ARTICLE

Rapid screening for residues of antibiotics in milk at the factory

W. Reybroeck

Published: March 05, 2004

SESSION 4. Chemical hazards and control measures

Abstract

The dairy industry is interested in screening incoming milk on the presence of residues of mainly b-lactam antibiotics in order to prevent technological problems (inhibition of fermentation processes) and to ensure that levels of residues in the tanker milk are not exceeding the safe/tolerance levels or Maximum Residue Limits (MRLs). Different commercial fast screening tests are commercially available now, giving a result within ten minutes; so tanker milk can be rejected at the entrance after a positive result.

Four different screening tests (receptor tests SNAP, Charm MRL Beta-lactam Test (ROSA), beta-s.t.a.r. and the immunoassay Parallux) are commonly used in Europe for screening purposes. All four tests were validated at the DVK-CLO. In this study, the sensitivity regarding the penicillins and cephalosporines present on the actual MRL list was tested, as well as the specificity, the ruggedness (influence of milk parameters such as somatic cells) and the occurrence of false positive/negative results. Also the applicability of the rapid tests for screening milk from animal species other than the cow (ewes and goats) was checked.

The results of this evaluation study can be very useful for dairy companies when choosing an appropriate test for controlling the milk at the milk reception.

KEYWORDS

Rapid tests, milk, b-lactam antibiotics, residues, screening.

INTRODUCTION

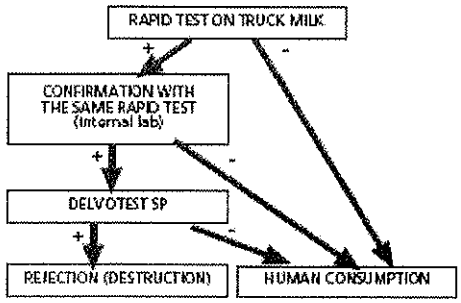
To ensure the technological and toxicological safety of milk and milk products a HACCP-like integrated system with shared responsibilities can be applied. In such a system milk processing establishments should make their own testing. Rapid tests are the methods by excellence to screen the incoming milk on antimicrobials (mainly b-lactam antibiotics) on MRL (Maximum Residue Limits) [1] or tolerance level. More than 90% of all loads with antimicrobial residues can be identified and rejected by checking the milk upon arrival for on the presence of b-lactams. In this way the most dominant cause of failures in the production of cheese and yoghurt can be prevented.

Four different screening tests (receptor tests SNAP, Charm MRL Beta-lactam Test (ROSA), beta-s.t.a.r. and the immunoassay Parallux), giving a result within ten minutes, are commonly used in Europe for screening purposes. All four tests were validated at the DVK-CLO. This paper willdiscuss the results of the validation studies.

In some countries the own checks by the dairy companies are prescribed by the local legislation. For example since October 15 2002 all in Belgium collected milk had to be checked for the presence of residues of b-lactam antibiotics before delivery at the dairy plant (Circular letter 523/275/7915b of August 23rd 2002). Since August 1st 2003 this obligation is temporarily withdrawn in order to gather information about the best way to deal with contaminated milk. A protocol was developed by the Belgian dairy sector in order to improve uniform testing among all dairies. Since the fact that not all b-lactams are detected by all four rapid tests at the same level and the fact that some cephalosporins are detected at levels far below their respective MRLs, the use of a microbiological test like the Dievotest SP is accepted for the final decision upon the rejection of a truckload. In figure 1 the Belgian sector protocol is described.

Since testing with a rapid test can result in the destruction of the whole tanker load, the accuracy of the test result is very important. For the daily check of the good performance of the tests (1st line control), standards of non-fortified blank and fortified milk can be used. The Belgian dairy companies also have the opportunity to participate in ring trials (3rd line control), organised by DVK-CLO.

FIGURE 1
Belgian dairy sector protocol for testing of incoming milk



DESCRIPTION OF THE RAPID TESTS

Parallux® Beta Lactam Assay

The Parallux (Idexx Laboratories Inc., Westbrook, ME) is a solid-phase fluorescence immunoassay for the detection of residues of b-lactam antibiotics in milk, performed in a fully automated instrument. Milk is added to antibodies (labelled with a fluorescent conjugate) in the reagent tray and incubated for three minutes. Drug residues, possibly present in the milk, will bind with the antibodies. Milk is aspired into the four capillaries of the cartridge. Remaining free antibodies will bind to drug coated upon each capillary. After a wash step the cartridge is put in a laser-based reader. The more fluorescence obtained the less drug is present in the sample. Each capillary acts as an independent assay. The total test time is five minutes. With the Beta Lactam Assay the following b-lactam antibiotics are detected: cloxacillin (channel 1), ceftiofur (channel 2), penicillin G, ampicillin and amoxicillin (channel 3) and cephapirin (channel 4). Besides the Beta Lactam Assay, different other cartridge configurations are available for the detection of b-lactams, tetracyclines and sulphonamides in milk.

SNAP® Beta Lactam Test Kit,beta s.t.a.r. and Charm MRL® Beta-lactam Test (ROSA)

These three receptor tests are based on the same principle: milk is incubated with a receptor. b-lactams, possibly present in the milk, will bind with the receptor. After migration over filtration paper in a cartridge or on a dipstick, the remaining quantity of free receptor is visually controlled by comparing the colour intensity of the sample spot (or test line) and the control spot (or control line). The colour intensity formed by the colour developer at the captation zone (test spot or test line) is inversely related to the concentration of b-lactam antibiotics in the milk sample. The reading can be done visually or by the use of a reader system. The total test time for the SNAP (Idexx Laboratories Inc., Westbrook, ME), the Charm MRL Test (ROSA) (Charm Sciences Inc., Lawrence, MA) and the beta s.t.a.r. (UCB- Bioproducts, Braine l'Aileud, BE) is nine, eight and five minutes respectively.

The SNAP and the Charm MRL Test (ROSA) also exist in a version for the detection of tetracyclines in milk.

EXPERIMENTAL: METHODS AND MATERIALS

All tests were performed according to the manufacturer's instructions. The beta-s.t.a.r. (25 tests version) results were obtained by a visual interpretation of the strips due to the fact that a reader system was not yet available during the period of the validation study. The results of SNAP, Charm MRL Beta-lactam Test (ROSA) and Parallux were obtained by an instrumental reading.

Test sensitivity

Milk free from antimicrobials (blank milk) and with a low bacterial load (< 5x.-104 CFU per ml) was collected from four cows in good health (< 1x.-106 somatic cells per ml). The milk was mixed and further used to prepare the test samples. For each veterinary drug substance involved in the validation study, a stock solution (weight calculated on basis of active substance) and dilution series were prepared in water. In the last two steps the dilution was made in milk. For the test sensitivity study different substance concentrations spiked in raw milk were tested on several days. The chosen concentration steps depended on the concentration and the expected detection level. The doped samples were blind coded before analysis.

For each tested concentration, the lowest concentration giving at least nine positive test results on ten test results (90 %) was determined and defined as the test sensitivity.

Test pecificity/cross-reactivity

Clavulanic acid, a b-lactamase inhibitor, was doped at ten, five and two times the respective MRL (200 µg/kg) and tested with the respective tests.

From the most important groups of anti-infectious agents (antibiotics and chemotherapeutics) different from the group of b-lactams, a substance was selected and doped at ten times the respective MRL and tested with the respective tests.

All b-lactam antibiotics on the present MRL list (EEC-Regulation 2377/90 and amendments), except for penethamate, were doped at 20, ten and two times the respective MRL and tested on each channel of the Parallux Beta Lactam Assay in order to test which substances are detected on each channel besides the substances claimed by the manufacturer.

Test ruggedness: impact of somatic cells

Thirty-six raw milk samples with a somatic cell count (SSC) > 1x.-106 per ml were tested with the respective tests.

Milk with a normal and an high SSC (> 1x.-106 per ml) was doped with penicillin G or ampicillin on the respective test sensitivity level (cfr Table 1). After testing, the obtained values were compared.

TABLE 1
Test sensitivity of the rapid tests for the most important ¯ -lactam compounds (penicillins and cephalosporins).
MRLs (EEC-Regulation 2377/90 and amendmends, situation March 2004) and test sensitivities are expressed in ¯ g/kg or ppb.

substance	MRL	Parallux	SNAP	beta-s.t.a.r.	Charm MRL Test
penicillin G	4	4	3	3	2
ampicillin	4	8	5	4	3
amoxicillin	4	6	7	4	3
cloxacillin	30	8	35	6	25
nafcillin	30	15*	80	14	45
ceftiofur	100	35	3	110	6
cefquinome	20	250*	20	10	14
cefazolin	50	-	15	60	10
cefapirin	60	16	3	12	5
cefacetril	125	-	12	40	6
cefoperazone	50	-	7	6	4
cefalexine	100	-	25	>1000	15
cefalonium	20	-	4	4	3

- : not detected;
* : cross-reactivity

Test ruggedness: milk from animal species other than the cow

Milk from ewes (30 samples) and milk from goats (30 samples) was tested with the respective tests in order to check if the tests are also suitable for the screening of milk of these animal species.

Test ruggedness: impact of a waiting time on the reading

The impact of a waiting time on the result obtained with the receptor tests was checked by testing blank milk and milk doped with penicillin G (4 µg/kg). The colour intensity of the sample spot (tests line) was interpreted directly after the test was finished and after 2, 5 and 10 minutes keeping the cartridges (test strips) at room temperature.

The effect of a waiting time on the Parallux result is not tested since this assay is performed automatically by an instrument.

Test ruggedness: occurrence of false-negative/ false-positive results

The rapid tests were integrated as screening tests in the monitoring programme of farm milk, tanker milk, consumption milk and milk powder.

The obtained results were compared with the results of microbiological screening tests like Delvotest MCS, Charm Aim-96 and CMT - Copan Milk Test.

Test ruggedness: ring trials

Blind coded raw milk samples (blank or fortified) were prepared and shipped to participating Belgian dairy companies. All participants were asked to perform the tests according to the manufacturer's instructions. The results were gathered at DVK-CLO.

EXPERIMENTAL: RESULTS AND DISCUSSION

Test sensitivity

The test sensitivity (the lowest concentration tested giving a positive test result at least nine upon ten 10 times) of the rapid tests for the most important ¯ -lactam compounds on the present European MRL list is summarized in table 1.

The Parallux is only detecting 3 cephalosporins due to the use of antibodies while the receptor tests are detecting all b-lactam compounds. No test is detecting all compounds at MRL, however the Charm MRL Test (ROSA) is only missing nafcillin at that level. In general the cephalosporins are detected by the receptor tests at a concentration far below their respective MRL.

Test specificity/cross-reactivity

All four tests showed a cross-reaction for clavulanic acid: a concentration of 1000 µg/kg clavulanic acid (MRL = 200 µg/kg) gave a positive result for all tests (for the Parallux Beta Lactam Assay only on channel 1); a concentration of 400 µg/kg was only detected by SNAP.

Even relatively high concentrations (10 times the MRL) of tetracyclines, sulphonamides, macrolides, aminoglycosides and quinolones never gave a positive result for any test, proving the good specificity of the rapid tests.

The manufacturer of the Parallux is only claiming the detection of four penicillins and two cephalosporins with the Beta Lactam Assay. The cross-reactivity of each channel for the other b-lactam substances was tested and is summarized in table 2. For channel 4 (= cefapirin) no cross-reaction for any other b-lactam compound was found.

Test ruggedness: impact of somatic cells

Thirty-six raw milk samples with a somatic cell count >1x.-106 per ml were tested. A few milk samples caused false-positive results (Parallux: one sample; SNAP: two samples) or an invalid reading (Charm MRL Test (ROSA): one sample). No false-positive results were obtained with the ¯ eta s.t.a.r..

Milk with a normal and an high somatic cell count (> 1x.-106 per ml) was doped with penicillin G or ampicillin on the respective test sensitivity level (cfr Table 1). After testing the obtained values were compared. The presence of a high number of somatic cells was not influencing the results obtained with the beta s.t.a.r. or the Charm MRL Test (ROSA). A slight tendency to a more positive result caused by a high somatic cell count was observed for the Parallux. The same effect was obtained for SNAP: with 3 µg/kg of penicillin G present in the milk, the ratio shifted from 1.4 to 2.2 by a high SSC, while the ratio measured for milk with 5 µg/kg of ampicillin changed from 1.03 to 1.4 by a high SSC.

It should be emphasized that tanker milk will never contain such a high number of somatic cells.

Test ruggedness: milk from animal species other than the cow

The protein and fat content of milk from ewes is normally high, while milk from goats sometimes contains a high fat content and a high SSC. Thirty samples of both milk types were tested with the respective tests. The analysis with the Parallux of both milk types was trouble free. Migration problems sometimes occurred with milk from ewes with the other tests: SNAP: two false-positive results (one of the two samples due to a longer migration time) and for another sample no result due to migration problems; beta s.t.a.r.: no result for one sample due to migration problems; Charm MRL Test (ROSA): one false-positive result due to migration problems.

The milk of goats also caused problems: SNAP: two false positive results (borderline results) and no result for one sample due to migration problems; Charm MRL Test (ROSA): four invalid readings due to migration problems. No problems occurred when testing goat's milk with the beta s.t.a.r..

Most of the migration problems with milk from animal species other than the cow over the filtration paper of the cartridge or dipstick can be eliminated by removal of the fat of the milk before testing (e.g. by centrifugation).

Test ruggedness: impact of a waiting time on the reading

When testing blank milk no impact of a waiting time (two, five or ten minutes) was noticed on the result obtained with the receptor tests. When the reading of the colour intensity of the sample and control spot of the SNAP is delayed, a tendency to a less positive result for milk doped with penicillin G (4 µg/kg) was noticed. For the Charm MRL Test (ROSA) a tendency in the opposite direction (towards a more positive result) was observed. The test line and control line of the beta s.t.a.r. were not influenced by a waiting time. The intensity of the lines on the used beta s.t.a.r. dipsticks does not change during storage at room temperature; so the dipsticks can be used as proof.

Test ruggedness: occurrence of false-negative/ false-positive results

In the framework of the monitoring of Belgian milk and milk powder for residues of antibiotics and chemotherapeutics no false-negative/false-positive results were observed for the rapid tests when integrated in the set of screening tests. Test ruggedness: ring trials The results of the ring trials organized by DVK-CLO showed that in general all participating dairy laboratories are capable to perform the screening tests in a correct way. A very good reproducibility was obtained for the rapid b-lactam screening tests. The use of a reader system for the interpretation of the colour forming is advisable instead of a visual reading [2].

CONCLUSION

Robust and reliable rapid tests are available for dairy companies for the control of milk on the presence of b-lactam antibiotics at the milk reception.

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IDF/FAO international symposium on dairy safety and hygiene Cape Town,
March 2–5, 2004,
South Africa