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Original Scientific Article

VALIDATION OF TWINSENSOR^{BT}, SCREENING TEST FOR THE DETECTION OF ß-LACTAMS AND TETRACYCLINES IN MILK, AND COMPARISON TO DELVOTEST® SP-NT

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Summary: Antimicrobial drugs have been widely used in dairy industry for more than five decades generally to prevent or treat mastitis. The detection of antibacterial residues in milk requires screening methods that are simple, quick and sensitive at antibiotic concentrations close to the maximum residue limit (MRL). A new competitive receptor test Twinsensor^{BT} was validated and compared with Delvotest®SP-NT an agar diffusion microbiological test. Both tests were designed for screening antimicrobial substances in milk. The performance criteria described by the Commission Decision 2002/657/EC, ISO 13969:2003, ISO 18330:2003 and Guide for analytical validation of screening methods (AFSSA Fougères) were used for the validation study. Validation was made on spiked samples of milk with 12 different ß-lactams (penicillin-G, ampicillin, amoxicillin, cloxacillin, nafcillin, cefapirin, cefalonium, cefazolin, cefoperazone, ceftiofur, cefalexin, cefquinome) and 4 tetracyclines (doxycycline, chlortetracycline, oxytetracycline, tetracycline). The Twinsensor^{BT} test was found to be easy to use, with very short incubation period (6 minutes), robust and sensitive to all certified ß-lactame and tetracycline antibiotics at or lower concentrations than EU maximum residue limits, except for nafcillin. The Delvotest®SP-NT on other hand has a longer incubation period (3 hours) and is less sensitive to oxytetracycline, but it can detect a wide range of other antimicrobial substances mostly at or below EU MRLs.

Key words: Twinsensor; Delvotest; milk; antibiotics; screening test; residues; food safety

Introduction

Antimicrobial drugs have been used in dairy industry for more than five decades. They are principally administered to prevent or treat udder infections, but are also applied for the treatment of other diseases. The presence of antimicrobial drug residues in milk is a public health issue (1, 2).

The dairy industry has always been interested in rapid tests to screen the incoming milk on residues of ß-lactam antibiotics in order to prevent technological problems in cheese or yoghurt production (3, 4, 5). To avoid the long incubation period inherent to microbiological inhibitor tests, enzymatic, receptor and immunological tests were developed for a rapid screening of foodstuffs of animal origin on the presence of antimicrobials. The first fast test developed for that aim was the Penzym Test, an enzymatic (carboxypeptidase) colorimetric test, producing a result in 20 minutes. In the late 80s and early 90s, several screening tests with a total test time below 10 minutes (receptor tests SNAP, Charm MRL Beta-lactam Test (ROSA) and βeta s.t.a.r. and immunoassays Lactek and Parallux) became commercially available for monitoring of raw milk on ß-lactams (3). More recently, some rapid tests (Charm MRL-3 and βeta s.t.a.r. 1+1) were adapted to give a test result within 3 minutes, allowing screening of milk at the farm before collection. Also rapid tests for the detection of tetracyclines (SNAP Tetracycline Test Kit, TetraSensor Milk, Charm Tetracyclines - ROSA), sulfamethazine (SNAP Sulfamethazine Test Kit, Charm SMZ), sulfadimethoxine and sulfamethazine (Charm SDSM), gentamicin (SNAP Gentamicin Test Kit), enrofloxacin (Charm ROSA Enrofloxacin) or for a simultaneous detection of ß-lactams and tetracyclines (TwinSensor Milk, Charm ROSA MRLBLTET-3

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and SNAP Duo) are present on the market. Parallux Milk Residue Testing System detects all six major ß-lactams, tetracyclines, spectinomycin, neomycin, streptomycin, spiramycin, sulfa drugs and quinolones in one test in 4 minutes (5).

The ideal screening method would detect most, if not all, antimicrobials at or below their permissible limits or maximum residue limits (MRLs) (1).

Within this paper an evaluation of the performance of the Twinsensor^{BT} (Unisensor Diagnostic Engineering, Belgium) β-lactam-tetracycline test is described and compared to the Delvotest®SP-NT (DSM, Delft, The Netherlands).

Material and methods

Bovine milk free of antimicrobial agents

UHT milk containing 3.5% of milk fat (Prekmurske mlekarne and Ljubljanske mlekarne - different batches) and different samples of raw milk from untreated cows were used. The milk samples were verified by microbiological (plate test acc. to KUNDRAT) and chemical method (LC-MS-MS) as being free of -lactam and tetracycline antibiotics prior to use in the validation study.

Standard solutions and spiked milk samples

Standard solutions and spiked milk samples were prepared in accordance to Commission Decision 2002/657/EC (6), ISO 13969:2003 (7), ISO 18330:2003 (8) and Guide for analytical validation of screening methods (9).

Analyte stock solutions (1 mg mL^{-1}) were prepared from reference standards on a weekly basis in water, methanol, phosphate buffer or DMSO as appropriate and stored at 2-8°C (unless stated otherwise).

Intermediate standard solutions were prepared freshly on a daily basis in distilled water.

Aliquots of the milk were spiked individually with antimicrobial substances on a daily basis. The addition of the spike was set at 0.5 mL of the intermediate standard and adjusted to 50 mL with milk (unless stated otherwise).

Apparatus

Heater Bloc: HeatSensor: 40±3°C

ReadSensor Version 2.1 (77 Elektronika KFT for Unisensor)

Water bath 64±0.5°C

Twinsensor^{BT}

Twinsensor^{BT} test kits were supplied by Unisensor Diagnostic Engineering (Belgium). Twinsensor^{BT} is a competitive test involving two receptors in one single operation. The test requires the use of two elements. The first element is a microwell containing a certain amount of both receptors and antibodies linked to gold particles and the second is a dipstick made of a set of membranes on capture lines. The "control" line printed in red is visible all the time and the other two are specific "test" lines placed on both sides of the control line. The unique line for ß-lactams (penicillins and cephalosporins) is located below the control line while the line relating to tetracyclines is located above it. The assay can either be read visually or instrumentally using the ReadSensor.

The test procedure

The test procedure was carried out exactly as per test kit instructions. 200 µL of the milk sample was applied into the microwell, mixed with the reagents in the microwell and incubated for 3 minutes. The dipstick was dipped into each of the microwell laid in the incubator and incubated for 3 more minutes. Dipsticks were read with a ReadSensor within 15 minutes of performing the test. Dipsticks were visually verified that strip has a valid development: central control line should be visible.

Interpretation of the ReadSensor readings

The visible valid dipstick was inserted into ReadSensor.

A) NEGATIVE readings (Beta: NEG, Tetra: NEG) indicate a negative result — no present ß-lactams or tetracyclines in a milk sample.

B) POSITIVE readings indicate a positive result:

- Beta: POS or LPOS, Tetra: NEG indicate a presence of ß-lactams;
- Beta: NEG, Tetra: POS or LPOS indicate a presence of tetracyclines;
- Beta: POS or LPOS, Tetra: POS or LPOS indicate a presence of ß-lactams and tetracyclines in a milk sample.

Validation experiments

Determination of detection capability CCβ

As screening methods only the procedures that do not exceed 5% of false negative results at concentration of interest can be used.

All 14 antimicrobial compounds specified in the manufacturer's manual and two additional cepha-

losporins used in Slovenia were included in the validation. Because the manufacturer gave a LOD interval (not fixed concentration), that is even variable with the test batch number, we decided to test the concentration at MRL (μgL^{-1}) or just a little bit lower – when possible for every substance. Doxycycline has no MRL set, so it was analyzed at the lowest LOD point specified by the manufacturer. Validation procedure was performed during the period from February 2009 to May 2010. At least twenty different blank milk samples were spiked (unless stated otherwise) and two different Test Kit Batch numbers and two analysts on different days were used for every substance mentioned in the Table 1.

Table 1: Antimicrobial agents included in the validation study

Compound	LOD [a]	MRL in milk [b]	Test conc.[c]
Penicillin-G	2-3	4	4
Ampicillin	3-5	4	4
Amoxicillin	3-5	4	4
Cloxacillin	6-8	30	20
Nafcillin	30-40	30	30
Cefapirin	6-8	60	60
Cefalonium	3-5	20	20
Cefazolin	18-22	50	50
Cefoperazone	3-4	50	50
Ceftiofur	10-15	100	50
Cefalexin	-	100	100
Cefquinome	-	20	20
Doxycycline	20-40	-	30
Chlortetracycline	45-55	100	100
Oxytetracycline	56-75	100	100
Tetracycline	75-100	100	100

[a] Limit of detection as stated in the test instructions Twinsensor^{BT} (μgL^{-1}) (10)
[b] Maximum residue limits: Commission Regulation No 37/2010 (μgL^{-1}) (11)
[c] The concentration we tested (μgL^{-1})

Specificity

Specificity means the ability of the method to discriminate between the tested analyte and other substances, which are chemically related or have a related effect (6).

We tested 12 different ß-lactams and 4 different tetracyclines in different concentrations to test the ability of the Twinsensor^{BT} test to discriminate between them. We also tested milk samples with standard additions of other antibiotics — representative for the antibiotic group according to the Guide for analytical validation of screening methods (9). They should not react positive on this test, because it is commercialized as specific for the screening of ß-lactams and tetracyclines. Compounds we analyzed: streptomycin, gentamicin, erythromycin, tylosin, lincomycin, enrofloxacin, trimethoprim, chloramphenicol and sulfathiazol. Working solutions in milk were made in concentrations: 500 and 250 mgL^{-1}.

Test robustness

Robustness of the method is defined as its susceptibility to minor changes in laboratory conditions (6).

The following robustness parameters were tested: incubation time, incubation temperature, different analysts, different test batch numbers, different bovine milk samples, milk with low pH (spoiled milk) and interfering substances: bronopol (BR).

To test the influence of incubation time we tested 6 different milk samples spiked with penicillin-G (concentration of 4 μgL^{-1}) and 6 blank milk samples. We prolonged first and second incubation period separately from 3 to 6 minutes.

To test the influence of the incubation temperature we changed the heating program on the HeatSensor to only alternative available: 50°C. The optimum temperature of incubation recommended by the manufacturer of the Twinsensor^{BT} Test is 40±3°C. 6 different spiked milk samples (penicillin-G, 4 μgL^{-1}) and 6 blank milk samples were analyzed.

During validation procedure we used 5 different test batch numbers, more than three different packages of the test per batch, changed 3 different analysts, 3 different people preparing spiked samples and the procedure was conducted in longer period of approximately one year.

At least two different test batch numbers were used and two different analysts on two or more different days analyzed altogether 20 or more different spiked milk samples of every substance and concentration validated.

We tested some milk samples with lower pH than normal: 2 different blank milk samples acidified with hydrochloric acid to pH 4.5 and 2 samples naturally spoiled - acidified.

Delvotest®SP-NT

Delvotest®SP-NT test kits were supplied by DSM (Netherlands). The antimicrobial compounds were purchased from Sigma and Aldrich / Fluka / Riedel De Haën Chemicals (Poole, Dorset, UK).

Delvotest®SP-NT is an agar diffusion test based on the inhibition of growth of *Bacillus stearothermophilus*, a thermophilic bacterium highly sensitive to many antimicrobials applied in the dairy industry. The agar contains a standardized number of bacterial spores, select nutrients and the pH indicator bromocresol purple. The assays can either be read visually or by using the AOAC-approved DelvosScan (1, 11).

The test procedure

The test procedure was carried out as per test kit instructions. 100 μL of milk sample was applied into the test ampoule and incubated for 3 hours in the water bath 64±0.5°C.

Interpretation of the results

Results were read visually within 5 minutes of performing the test: yellow colour was interpreted as a negative result, 50% or more intensive purple colour was recorded as a positive test result.

Additional tests

The validation study for Delvotest®SP-NT was already conducted in our laboratory. We made additional tests to validation and used some data from the literature (1, 11).

Results of the validation of the Twinsensor^{BT} and comparison to Delvotest®SP-NT

Determination of detection capability CCβ

We analyzed 69 different samples of antibiotic free (blank) milk and the same ones with a standard addition of antibiotic substances named in the Table 1 (n = 16). The replicates were made on different days, through the period of approximately one year of validation study, with three different analysts and five different batches of the Twinsensor^{BT} test.

Concentrations applied within the validation study were first set at MRL-s (μgL^{-1}) except for the doxycycline (20 μgL^{-1}).

The test showed lower ability to detect the amoxicillin and poor ability to detect nafcillin, doxycycline and cefalexin residues in milk (Figure 1). After the first two applications of doxycycline (20 μgL^{-1}), nafcillin (30 μgL^{-1}) and cefalexin (100 μgL^{-1}) that gave negative result we stopped the validation procedure for those antibiotics. They were additionally tested on different day, with new standard solutions made and different analyst. After two more results for every one of them under these conditions were obtained we analyzed nafcillin and doxycycline in higher concentrations (Table 2). In newer batch numbers of the Twinsensor^{BT} test the LOD for cefalexin was set at more than 750 μgL^{-1} which is much higher than MRL (100 μgL^{-1}) so we decided to stop the validation procedure for this substance.

Doxycycline has no MRL set and LOD of the Twinsensor^{BT} test is around 30 μgL^{-1}.

With exception of nafcillin (LOD is around 60 μgL^{-1}) and cefalexin all the antibiotic compounds listed on the manufacturer's certificate were detected at estimated detection limits and at or below the EU MRLs.

The Twinsensor^{BT} test has a relatively narrow spectrum of antibiotic substances it is sensitive to in comparison to Delvotest®SP-NT (Table 3).

Regarding tetracyclines and ß-lactams used in Slovenia, Twinsensor^{BT} test is more sensitive. Both tests are able to detect those substances at

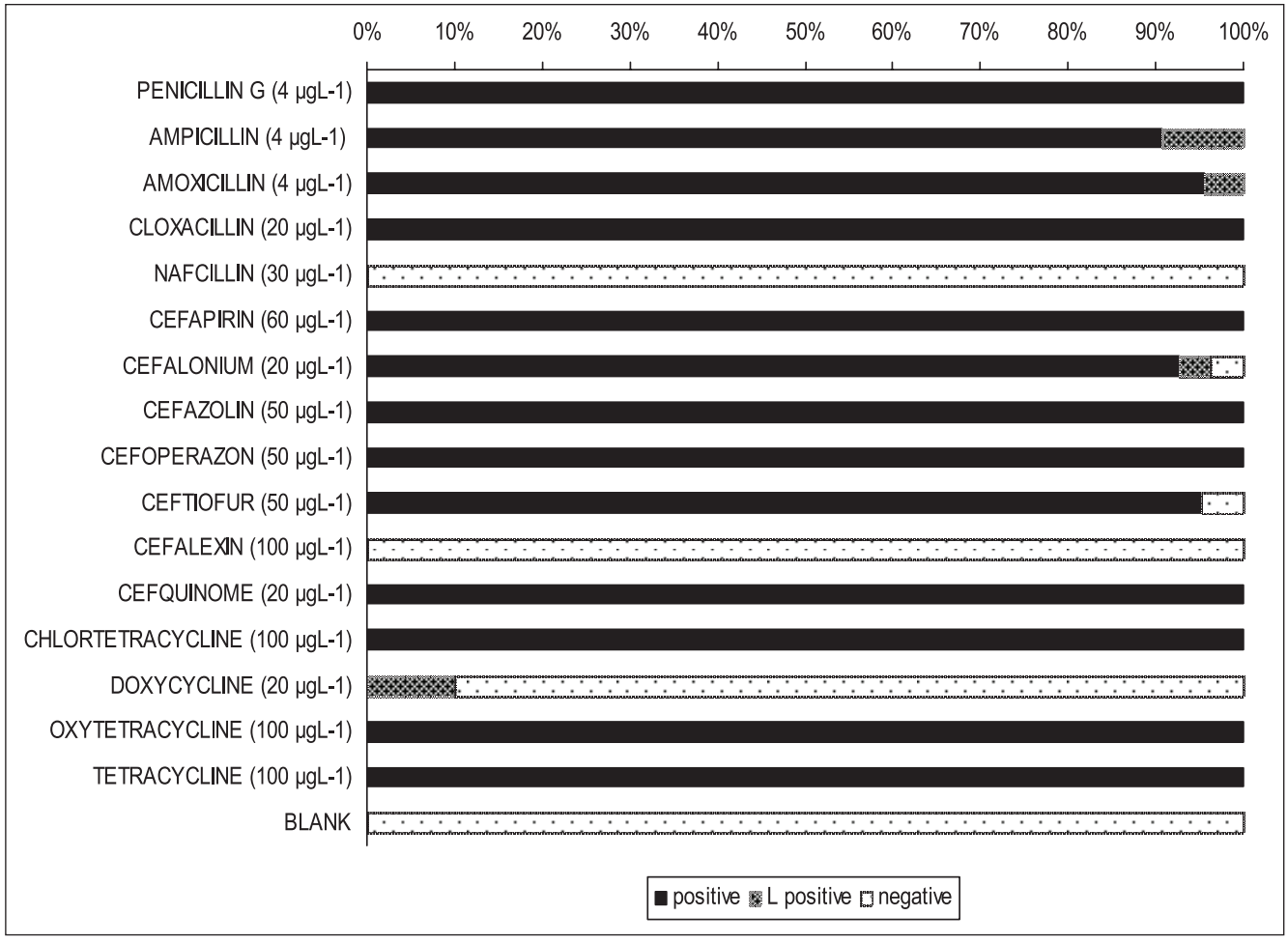

Figure 1: Detection pattern of antimicrobial substances

Table 2: Nafcillin and doxycycline tested in higher concentrations

Compound	LOD [a] (μgL^{-1})	MRL [b] (μgL^{-1})	T. conc. [c] (μgL^{-1})	POS	LPOS	NEG	n
NAFCILLIN	30-40	30	30	-	-	4	4
			40	-	-	4	4
			50	4	1	3	8
			60	20	2	-	22
			80	2	-	-	2
			100	2	-	-	2
DOXYCYCLIN	20-40	-	10	-	-	1	1
			20	-	2	18	20
			30	20	1	1	22
			40	1	-	-	1
			50	1	-	-	1

[a] Limit of detection as stated in the test instructions (10)
[b] Maximum residue limits (MRL) (11)
[c] The concentration we tested (µgL-1)

POS – Number of positive results
NEG – Number of negative results
LPOS – Number of low positive results
n – Number of all tested spiked milk samples

Table 3: Sensitiveness and capability of achieving MRL in milk by Twinsensor^{BT} and Delvotest®SP-NT regarding results of validation and information from the literature - of antibiotic substances allowed for use in Veterinary medicine in Slovenia to treat lactating cows (14)

Antibiotic group	For i/mam application	Other applications	MRL in milk (μgL^{-1})	LOD Delvo (μgL^{-1})*	LOD Twin (μgL^{-1})**
PENICILLINS	Ampicillin	Ampicillin	4	4	3-5
	Amoxicillin	Amoxicillin	4	4	3-5
	Cloxacillin		30	20	6-8
	Penicillin-G	Penicillin-G	4	2	2-3
CEPHALOSPORINS	Cefacetril		125	20	30-40
	Cefalexin	Cefalexin	100	100	> 750
	Cefalonium		20	5-10	3-5
	Cefapirin	Cefapirin	60	10	6-8
	Cefquinome	Cefquinome	20		20-30
		Ceftiofur	100	100	10-15
	Cefoperazone		50	40	3-4
AMINO-GLYCOSIDES	Dihydro-streptomycin	Dihydro-streptomycin	200	300-500	NS
		Gentamycin	100	100	NS
	Kanamycin		150	2500	NS
	Neomycin	Neomycin	500	250	NS
	Streptomycin		200		LS
TETRACYCLINES		Oxytetracyclin	100	250	60-80
	Tetracycline		100	100	80-100
QUINOLONES		Danofloxacin	30		NS
		Enrofloxacin	100		NS
		Marbofloxacin	75		NS
SULFONAMIDES		Sulfadiazin		50	NS
		Sulfadimidin	100		NS
		Sulfadoxin			NS
OTHERS	Bacitracin		100	50	NS
	Lincomycin		150	100	NS
	Novobiocin		50	25	NS
		Tylosin	50	10-20	NS
		Trimethoprim	50	50	LS

* Results read at first Control Time - after 2.5h of incubation (1, 12)

** Concentrations given in the instructions 2010 (10) – corrected when proved different in our validation (in bald)

NS - Not sensitive

LS - Very low sensitivity

concentration at or lower than MRL (except from Delvotest®SP-NT that detects oxytetracycline at levels two to three times higher than EU MRL).

Specificity

The Twinsensor^{BT} test can distinguish between the tetracyclines and ß-lactams which are the two most commonly used antibiotics in treating lactating cows. It is unable to detect any other inhibitory substance in milk. Delvotest®SP-NT on other hand is sensitive to almost all antibiotic substances (at or below the MRL) used in Slovenia — except of oxytetracycline, dihydrostreptomycin, kanamycin and quinolones, but also detects other inhibitory substances which can be present in milk (naturally occurring inhibitory substances, bronopol, ...) and can give false positive results.

The false positive rate of Twinsensor ^{BT} test was determined as 0%. 69 different blank milk samples

were tested (all detected NEG) and all milk samples spiked with tetracyclines or ß-lactams were all detected correctly as Tetra POS or Beta POS.

Two different blank milk samples were spiked with different concentrations of antimicrobial compounds to which Twinsensor^{BT} test should not be sensitive to and tested. Results are presented in Table 4.

Table 4: Test of the sensitivity of the Twinsensor^{BT} test to other antibiotics than β-lactams and tetracyclines

Compound	500 mgL^{-1}		250 mgL^{-1}	
	BETA	TETRA	BETA	TETRA
Streptomycin	POS POS	NEG NEG	POS POS	NEG NEG
Gentamicin	NEG NEG	NEG NEG	NEG NEG	NEG NEG
Erythromycin	POS POS	NEG NEG	NEG NEG	NEG NEG
Tylosin	NEG NEG	NEG NEG	NEG NEG	NEG NEG
Lincomycin	NEG NEG	NEG NEG		
Enrofloxacin	NEG NEG	NEG NEG		
Trimethoprim	POS LPOS	NEG NEG	NEG NEG	NEG NEG
Chloramphenicol	NEG NEG	NEG NEG		
Sulfathiazol	NEG NEG	NEG NEG		
Bacitracin	NEG NEG	NEG NEG	NEG NEG	NEG NEG
Novobiocin	NEG NEG	NEG NEG	NEG NEG	NEG NEG

POS – Positive results
NEG – Negative results
LPOS – Low positive results
BETA – Beta lactams
TETRA – Tetracyclines

The test reacted Beta POS to high concentrations (500 mgL^{-1}) of streptomycin, erythromycin and trimethoprim and to streptomycin at concentration 250 mgL^{-1}. The Twinsensor^{BT} test is a specific test that detects tetracyclines and ß-lactams in milk samples and can distinguish between those two groups, but can react falsely positive to the high concentrations of some other antibiotics.

Delvotest®SP-NT is a very broad spectrum screening test which can detect almost every inhibitory substance at quite low concentrations but can not distinguish between groups of antibiotics nor between antibiotics and other inhibitory substances.

Robustness

Incubation period

6 different milk samples spiked with penicillin-G (4 µgL^{-1}) and 6 blank milk samples were applied and analysed. The same samples were tested again with the prolongation of the first incubation period from 3 to 6 minutes. Second incubation was not changed. Than the experiment was repeated with the same

samples and only the second incubation was prolonged from 3 to 6 minutes. No change in negative and positive results was observed.

The manufacturer warrants that prolonged first incubation could lead to false positive results. Prolongation of the second incubation period is sometimes needed if the milk sample is very thick and therefore the flow up the test dipstick is slow. The liquid should reach the upper filter paper on the test dipstick to achieve valid results. If you shorten the second incubation, you can obtain false positive results (Tetra LPOS).

When working with Delvotest®SP-NT prolonged incubation can lead to false negative results.

Incubation temperature

The optimum temperature of incubation recommended by the manufacturer of the Twinsensor^{BT} test 40±3°C was changed to 50°C (the only alternative option on HaetSensor). Six different blank milk samples and the same milk samples with standard addition of penicillin-G (4 μgL^{-1}) were applied. Higher temperature of incubation results in false positive reading – all samples applied were Beta: POS.

Incubation temperature recommended by the manufacturer of Delvotest®SP-NT is 64±0.5°C. At temperatures below 64°C, the incubation time was found to increase. At temperatures above the 66°C the blank milk response was found to be positive.

Different analyst

Three different analysts were included in the validation study. Every substance validated was tested at least with two different analysts on different days. No deviation in results when changing analyst was observed.

Different batch numbers

During the validation study of the Twinsensor^{BT} test five different batch numbers of the kit were used. More than three test boxes of each batch number were used and every antibiotic substance was tested with at least two different test bach numbers.

Milk with low pH (spoiled milk)

Two blank milk samples were acidified with hydrochloric acid to pH 4.5. The acidified sample and 2 blank milk samples were tested. Acidified milk gave a Beta and Tetra: POS result at the Twinsensor^{BT} test and a positive response at Delvotest®SP-NT. Two more samples of naturally spoiled milk were applied and gave Beta: POS result at the Twinsensor^{BT} test.

The finding demonstrates that neither of the tests is applicable for the analysis of spoiled milk.

Susceptibility to bronopol (BR)

BR is used as the milk sample preservative (13). The concentration of 0.2% BR in two different blank milk samples was made and applied on both tests. The Twinsensor^{BT} test is obviously not sensitive to BR at this concentration as the results were negative. Delvotest®SP-NT on other hand reacted positive.

Discussion

The Twinsensor^{BT} is a rapid and low cost screening test for the direct detection of tetracycline and ß-lactam antibiotics in milk samples. The test is easy to perform: samples are applied directly; incubation is very short (6 minutes) and the results can be read visually or instrumentally.

In comparison to Delvotest®SP-NT, fewer samples can be done in one step when performing Twinsensor^{BT} (maximum 8 samples), but the incubation is longer at Delvotest®SP-NT (3 hours).

Delvotest®SP-NT detects almost all important antimicrobial substances at or below EU MRLs. Twinsensor^{BT} test on other hand is applicable only for screening tetracycline and ß-lactam antibiotics in milk, but it was seen to have better LODs than Delvotest®SP-NT when detecting tetracyclines in milk samples. For some antibiotics in the group of ß-lactams, the LODs can be low to the point of disturbing, because there are many positive responses even if the antibiotic residues present in the sample are way below the MRLs. Twinsensor^{BT} test is not suitable for detection of nafcillin and cefalexin in milk samples (LOD is higher than MRL).

The most important advantage of the Twinsensor^{BT} test is that the test can distinguish between tetracycline and ß-lactam antibiotics. Delvotest®SP-NT gives us only a positive or negative response.

Neither of the tests is applicable for screening of the spoiled milk (low pH), but both tests were found to be easy to perform and robust in terms of incubation period, different analysts and test batches.

References

1. Stead SL, Ashwin H, Richmond SF et al. Evaluation and validation according to international standards of the Delvotest®SP-NT screening assay for antimicrobial drugs in milk. Int Dairy J 2008; 18: 3–11.

2. Pengov A, Kirbis A. Risks of antibiotic residues in milk following intramammary and intramuscular treatments in dairy sheep. Anal Chim Acta 2009; 637: 13–7.

3. De Brabander HF et al. Residue analysis: future trends from a historical perspective. J Chromatogr A 2009; 46: 7964–76.

4. EMEA. Committee for Veterinary Medical Products. Pennicillins: summary

5. report. London: European Medicines Agency, Veterinary Medicines and Inspections, 2008: 2 str. http://www.emea.europa.eu/pdfs/vet/mrls/penicillins.pdf (24. 9. 2009)

6. Suhren, G. Influence of antimicrobials on starter cultures in milk – model trials. In: 3rd Karlsruhe Nutrition Symposium: European research towards safer and better food. Karlsruhe, Germany, 1998: II, 3-9.

7. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC establishes criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories. Off J Eur Commun 2002; L 221(17. 8. 2002): 8–36.

8. SIST EN ISO 13969: 2003. Milk and milk products – Guidelines for a standardized description of microbiological inhibitor tests. Geneve: International Organization for Standardization, 2004: 1–24 .

9. EN ISO 18330: 2003. Milk and milk products – Guidelines for a standardized description of immunoassays or receptor assays for the detection of antimicrobial residues.

10. Gaudin V, Sanders P. Draft guide for analytical validation of screening methods, version 2, CRL internal document 19/10/2005. AFSSA Foug res: Community Reference Laboratory for Antimicrobial Residues in Food , 2005.

11. Commission Regulation No 37/2010, of 22. December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs on animal origin. Off J Eur Commun 2010; L 15(20. 1. 2010): 72 str.

12. Le Breton MH, Savoy-Perroud MC, Diserens JM. Validation and comparison of the Copan Milk Test and Delvotest SP-NT for the detection of antimicrobials in milk. Anal Chim Acta 2007; 586: 280-3.

13. Seznam zdravil za uporabo v veterinarski medicini, za katera je bilo izdano dovoljenje za promet. Ljubljana: Javna agencija republike Slovenije za zdravila in medicinske pripomočke, 2008 http://www.jazmp.si/files/ijz/ZdravilaVet.pdf (24. 12. 2009)

14. Kroger M. Milk sample preservation. J Dairy Sci 1985; 68: 783–7.

PREVERJANJE TESTA TWINSENSOR^{BT}, PRESEJALNE METODE ZA UGOTAVLJANJE PRISOTNOSTI ß-LAKTAMOV IN TETRACIKLINOV V MLEKU, IN PRIMERJAVA Z DELVOTESTOM®SP-NT

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Povzetek: Preverjali smo nov kompetitivni receptorski test, imenovan Twinsensor^{BT} in ga primerjali z mikrobiološkim agarsko-difuzijskim testom Delvotest®SP-NT. Oba sta namenjena ugotavljanju prisotnosti zaviralnih substanc v mleku. Pri preverjanju smo upoštevali priporočila odločbe 2002/657/EC, standardov ISO 13969:2003, ISO 18330:2003 in Vodilo za validacijo presejalnih metod (AFSSA Fougères).
Ugotovili smo, da je test Twinsensor^{BT} enostaven za uporabo. Čas inkubacije je zelo kratek (6 minut), metoda je robustna in občutljiva na vse na navodilu proizvajalca navedene β-laktamske in tetraciklinske antibiotike v koncentracijah, ki so nižje ali enake kot so določene s strani Evropske unije (maksimalna koncentracija ostanka antibiotika v hrani - MRL), razen za nafcilin. V primerjavi s testom Twinsensor^{BT} ima Delvotest®SP-NT daljšo dobo inkubacije (3 ure) in je manj občutljiv na oksitetraciklin, po drugi strani pa zazna zelo širok spekter ostalih antibiotikov in zaviralnih substanc, večino v koncentracijah, nižjih od predpisanih MRL vrednosti.

Ključne besede: Twinsensor; Delvotest; mleko; antibiotiki; presejalni test; ostanki; varna hrana