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REPORT RING TEST

"SCREENING FOR ANTIBIOTICS AND SULFONAMIDES IN RAW GOATS' MILK"

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1. INTRODUCTION

As National Reference Laboratory (NRL) Chemistry 96/23, the Technology and Food Science Unit of the Institute for Agricultural and Fisheries Research (ILVO-T&V) organised in November 2013 a ring test "screening for antibiotics and sulfonamides in raw goats' milk", in collaboration with the Federal Agency for the Safety of the Food Chain (FASFC). A first ring test was already organised in September 2012 by ILVO-T&V as NRL Milk and Milk Products [1] but was reorganised on request of the Interprofessional Organisms, MCC-Vlaanderen and Comité du Lait.

This proficiency test was obligatory for MCC-Vlaanderen and Comité du Lait and also for the laboratory of ILVO-T&V. The laboratories were free to use one or more screening tests of their choice.

2. PLANNING OF THE RING TEST

On September 24, 2013 the above mentioned laboratories and also some private laboratories were invited to participate. Finally, 4 laboratories subscribed to the ring test.

On November 5, a parcel containing 10 randomly coded raw goats' milk samples was shipped on dry ice by DHL to the participants. The participants were asked to store the samples refrigerated (below 6°C) upon arrival and to analyse the samples as they were routine samples and not later than November 7.

It was asked to return the results before November 15 using the specific results form.

3. SAMPLES

3.1 PREPARATION AND TESTING OF THE SAMPLES

The milk samples were prepared at ILVO-T&V on the 5th of November.

Each sample might contain one or more antibiotic(s) and/or sulfonamide(s). The antibiotics were chosen according to the active substances of veterinary drugs registered in Belgium for dairy production [2]; the spiking concentrations were chosen in relation to the respective Maximum Residue Limits (MRLs) (for bovine milk) [3].

Fresh raw milk, originating from goats that were not treated with veterinary drugs during the last eight weeks, was used as blank. On the day of preparation, the blank milk was checked on the presence of ß-lactam antibiotics (ßeta-s.t.a.r., Neogen Corporation), inhibitory substances (Delvotest T, DSM Food Specialties bv) and ß-lactamases (own method based on the Delvotest T). Also the doped milk samples were verified on the same day by the most appropriate test(s) (ßeta-s.t.a.r., Delvotest T and/or CMT Milk Test (DSM Food Specialities bv)).

The individual codes of the milk samples, each corresponding to a general sample code, are presented in Table 1.

Table 1. Codification of the milk samples.

Spiked substance	Concentration (µg/kg)	MRL (µg/kg)	General code	Code lab 1	Code lab 2	Code lab 3	Code lab 4
Tylosin A	50	50	A	2	12	30	31
Blank milk	---	---	B	10	13	29	33
Chlortetracycline	100	100	C	6	20	28	35
Amoxicillin	4	4	D	5	11	22	32
Sulfadiazine	100	100	E	7	14	27	37
Cefalexin	40	---*	F	1	15	26	38
Blank milk	---	---	G	9	17	21	39
Cloxacillin	30	30	H	8	19	23	36
Benzylpenicillin	-	4	I	3	18	24	34
Cefoperazone	25	---*	J	4	16	25	40

** MRL of 100 µg/kg cefalexin in bovine milk; ** MRL of 50 µg/kg cefoperazone in bovine milk.*

3.2 HOMOGENEITY OF THE SAMPLES

Ad randomly 3 series of samples (A-J) were analysed in double with the Delvotest T. The Z-values obtained give an estimation of the homogeneity of each doped milk sample (Table 2).

Table 2. Homogeneity of the samples: average Z-values, standard deviations and minimum and maximum Z-values for 3 series of milk samples (A-J) analysed in double with the Delvotest T (cut-off Z-value = -3.00).

CODE	SUBSTANCE	CONCENTRATION (µg/kg)	Z-VALUES Delvotest T			
			average	stdev	min	max
A	Tylosin A	50	-1.11	0.50	-1.76	-0.64
B	Blank milk	---	-7.40	0.86	-8.37	-6.38
C	Chloortetracycline	100	-0.52	0.92	-1.83	0.40
D	Amoxicillin	4	11.03	0.51	10.30	11.70
E	Sulfadiazine	100	10.01	0.33	9.58	10.48
F	Cefalexin	40	9.11	1.58	6.67	10.92
G	Blank milk	---	-6.11	1.03	-7.34	-4.97
H	Cloxacillin	30	10.88	1.08	9.21	11.98
I	Benzylpenicillin	4	12.36	0.86	11.02	13.39
J	Cefoperazone	25	8.71	0.75	7.34	9.38

The standard deviations were in the range that could be expected for Delvotest T duplicate test values. Hence, it can be concluded that the homogeneity of all doped samples is good.

3.3 STABILITY OF THE DOPED SAMPLES

In the framework of a proficiency study organised by the Community Reference Laboratory of Fougères (France), the stability at -20°C of benzylpenicillin, cloxacillin, tylosin, a sulfonamide (sulfamethazine) and two tetracyclines (tetracycline and oxytetracycline) doped in milk was checked using a LC-MS/MS procedure on a period of 48 to 55 days. No problem of depletion was observed by Fuselier *et al.* [4]. In the stability study of another proficiency test in raw milk (-20°C, 1 month), organised by the same laboratory, no significant degradation was observed by LC-MS/MS for benzylpenicillin, cloxacillin and two cephalosporins (cefalonium and cefquinome) [5].

In general, penicillins and cephalosporins are rather stable in milk when kept at -20°C. However, the main problem for the stability of doped milk samples is the presence of (natural) ß-lactamases in the milk. Therefore, the blank milk was tested at ILVO-T&V on the presence of ß-lactamases and no presence of ß-lactamases was noticed. Hence, no problems of stability were expected for the samples doped with ß-lactam compounds. Summarised, no stability problems could be expected for all compounds used in this study.

3.4 RECEPTION OF THE SAMPLES

Only laboratories 1, 3 and 4 sent back the form "acknowledgement of receipt of samples" upon arrival of the samples. They confirmed that the samples arrived in time and in good condition.

4. APPLIED SCREENING METHODS

An overview of the applied screening methods is presented in Table 3.

Labs 2 and 4 applied the screening tests approved by the FASFC for antimicrobial testing in the framework of the determination of the quality of raw farm milk [6, 7 and 8]. According to this test scheme, all milk samples are first screened for inhibitory substances with the Delvotest T and positive screened samples are successively screened for ß-lactam antibiotics with the βeta-s.t.a.r. 25, for (ß-lactam antibiotics,) sulfonamides and tetracyclines with the Trisensor (Milk) (Unisensor s.a.), for aminoglycosides with the 4-Aminosensor (Milk) (Unisensor s.a.), for tylosin with the

Tylosensor (Milk) (Unisensor s.a.) and for fluoroquinolones with the Quinosensor (Milk) (Unisensor s.a.). The other laboratories applied the screening tests they use in routine for the analysis of goats' milk. Lab 1 also participated in this ring test with the Delvotest T to investigate if this test can be used to analyse goats' milk samples in the framework of monitoring programmes.

Table 3. Overview of the applied screening methods.

Lab	Test(s) used for the screening for					
	ß-lactams	**Sulfonamides**	**Tetracyclines**	**Macrolides**	**Aminoglycosides**	**Quinolones**
1	CMT Milk Test Delvotest T (3h) (defatted & preheated sample and sample treated with ß-lactamase ES) ßeta-s.t.a.r. (sample treated with penase) SNAP	CMT Milk Test Delvotest T (3h) (defatted & preheated sample and sample treated with PABA)	*B. cereus*-test Delvotest T (3h) (defatted & preheated sample) ßeta-s.t.a.r. Combo	CMT Milk Test Delvotest T (3h) (defatted & preheated sample) CharmII Macrolides Tylosensor	CMT Milk Test Delvotest T (3h) (defatted & preheated sample)	*E. coli*-test (defatted & preheated sample)
2	Delvotest T (3h10min) ßeta-s.t.a.r. 25 (Trisensor (BL))	Delvotest T (3h10min) Trisensor (S)	Delvotest T (3h10min) Trisensor (T)	Delvotest T (3h10min) Tylosensor	Delvotest T (3h10min) 4-Aminosensor	Delvotest T (3h10min) (Quinosensor)
3	*G. stearothermophilus* var. *calidolactis* pH 7.0	*B. pumilus* pH 7.0	*B. cereus* pH 6.0	*K. rhizophila* pH 8.4	*B. megatherium* pH 8.5	*E. coli* pH 8.0
4	Delvotest T (3h15min) ßeta-s.t.a.r. 25 (Trisensor (BL))	Delvotest T (3h15min) Trisensor (S)	Delvotest T (3h15min) Trisensor (T)	Delvotest T (3h15min) (Tylosensor)	Delvotest T (3h15min) (4-Aminosensor)	Delvotest T (3h15min) (Quinosensor)

** Lab 3 analysed the samples with a microbiological multi-plate method. For the screening of novobiocin and rifamycin, S. epidermis pH 6.0 test plates were used.*
BL: ß-lactam; S: sulfonamides; T: tetracyclines.
Cut-off Z-value DelvoScan for Delvotest T: -3.00; cut-off CIF-value C-Scan for CMT Milk Test: 4.50.
Cut-off ratio AccuScan III Reader for ßeta-s.t.a.r.: 1.000. Cut-off ratio ReadSensor for Trisensor, 4-Aminosensor, Tylosensor and Quinosensor: 1.10.
Cut-off ratio Snapshot Reader for SNAP: 1.00.

5. RESULTS AND DISCUSSION

All laboratories analysed the samples within the indicated timeframe.

5.1 Sample A

Table 4. Results of sample A, spiked with 50 µg/kg tylosin A (MRL milk = 50 µg/kg).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (0.48) CMT Milk Test: NEG (3.8) Charm II Macrolides Milk: POS Tylosensor: POS (0.19)	Delvo T: POS (-1.1) 4-aminosensor: POS (0.53 genta; 0.80 strepto)	POS	Delvo T: NEG (-3.19)
INTERPRETATION	Tylosin	Gentamycin Streptomycin	Macrolide	No traces of inhibitory substances detected

Sample A was doped with 50 ppb tylosin A and was screened positive by laboratories 1, 2 and 3. Lab 3 was able to specify the group of inhibitory substances as macrolides. Lab 1 was able to identify the inhibitory substance as tylosin.

Lab 2 obtained a positive screening result for this milk sample with the 4-Aminosensor on both the gentamycin line and the streptomycin line. So, **false positive results** were obtained by lab 2 with the 4-Aminosensor on both lines for the milk sample doped with 50 ppb tylosin A.

Extra tests at ILVO-T&V confirmed that false positive results can be obtained with the 4-Aminosensor for goats' milk spiked with 50 ppb tylosin A.

Note that the results obtained by lab 2 are wrongly reported as gentamycin and streptomycin since also neomycin and kanamycin are detected on the gentamycin line and also dihydrostreptomycin is detected on the streptomycin line.

Lab 4 reported a (borderline) negative result for the sample spiked with 50 ppb tylosin A (Z-value: -3.19) which is indicating that the incubation time of the Delvotest T plates was too long. A shorter incubation time of the Delvotest T plates however should have resulted in a false positive result for the blank milk sample (see results for sample B). For raw cows' milk, a detection capability of 45 µg/kg was obtained with the Delvotest T for tylosin A (validation study ILVO-T&V, not published yet).

5.2 Sample B

Table 5. Results of sample B, a blank milk sample.

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: NEG (-6.62) CMT Milk Test: NEG (1.2) *B. cereus*-test: NEG *E. coli*-test: NEG ßeta-s.t.a.r.: NEG (6.904)	Delvo T: NEG (-7.1)	NEG	Delvo T: NEG (-3.42)
INTERPRETATION	Antibiotics or sulfonamides not detected	Negative		No traces of inhibitory substances detected

Sample B was a raw milk sample free from inhibitory substances.

All laboratories found a negative result for this blank milk sample. Hence, no false positive results were obtained.

However, the Z-value obtained by lab 4 (-3.42) for the blank milk sample is too close to the cut-off Z-value (-3.00) which is indicating that the incubation time of the Delvotest T plates was too short. This is in contradiction with the results obtained for the sample spiked with 50 ppb tylosin A (sample A).

5.3 Sample C

Table 6. Results of sample C, spiked with 100 µg/kg chloortetracycline (MRL milk = 100 µg/kg).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (0.38) CMT Milk Test: NEG (0.9) *B. cereus*-test: POS ßeta-s.t.a.r. Combo: POS TETRA (0.205)	Delvo T: POS (-1.7) Trisensor TET: POS (0)	POS	Delvo T: POS (-1.81) Trisensor TET: POS (0.00)
INTERPRETATION	Tetracyclines (group)	Tetracycline	Tetracyclin	Inhibitory substances (tetracyclines)

Sample C was spiked with 100 ppb chloortetracycline and was screened positive for tetracyclines by all laboratories.

5.4 Sample D

Table 7. Results of sample D, spiked with 4 µg/kg amoxicillin (MRL milk = 4 µg/kg).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (13.14) CMT Milk Test: POS (8.9) ßeta-s.t.a.r.: POS (0.128) ßeta-s.t.a.r. + penase: NEG (10.076)	Delvo T: POS (11.5) ßeta-s.t.a.r. 25: POS (0.173)	POS	Delvo T: POS (14.40) ßeta-s.t.a.r. 25: POS (0.979)
INTERPRETATION	Benzylpenicillin, ampicillin and/or amoxicillin	ß-lactam	ß-lactam	Inhibitory substances (ß-lactam)

Sample D was raw milk spiked with 4 ppb amoxicillin and was screened positive for ß-lactam antibiotics by all laboratories.

Lab 1 was able to specify that the ß-lactam antibiotic was either benzylpenicillin, ampicillin and/or amoxicillin.

5.5 Sample E

Table 8. Results of sample E, spiked with 100 µg/kg sulfadiazine (MRL milk = 100 µg/kg).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (9.26) Delvo T + PABA: NEG (-4.07) CMT Milk Test: POS (7.4)	Delvo T: POS (9.5) Trisensor SULF: POS (0)	POS	Delvo T: POS (12.30) Trisensor SUL: POS (0.00)
INTERPRETATION	Sulfonamide	Sulfamide	Sulfonamide	Inhibitory substances (sulfonamides)

Sample E was a raw milk sample spiked with 100 ppb sulfadiazine and was screened positive for sulfonamides by all laboratories.

5.6 Sample F

Table 9. Results of sample F, spiked with 40 µg/kg cefalexin (no MRL in caprine milk; MRL of 100 µg/kg in bovine milk).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (9.60) Delvo T + ß-lactamase ES: POS (0.62) CMT Milk Test: POS (6.0) ßeta-s.t.a.r.: NEG (8.001) SNAP: POS (2,53)	Delvo T: POS (9.1) ßeta-s.t.a.r. 25: NEG (7.72) Delvo T + penicillinase: POS (-2.88) Trisensor BL: NEG (2.45) 4-aminosensor: POS (0.58 genta; 0.83 strepto)	NEG	Delvo T: POS (8.45) ßeta-s.t.a.r. 25: NEG (10.223) Delvo T + penicillinase: POS (-1.11) Trisensor BL: NEG (2.37)
INTERPRETATION	ß-lactam	Gentamycin Streptomycin		No traces of inhibitory substances detected

Sample F, spiked with 40 ppb cefalexin was screened positive by labs 1, 2 and 4.

Lab 3 reported a negative result for this milk sample. Note that the detection capability claimed by this lab for cefalexin is only 75 ppb, which is rather high for a compound that is not allowed in raw goats' milk.

Lab 1 was able to specify the group of inhibitory substances as ß-lactam antibiotics. Since a decrease of the Z-value was noticed with the Delvotest T after addition of ß-lactamase ES and taking into account that the ßeta-s.t.a.r. is completely missing cefalexin at the MRL of bovine milk [9], an additional SNAP Test, resulting in a positive result, was performed.

Labs 2 and 4 screened this sample as positive with the Delvotest T but were not able to specify that the inhibition was caused by a ß-lactam antibiotic. Both labs neglected the fact that the addition of penicillinase to this sample resulted in a serious drop in inhibition (decrease of more than 9 Z-values), which is indicating the presence of ß-lactam antibiotics. It is known that the ßeta-s.t.a.r. is not able to detect concentrations below 1,000 µg/kg of cefalexin in bovine milk [9].

Despite the positive Delvotest T indicating the presence of inhibitory substances, lab 4 wrongly reported that no traces of inhibitory substances were detected in this sample.

Lab 2 obtained positive screening results for this milk sample with the 4-Aminosensor on both the gentamycin line and the streptomycin line. So, **false positive results** were obtained by lab 2 with the 4-Aminosensor on both lines for the milk sample doped with 40 ppb cefalexin.

Extra tests at ILVO-T&V confirmed that false positive results can be obtained with the 4-Aminosensor for goats' milk spiked with 40 ppb cefalexin.

Note that the results obtained by lab 2 are wrongly reported as gentamycin and streptomycin since also neomycin and kanamycin are detected on the gentamycin line and also dihydrostreptomycin is detected on the streptomycin line.

Sample G

Table 10. Results of sample G, a blank milk sample.

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: NEG (-5.11) CMT Milk Test: NEG (1.4) *B. cereus*-test*: NEG* *E. coli*-test: NEG ßeta-s.t.a.r.: NEG (7.792)	Delvo T: NEG (-7.9)	NEG	Delvo T: NEG (-6.84)
INTERPRETATION	Antibiotics or sulfonamides not detected	Negative		No traces of inhibitory substances detected

Sample G was another blank milk sample.

All laboratories found a negative result for this blank milk sample. Hence, no false positive results were obtained.

Remark that lab 4 obtained a normal Z-value (-6.84) for this second blank milk sample with the Delvotest T.

5.8 Sample H

Table 11. Results of sample H, spiked with 30 µg/kg cloxacillin (MRL milk = 30 µg/kg).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (11.25) CMT Milk Test: POS (9.0) ßeta-s.t.a.r.: POS (0.049) ßeta-s.t.a.r. + penase : POS (0.012)	Delvo T: POS (11.4) ßeta-s.t.a.r. 25: POS (0.008)	POS	Delvo T: POS (14.40) ßeta-s.t.a.r. 25: POS (0.034)
INTERPRETATION	ß-lactam	ß-lactam	ß-lactam	Inhibitory substances (ß-lactam)

Sample H was raw milk spiked with 30 ppb cloxacillin.

This sample was screened positive for ß-lactam antibiotics by all laboratories.

5.8 Sample I

Table 12. Results of sample I, spiked with 4 µg/kg benzylpenicillin (MRL milk = 4 µg/kg).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (12.16) CMT Milk Test: POS (9.0) ßeta-s.t.a.r.: POS (0.002) ßeta-s.t.a.r. + penase: NEG (10.395)	Delvo T: POS (11.9) ßeta-s.t.a.r. 25: POS (0.006)	POS	Delvo T: POS (13.96) ßeta-s.t.a.r. 25: POS (0.014)
INTERPRETATION	Benzylpenicillin, ampicillin and/or amoxicillin	ß-lactam	ß-lactam	Inhibitory substances (ß-lactam)

Sample I was raw milk spiked with 4 ppb benzylpenicillin and was screened positive for ß-lactam antibiotics by all laboratories.

Lab 1 was able to specify that the ß-lactam antibiotic was either benzylpenicillin, ampicillin and/or amoxicillin.

5.9 Sample J

Table 13. Results of sample J, spiked with 25 µg/kg cefoperazone (no MRL in caprine milk; MRL of 50 µg/kg in bovine milk).

	LAB 1	LAB 2	LAB 3	LAB 4
RESULT	Delvo T: POS (9.76) CMT Milk Test: POS (5.9) ßeta-s.t.a.r.: POS (0.000) ßeta-s.t.a.r. + penase: POS (0.046)	Delvo T: POS (8.0) ßeta-s.t.a.r. 25: POS (0.001)	POS	Delvo T: POS (6.43) ßeta-s.t.a.r. 25: POS (0.003)
INTERPRETATION	ß-lactam	ß-lactam	ß-lactam	Inhibitory substances (ß-lactam)

Sample J was doped with 25 ppb cefoperazone and was found positive for ß-lactam antibiotics by all laboratories.

6. CONCLUSIONS

No false positive results were obtained by **lab 1** for the blank milk samples and, for all doped samples, the group of antibiotics or sulfonamides could be identified. Since the Delvotest T is detecting in raw goats' milk the investigated penicillins, tylosin A, chlortetracycline and sulfadiazine at their respective MRL and also the investigated cephalosporins, it can be concluded that the Delvotest T can replace the CMT Milk Test, the *B. cereus*-test and the Charm II Sulfa Drugs Test to analyse goats' milk samples in monitoring programmes.

No false positive results were obtained by **lab 2** for the blank milk samples.

With exception of the negative result obtained for the sample doped with 40 ppb cefalexin (sample F), the groups of anti-infectious agents could be correctly identified for all other doped samples.

Lab 2 neglected the fact that the addition of penicillinase to the sample spiked with 40 ppb cefalexin (sample F) resulted in a serious drop in inhibition (decrease of more than 11 Z-values), which is indicating the presence of penicillins or cephalosporins. It is known that the ßeta-s.t.a.r. is not able to detect concentrations below 1,000 µg/kg of cefalexin in bovine milk [9].

Lab 2 obtained false positive results with the 4-Aminosensor, on both the gentamycin line and the streptomycin line, for the sample spiked with 50 ppb tylosin A (sample A) and for the sample spiked with 40 ppb cefalexin (sample F). Extra tests at ILVO-T&V confirmed that false positive results can be obtained with the 4-Aminosensor for goats' milk.

Lab 2 wrongly reported the results obtained with the 4-Aminosensor as gentamycin and streptomycin since also neomycin and kanamycin are detected on the gentamycin line and also dihydrostreptomycin is detected on the streptomycin line.

No false positive results were obtained by **lab 3** for the blank milk samples.

With exception of the negative result obtained for the sample doped with 40 ppb cefalexin (sample F), the groups of anti-infectious agents could be correctly identified for all other doped samples. Note that the detection capability claimed by lab 3 for cefalexin is only 75 ppb, which is rather high for a compound that is not allowed in raw goats' milk.

No false positive results were obtained by **lab 4** for the blank milk samples. However, lab 4 obtained a too low Z-value with the Delvotest T for the first blank milk sample (sample B).

With exception of the negative results obtained for the sample doped with 50 ppb tylosin A (sample A) and the sample spiked with 40 ppb cefalexin (sample F), the groups of anti-infectious agents could be correctly identified for all other doped samples.

The borderline negative result obtained for the sample spiked with 50 ppb tylosin A is indicating that the incubation time of the Delvotest T plates was too long. In contrast, a shorter incubation time of the Delvotest T plates should have resulted in a false positive result for the first blank milk sample.

Lab 4 neglected the fact that the addition of penicillinase to the sample spiked with 40 ppb cefalexin (sample F) resulted in a serious drop in inhibition (decrease of more than 9 Z-values), which is indicating the presence of ß-lactam antibiotics. It is known that the ßeta-s.t.a.r. is not able to detect concentrations below 1,000 µg/kg of cefalexin in bovine milk [9].

Despite the positive Delvotest T indicating the presence of inhibitory substances in the sample spiked with 40 ppb cefalexin (sample F), lab 4 wrongly reported the obtained results as "no traces of inhibitory substances detected".

7. REFLECTIONS

Labs 2 and 4 used the same screening tests and the same test scheme as in the previous ring trial for antibiotics and sulfonamides in raw goats' milk but in this ring test they did not decide about penalization.

It is remarkable that, in this ring trial, there were no false positive results obtained with the Delvotest T.

First of all, it was advised to the Interprofessional Organisms to analyse control samples of raw goats' milk (same animal species !) in order to determine the correct incubation time for raw goats' milk for the batch of Delvotest T used (often exceeding 3 hours for raw goats' milk) and hence to limit the number of false positive results.

In the previous ring trial, it was also stated that the rate of false positive results obtained with a microbiological inhibitor test could be decreased by defatting and preheating the milk sample (10 minutes at 80°C) before testing. It is not known if labs 2 and 4 also defatted and preheated the milk samples before analysis.

Finally, it was noticed in 2013 at ILVO-T&V that the Delvotest T plates have become more stable and that less false positive results were generated.

In this ring test, false positive results are obtained with the 4-Aminosensor for goats' milk. So, positive results should always be handled with care and have to be confirmed with another test (by preference based on another principle or by chromatographic methods). Remark that Unisensor s.a. is not claiming that the 4-Aminosensor is also suited for goats' milk.

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