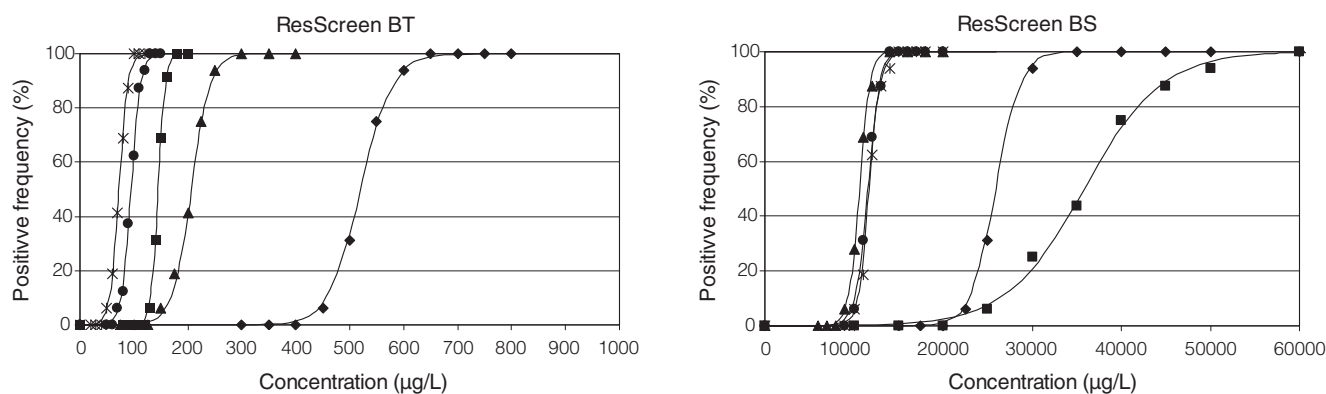
With regard to tetracyclines, Table 2 indicates how the BT ResScreen<sup>®</sup> method presented detection limits near at the MRLs, unlike the BS method which required higher concentrations of these antibiotics for them to be detected.

The detection limits calculated for the three tetracyclines with the BT ResScreen<sup>®</sup> method were lower than those reported by other authors with the BRT<sup>®</sup> AiM<sup>(22,23)</sup>, Charm<sup>®</sup>

AIM-96<sup>(24)</sup>, Delvotest<sup>®</sup> SP<sup>(22,25)</sup> and Eclipse<sup>®</sup> 100ov<sup>(26)</sup> methods due to improved sensitivity from adding chloramphenicol to the culture medium<sup>(16)</sup>.

Also, Table 2 indicates how the detection limits of sulfonamides for BS ResScreen<sup>®</sup> approached their MRLs, while the BT method was not sensitive enough to detect these drugs in milk (detection limits higher than 12,000 µg/L).

The BS ResScreen<sup>®</sup> method detection limits of



**Figure 4.** Dose-response curves for different sulphamide concentrations in milk analyzed by the Rescreen<sup>®</sup> system (■: sulfadiazine, ▲: sulfadimethoxine, ◆: sulfamethazine, ●: sulfamethoxazole, X: sulfathiazole).

**Table 2.** The ResScreen<sup>®</sup> system detection limits (µg/L) for antibiotics in milk

Antibiotics	ResScreen <sup>®</sup>		BRT <sup>®</sup> AiM		Delvotest <sup>®</sup> SP		Charm <sup>®</sup> AIM	Eclipse <sup>®</sup> 100ov	MRLs <sup>a</sup>
	BT	BS	Charm y Ruth (1993) <sup>(22)</sup>	Heeschen <i>et al.</i> (1995) <sup>(23)</sup>	Charm y Ruth (1993) <sup>(22)</sup>	Althaus <i>et al.</i> (2002) <sup>(25)*</sup>	Linage <i>et al.</i> (2007) <sup>(24)*</sup>	Montero <i>et al.</i> (2005) <sup>(26)*</sup>	
<i>Beta-lactams</i>									
Amoxicillin	8	5	5	---	10	5	---	7	4
Ampicillin	7	4	10	5	10	3	6	---	4
Cloxacillin	42	40	100	35	50	23	42	68	30
Oxacillin	17	16	---	---	---	---	---	28	30
Penicillin-G	3	3	10	1.5	2.5	1.4	4	5	4
Cefadroxil	159	190	---	---	---	63	---	86	---
Cephalexin	99	160	---	---	---	68	202	115	100
Cefoperazone	62	94	---	---	---	41	82	110	50
Ceftiofur <sup>®</sup>	105	115	100	---	50	59	107	---	100
Cefuroxime	42	170	---	---	---	41	---	85	---
<i>Tetracyclines</i>									
Clortetracycline	275	3600	>1000	---	420	---	3989	1500	100
Oxytetracycline	150	850	1000	---	200	420	501	560	100
Tetracycline	158	720	1000	450	420	450	257	480	100
<i>Sulfonamides</i>									
Sulfadiazine	49000	164	1000	100-1000	>1000	260	---	---	100
Sulfadimethoxine	12000	260	100	100-1000	>1000	---	119	170	100
Sulfamethoxazole	14000	120	---	---	---	110	---	---	100
Sulfathiazole	13000	100	1000	100-1000	>1000	---	151	250	100

<sup>a</sup> MRLs (µg/L), EU maximum residue limits, \* Decision limits in ewe milk.

sulfonamides were slightly higher than those observed for BRT<sup>®</sup> AiM<sup>(27)</sup>, although other authors have reported higher detection limits for BRT<sup>®</sup> AiM<sup>(22,23)</sup>, Delvotest<sup>®</sup> SP<sup>(22,25)</sup> and Eclipse<sup>®</sup> 100<sup>(26)</sup>.

## II. Cross Specificity

The detection limits calculated by the logistic model for other antimicrobial agents (aminoglycosides, macrolides and quinolones) with the ResScreen<sup>®</sup> system are provided in

Table 3. Of all these antibiotics, only neomycin, lincomycin and tylosin residues were detected by the ResScreen<sup>®</sup> system at levels approaching their MRLs.

Various authors have indicated similar detection limits to those calculated in Table 3 by other methods using *G. steierthermophilus* subsp. *calidolactis*, such as BRT<sup>®</sup> AiM<sup>(22,23)</sup>, Charm<sup>®</sup> AIM-96<sup>(24)</sup> and Delvotest<sup>®</sup> SP<sup>(22,25)</sup> indicating good sensitivity to these three antibiotics (neomycin, lincomycin, tylosin) and a low detection capacity for the rest of antimicrobials.

**Table 3.** The ResScreen<sup>®</sup> system detection limits (µg/L) for other antimicrobials in milk

Other antimicrobials	ResScreen <sup>®</sup>		BRT <sup>®</sup> AIM		Delvotest <sup>®</sup> SP		Charm <sup>®</sup> AIM	Eclipse <sup>®</sup> 1000v	MRLs <sup>a</sup>
	BT	BS	Charm y Ruth (1993) <sup>(22)</sup>	Heeschen <i>et al.</i> (1995) <sup>(23)</sup>	Charm y Ruth (1993) <sup>(22)</sup>	Althaus <i>et al.</i> (2002) <sup>(25)*</sup>	Linage <i>et al.</i> (2007) <sup>(24)*</sup>	Montero <i>et al.</i> (2005) <sup>(26)*</sup>	
<i>Aminoglycosides</i>									
Gentamycin	320	530	>500	---	150	1200	382	3140	100
Kanamycin	5600	6200	---	---	---	---	---	18700	150
Neomycin	600	1200	>500	300	150	3300	1084	9100	1500
Streptomycin	2300	3600	>1000	---	>1000	10000	3593	10100	200
<i>Macrolides</i>									
Erythromycin	210	190	1000	2250	400	980	522	750	40
Lincomycin	150	220	---	---	---	---	---	---	150
Tylosin	74	50	50	---	100	120	51	230	50
Spiramycin	3400	2600	---	---	---	---	1346	18100	200
<i>Quinolones</i>									
Ciprofloxacin	1750	1710	---	---	---	---	---	5100	100
Enrofloxacin	2000	2300	---	---	---	---	46000	4000	100
Marbofloxacin	2700	4400	---	---	---	---	---	---	75
Norfloxacin	7100	6800	---	---	---	---	---	9500	---

<sup>a</sup> MRLs (µg/L), EU maximum residue limits, \* : Decision limits in ewe milk.

### III. Identification of Antibiotic Residues by the ResScreen<sup>®</sup> System

Table 4 summarizes the results of Table 2 and Table 3 by collectively and simply presenting the interpretation of the results of both bioassays.

Milk samples that led to changes in color of both bioassays indicate the absence of antimicrobials (or substances that were not detected by this system). Beta-lactam antibiotics were identified by the persistence of both methods' original colors. The fact that the original color of the BT bioassay remained and the original color of the BS bioassay changed denotes the presence of tetracycline residues. Conversely, milk samples that have sulfamides brought about a change in the color of the BT method but maintained the color of the BS method.

Finally, those milk samples containing neomycin, lincomycin or tylosin residues were detected by the ResScreen<sup>®</sup> system, but were identified as beta-lactams because the BT and BS bioassays were sensitive enough to detect such substances (Table 3). The difficulty owing to the cross specificity of the ResScreen<sup>®</sup> system could be resolved by implementing subsequent tests with penicillinase and cephalosporinase enzymes.

### CONCLUSIONS

To summarize, the ResScreen<sup>®</sup> system uses only two bioassays and provides a simple, economical solution to identify residues in milk. Moreover, this microbiological system identifies a larger number of antibiotic families

**Table 4.** Interpretation of the ResScreen<sup>®</sup> system results

Antibiotics	Results	
	ResScreen <sup>®</sup> BT	ResScreen <sup>®</sup> BS
Absence (or not detect)	-	-
Betalactams	+ <sup>(*)</sup>	+ <sup>(*)</sup>
Tetracyclines	+	-
Sulfamides	-	+

(\*) Interference due to neomycin, lincomycin and tylosin.

(beta-lactams, tetracyclines and sulfamides) compared with current penicillinase and p-aminobenzoic acid methodologies (beta-lactams and sulfamides).

In the future, new bioassays can be incorporated into the ResScreen<sup>®</sup> system in order to increase its identification capacity to other antibiotic groups (macrolides, aminoglycosides or quinolones).

### ACKNOWLEDGMENTS

This research work has been carried out as part of the CAI+D'09/11 Project (No. 033-173 Res.C.D. No. 100/09) and supported by financial assistance from the Universidad Nacional del Litoral (Santa Fe, República Argentina).

### REFERENCES

- Demoly, P. and Romano, A. 2005. Update on beta-lactam allergy diagnosis. *Curr. Allergy Asthma Rep.* 5: 9-14.

2. Wilke, M. S., Andrew, L., Lovering, A. L. and Strynadka, N. C. 2005.  $\beta$ -Lactam antibiotic resistance: a current structural perspective. *Curr. Opin. Microbiol.* 8: 525-533.
3. Packham, W., Broome, M. C., Limsowtin, G. K. and Roginski, R. 2001. Limitations of standard antibiotic screening assays when applied to milk for cheese-making. *Australian. J. Dairy Tech.* 56: 15-18.
4. Berruga, M. I., Molina, M. P., Noves, B., Roman, M. and Molina, A. 2007. *In Vitro* study about the effect of several penicillins during the fermentation of yogurt made from ewe's milk. *Milchwissenschaft* 62: 303-305.
5. Bradley, A. J. and Green, M. D. 2009. Factors affecting cure when treating bovine clinical mastitis with cephalosporin-based intramammary preparations. *J. Dairy Sci.* 92: 1941-1953.
6. Diserens, J., Beck Henzelin, A., Le Breton, M. and Savoy Perroud, M. 2005. Antibiotics in milk: Actual situation & compilation of commercial available screening methods for the detection of inhibitors/antibiotics residues in milk. Informe técnico: Quality & Safety Department, Nestlé Research Center. p.186. Lausanne, Switzerland.
7. Toldra, F. and Reig, M. 2006. Methods for rapid detection of chemical and veterinary drug residues in animal foods. *Trends Food Sci. Technol.* 17: 482-489.
8. Kelly, W. N. 1982. Qualitative ampule and multitest for beta-lactam residues in fluid milk products: collaborative study. *J. AOAC* 65: 1193-1207.
9. Müller, F. and Jones, A. 1993. BR-Test and BRT-AS methods. In *Inhibitory Substances in Milk- Current Analytical Practice*. IDF Bull. No. 283 ed. pp. 24-28. International Dairy Federation. Secretariat General. Brussels, Belgium.
10. Zeu-Inmunotec. 2003. Screening Plus. Informe técnico. Ed. Zeu-Inmunotec S. L. Zaragoza, España.
11. Zomer, E. and Lieu, T. 1995. AIM-96, a broad spectrum antimicrobial drug inhibition monitoring assay: advantages and enhancement of an integrated monitoring system. In *Residues of antimicrobial drugs and other inhibitors in milk*. IDF S. I. No. 9505 ed. pp. 192-194. International Dairy Federation. Secretariat General. Brussels, Belgium.
12. Botsoglou, N. and Fletouris, J. 2001. Drugs residues in foods: Pharmacology, food safety, and analysis. In *Serie Food Science and Technology*. Marcel Dekker, Inc. New York, U.S.A.
13. Yamaki, M., Berruga, M. I., Althaus, R. L., Molina, M. P. and Molina, A. 2004. Occurrence of antibiotic residues in milk from manchega ewe dairy farms. *J. Dairy Sci.* 87: 3132-3137.
14. Althaus, R. L., Nagel, O. N. Bioensayo para la identificación de agentes antimicrobianos en muestras fluidas y método de identificación de dichos agentes antimicrobianos. Argentina. N° de solicitud: p-060104535 del 18 de octubre de 2006.
15. International Standardization Organization. 2006. Milk and milk products - Determination of antimicrobial residues - Tube diffusion test. Reference numbers ISO/TS 26844: 2006 (E) - IDF/RM 215: 2006(E). pp 13. Genève, Switzerland.
16. Nagel, O., Zapata, M. L., Basilico, J. C., Molina, P. and Althaus, R. 2009. Effect of Chloramphenicol on a bioassay response for the detection of tetracycline residues in milk. *J. Food Drug Anal.* 17: 36-42.
17. Nagel, O. G. 2009. Diseño de un sistema microbiológico en microplacas ELISA (SMmp) para la detección e identificación de residuos de antimicrobianos en la leche. Ph.D. Diss. Universidad Nacional del Litoral, Argentina, 12/09/2009, p. 271.
18. Codex Alimentarius. 2010. Codex committee on residues of veterinary drugs in foods. 9th session of the 30 August-3 September 2010. Discussion paper on methods of analysis for residues of veterinary drugs in foods (CX/RVDF 10/19/6). Vermont, U.S.A.
19. Suhren, G., Reichmuth, J. and Walte, H. G. 1996. Detection of  $\beta$ -lactam antibiotics in milk by the Penzym-test. *Milchwissenschaft* 51: 269-273.
20. SAS. 2001. SAS Users guide: statistics version 9.1. Cary: SAS® Institute Inc.
21. International Dairy Federation (IDF). 1999. Guidance for the standardized evaluation of microbial inhibitor test. IDF Standard N° 183. Interanational Dairy Federation, Brussels, Belgium.
22. Charm, S. E. and Ruth, G. P. 1993. Advances in charm technology for antimicrobial residues in milk. In *Inhibitory Substances in Milk-Current Analytical Practice*. IDF Bull. No. 283 ed. pp. 32-46. International Dairy Federation. Secretariat General. Brussels, Belgium.
23. Heeschen, W. H. and Blüthgen, A. 1991. Veterinary drugs and pharmacologically active compounds. In *Residues and contaminants in milk products*. IDF S. I. No. 9101 ed. pp. 16-39. International Dairy Federation. Secretariat General. Brussels, Belgium.
24. Linage, B., Gonzalo, C., Carriedo, J., Asensio, J., Blanco, M., De la Fuente, L. and San Primitivo, F. 2007. Performance of Blue-Yellow Screening test for antimicrobial detection in ovine Milk. *J. Dairy Sci.* 90: 5374-5379.
25. Althaus, R., Peris, C., Montero, A., Torres, A., Molina, M. P. and Fernandez, N. 2002. Detection limits of antimicrobial agents in ewe milk by Delvotest®. *Milchwissenschaft*. 57: 660-664.
26. Montero, A., Althaus, R., Molina, A., Berruga, I. and Molina, M. 2005. Detection of antimicrobial agents by specific microbiological method (Eclipse 100) for ewe milk. *Small Rum. Res.* 57: 229-237.
27. Adriany, A., Märtilbauer, E. and Zaadhof, K. 1995. A modified brilliant black-reduction test (BRT) with improved sensitivity for tetracyclines and sulfonamides. pp. 172-174. In *Proceedings of the IDF-Symposium Residues of antimicrobial drugs and other inhibitors in milk*. pp. 28-31. Kiel, Germany.