FLANDERS RESEARCH INSTITUTE FOR AGRICULTURE, FISHERIES AND FOOD
TECHNOLOGY AND FOOD SCIENCE UNIT

REPORT RING TEST

"SCREENING FOR ANTIMICROBIAL SUBSTANCES WITH THE NEW BELGIAN KIDNEY TEST (NBKT)"

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Sigrid Ooghe & Wim Reybroeck
ILVO

1. INTRODUCTION

The Flanders Research Institute for Agricultural, Fisheries and Food (ILVO) organised in November 2016 as National Reference Laboratory (NRL) Chemistry for substances with anabolic effect and veterinary drugs - in consortium with CER-Groupe - a third ring test "Screening for antimicrobial substances with the New Belgian Kidney Test", in collaboration with the Federal Agency for the Safety of the Food Chain (FASFC).
This ring test was organised to follow up the unsatisfactory results that some laboratories obtained for the previous ring test(s) [1] & [2].
This proficiency test was obligatory for the following approved laboratories: CARAH, EURACETA, Eurofins Food Testing Belgium (EUROFINS-FOOD), Servaco Food Control (FOOD CONTROL), Hainaut Vigilance Sanitaire (HVS), LARECO, LOVAP and Quality Partner (QP).
The labs were asked to perform the NBKT as described in the Ministerial Decree of 19 June 1995 [3] which is an amendment of the Ministerial Decree of 18 December 1973 [4].

2. PLANNING OF THE RING TEST

On 12 October 2016, the above mentioned laboratories and also some private laboratories and slaughterhouses were invited to participate. Finally, 12 laboratories subscribed to the ring test. Only labs 12 and 14 of the last ring test didn't subscribe. In order to facilitate the comparison of the results of the labs for the 3 ring tests, the anonymous codes of the labs were retained.
On 22 November, a parcel containing 8 blind coded antibiotic disks (in double) was sent to the participants by postal service. The participants were asked to store the disks refrigerated (below 6°C) upon arrival and to analyse the disks in week 47.
It was asked to return the results and the interpretation of the results before 2 December using the specific results form.

3. SAMPLES

Since the results of the previous ring tests were not very satisfactory, it was decided to send again (spiked) antibiotic disks to the participants and no kidney material to avoid discussions about matrix homogeneity or interferences by kidney juice.

The disks were prepared and tested at ILVO on 21 November. The antibiotics were chosen according to the active substances of veterinary drugs registered for pork in Belgium [5].

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

Table 1. Codification of the antibiotic disks.

Blind coded disk spiked with	CODE												
	General	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11	Lab 13
0.5 µg sulfadimethoxine	A	3	15	24	25	39	48	51	59	67	75	81	91
1 µg lincomycin	B	1	12	21	29	34	42	49	64	68	76	87	89
0.1 µg oxytetracycline	C	6	10	17	26	38	43	53	60	72	77	84	96
2 µg ceftiofur	D	2	16	18	32	35	47	55	62	65	79	82	93
0.5 µg amoxicillin	E	8	11	22	31	37	44	50	58	66	73	86	90
1 µg sulfadiazine	F	5	13	23	27	40	41	56	63	71	74	85	92
0.1 µg enrofloxacin	G	4	14	20	28	33	45	52	61	69	78	88	95
BLANK DISK	H	7	9	19	30	36	46	54	57	70	80	83	94

All labs received the disks on 23 November. Only laboratory 13 did not return the form "acknowledgement of receipt of samples", confirming that the samples arrived in good condition.

4. SCREENING METHOD

The procedure, as described in the national legislation, recommends the use of Test Agar pH 7.2 (Merck 15/87 or equivalent), addition of 0.4% dextrose, sterilization at 121°C during 15 minutes, adjustment of the pH to 7.2 and addition of 0.2 µg of trimethoprim per ml agar and 0.1% (V/V) of a spore solution of 10^7 spores of *Bacillus subtilis* BGA per ml. A layer of 2 mm agar is obtained by pouring 14 ml agar medium in petri dishes of 9 cm diameter. Petri dishes have to be incubated at 30°C during 16-24 hours.

Since Test Agar pH 7.2 became no longer commercially available, two alternatives were suggested by Cornet *et al.* [6] namely Standard II Nutrient Agar from Merck (commercially available at VWR Int.) and Niertest Agar (Base) from Biotrading (Tritium Microbiologie (NL),

commercially available at Led Techno and Bio-Rad Laboratories). The medium of Tritium Microbiologie is available in ready-to-use plates (N001) and in bottles to which trimethoprim and spores have to be added (N003). Last year, the composition of the ready-to-use plates has been changed by Tritium Microbiologie (available as N022).

These alternative media are slightly different in composition compared to Test Agar pH 7.2.

The labs are also allowed to compose their own agar medium out of single ingredients.

ILVO and labs 1, 2, 3, 4, 8 and 11 prepared their own "Test Agar pH 7.2" medium in the right proportions starting from Bacto Agar and they added Bacto Peptone, Bacto Casitone, NaCl and dextrose. Lab 9 used petri dishes prepared by lab 8.

Labs 5 and 7 used Standard II Nutrient Agar and added dextrose.

Labs 6, 10 and 13 used the Niertest Agar Base of Tritium Microbiologie (N003).

There were no more labs that used the ("improved") ready-to-use Niertest Agar plates of Tritium Microbiologie (N001 or N022).

Labs using the same test medium are marked in the same colour in Tables 2 till 10 in order to facilitate the interpretation of the results.

All labs seeded their agar medium with the right concentration of *Bacillus subtilis* spores and added trimethoprim to their medium, however in different concentrations ranging from the recommended 0.2 µg trimethoprim per ml of agar till 3 µg trimethoprim per ml of agar. It is known that an increase of the concentration of trimethoprim is resulting in larger inhibition zones, especially for sulphonamides.

Finally, labs 4 and 9 did not analyse the samples in week 47, but later.

5. HOMOGENEITY OF THE SAMPLES

Of each sample 6 disks were randomly analysed at ILVO on different plates and the mean diameter and the standard deviation were calculated. The values for 0.5 µg sulfadimethoxine, 1 µg lincomycin, 0.1 µg oxytetracycline, 2 µg ceftiofur, 0.5 µg amoxicillin, 1 µg sulfadiazine and 0.1 µg enrofloxacin were 27.4±0.5, 23.5±0.7, 16.9±0.3, 23.3±0.9, 28.6±0.2, 28.0±0.6 and 24.4±0.3 mm, respectively (after 17 hours of incubation). No inhibition zones were obtained for the blank disks.

6. RESULTS AND DISCUSSION

In order to facilitate the comparison of the results of the ring tests, the results of the previous ring tests (if performed) are mentioned in between brackets after the current results, whereas the first inhibition zone is the result of the ring test of 2015 and the second inhibition zone the result of the ring test of 2014.

Table 2 gives an overview of the results that the labs obtained for their control antibiotic disks.

Table 2. Inhibition zones (in mm) obtained for the control antibiotic disks.

LAB	Inhibition zone (mm)			
	Sulfadimidine 1 µg (≥17 mm)	Oxytetracycline 1 µg (≥18 mm)	Streptomycin 1 µg (≥20 mm)	Tylosin 1 µg (≥20 mm)
ILVO	20.8 (23.9; 23.5)	25.8 (27.7; 29.1)	22.1 (23.2; 23.8)	27.7 (29.3; 30.3)
1	22.79 (19.81; 22.8)	25.95 (30.73; 25.0)	23.18 (15.91; 24.1)	30.54 (26.66; 29.1)
2	21.1 (20.4; 23.2)	30.3 (28,5; 32.6)	24.3 (22.2; 25.8)	31.0 (27.7; 31.5)
3	18.8 (20.4; 17.6)	24.3 (30.3; 23.2)	25.1 (20.1; 24.2)	30.3 (24.0; 24.3)
4	19.0 (20.5; negative)	27.5 (27.5; 31)	24.5 (22; 26)	29.5 (30; 30)
5	21.2 (19.6; 17)	31.3 (36.0; 34)	27.2 (30.5; 25)	30.1 (33.4; 32)
6	20.6 (20.9; 20.65)	25.6 (26.2; 25.75)	20.2 (20.4; 20.95)	26.6 (23.4; 25.25)
7	22 (20.0; 21.63)	31 (30.3; 35.62)	29 (25.5; 36.47)	32 (29.8; 34.89)
8	22.9 (22.0; 24.99)	23.4 (25.2; 30.13)	26,1 (26.0; 22.92)	30.8 (30.5; 26.17)
9	22.4 (21.7; not used)	25.8 (21.6; not used)	28.7 (25.4; not used)	32.5 (31.2; not used)
10	28.63 (21.13; 26.15)	30.66 (29.18; 32.83)	22.79 (20.69; 21.52)	28.63 (26.34; 29.44)
11	18.20 (16.60; 12.70)	26.40 (32.90; 32.30)	24.10 (18.20; 26.30)	28.55 (24.70; 31.40)
13	17 (18; 25)	21 (21; 31.5)	26 (25; 21)	32 (29; 27)

Note: diameter of paper disk = 12.7 mm; results of previous ring tests in between brackets.

All control disks are meeting the criteria.

Lab 3 interpreted the result of their sulfadimidine control disk as negative although an inhibition zone of 18.8 mm (≥17 mm) was obtained. This lab was probably confusing the cut-off of 17 mm for the sulfadimidine control disk with the cut-off of 20 mm applied for samples.

Labs 4 and 7 didn't interpret the results of their control disks.

The inhibition zone obtained by lab 13 for the sulfadimidine control disk is in fact too small and hence the sensitivity of their test plates is too low for the group of sulphonamides. See also the results of lab 13 for disks A and F.

6.1 Disk A

Table 3. Results of disk A, spiked with 0.5 µg sulfadimethoxine.

LAB	Inhibition zone (mm)				Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	CONTROL DISK sulfadimidine 1 µg	DISK A			
		1	2	Average	
ILVO	20.8 (23.9; 23.5)	27.2	27.4	27.3	POS
1	22.79 (19.81; 22.8)	26.02	25.39	25.71	POS
2	21.1 (20.4; 23.2)	25.0	26.0	25.5	POS
3	18.8 (20.4; 17.6)	21.9	22.2	22.1	POS
4	19.0 (20.5; NEG)	22	22	22	POS
5	21.2 (19.6; 17)	23.3	24.3	23.8	POS
6	20.6 (20.9; 20.65)	24.1	24.1	24.1	POS
7	22 (20.0; 21.63)	27.1	27.1	27	POS
8	22.9 (22.0; 24.99)	24.8	24.2	24.5	POS
9	22.4 (21.7; not used)	24.1	24.2	24.2	POS
10	28.63 (21.13; 26.15)	27.71	28.16	27.94	POS
11	18.20 (16.60; 12.70)	21.00	20.90	20.95	POS
13	17 (18; 25)	**18**	**17**	**17.5**	**NEG**

Note: diameter of paper disk = 12.7 mm.

Lab 13, that reported a borderline positive result (17 mm) for their control disk spiked with 1 µg sulfadimidine, reported a negative result for disk A. Hence, lab 13 obtained a **false negative result** for disk A and is not screening sensitively the group of sulphonamides with their plates.

All other labs reported a positive result for disk A.

Remark also that labs 3, 4 and 11 that obtained smaller inhibition zones for their control disk spiked with 1 µg sulfadimidine, also obtained smaller inhibition zones for disk A.

6.2 Disk B

Table 4. Results of disk B, spiked with 1 μg lincomycin.

LAB	Inhibition zone for DISK B (mm)			Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	1	2	Average	
ILVO	23.9 (29.7)	22.5 (27.2)	23.2 (28.5)	POS (POS)
1	22.57 (24.7)	19.51 (23.5)	21.04 (24.1)	POS (POS)
2	24.5 (26.6)	24.0 (26.7)	24.3 (26.65)	POS (POS)
3	24.8 (<15)	24.9 (<15)	24.9 (<15)	POS (NEG)
4	23 (26)	23 (26)	23 (26)	POS (POS)
5	26.3 (20)	26.5 (21)	26.4 (21)	POS (POS)
6	**15.8 (16.1)**	**15.8 (16.0)**	**15.8 (16.05)**	**NEG (NEG)**
7	27.7 (30.17)	28.3 (31.05)	28 (30.61)	POS (POS)
8	25.8 (18.51)	25.0 (19.07)	25.4 (18.79)	POS (NEG)
9	25.2 (13)	25.9 (/)	25.6 (13)	POS (NEG)
10	**16.53 (19.36)**	**17.16 (19.34)**	**16.85 (19.35)**	**NEG (NEG)**
11	26.30 (31.00)	23.85 (29.00)	25.10 (30.00)	POS (POS)
13	31 (21)	31 (21)	31 (21)	POS (POS)

Note: diameter of paper disk = 12.7 mm; results of the first ring test in between brackets.

Labs 6 and 10 reported a negative result for disk B. the other labs reported a positive result for disk B. Hence, labs 6 and 10 obtained **false negative results**.

It's worth noting that labs 6 and 10 were both using Niertest Agar Base. On the other side, the largest inhibition zones were obtained by lab 13, also using the same test medium.

6.3 Disk C

Table 5. Results of disk C, spiked with 0.1 μg oxytetracycline.

LAB	Inhibition zone (mm)				Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	CONTROL DISK Oxytetracycline 1 μg	DISK C			
		1	2	Average	
ILVO	25.8 (27.7; 29.1)	15.9	16.3	16.1	NEG
1	25.95 (30.73; 25.0)	16.94*	16.45*	16.69	NEG
2	30.3 (28.5; 32.6)	21.9	21.9	21.9	POS
3	24.3 (30.3; 23.2)	16.0	16.0	16.0	NEG
4	27.5 (27.5; 31)	16.5	17	16.5	NEG
5	31.3 (36.0; 34)	24.7	25.5	25.1	POS
6	25.6 (26.2; 25.75)	17.2	17.2	17.2	NEG
7	31 (30.3; 35.62)	24	24.6	24	POS
8	23.4 (25.2; 30.13)	14.9	13	14.0	NEG
9	25.8 (21.6; not used)	13	13	13	NEG
10	30.66 (29.18; 32.83)	20.63	20.30	20.47	POS
11	26.40 (32.90; 32.30)	14.70	13.25	14.00	NEG
13	21 (21; 31.5)	13	13	13	NEG

Note: diameter of paper disk = 12.7 mm; *: difficult to measure the inhibition zone (no clear lining).

Disk C should give small inhibition zones and hence a negative result but inhibition zones (slightly) bigger than 20 mm (and hence a positive result) are unavoidable. Remark that homogeneity testing at ILVO resulted in inhibition zones of 16.9±0.3 mm.

With the exception of labs 2, 5, 7 and 10 that reported a positive result for disk C, all other labs reported a negative result. Remark that labs 2, 5, 7 and 10 also obtained large inhibition zones (> 30.0 mm) for their control disk spiked with 1 μg oxytetracycline. Hence, labs 2, 5, 7 and 10 are screening more sensitively the group of tetracyclines with their plates. Remark also that the test medium used by labs 5 and 7 is resulting in larger inhibition zones for tetracyclines.

6.4 Disk D

Table 6. Results of disk D, spiked with 2 µg ceftiofur.

LAB	Inhibition zone for DISK D (mm)			Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	1	2	Average	
ILVO	23.1 (26.7; 24.1)	21.9 (25.7; 25.0)	22.5 (26.2; 24.6)	POS (POS; POS)
1	**19.68*** (26.2; 24.4)	20.09* (25.63; 26.6)	**19.88** (25.81; 25.5)	**NEG** (POS; POS)
2	**18** (17.4; 20.9)	**18** (18.3; 21.4)	**18** (17.8; 21.15)	**NEG** (NEG; POS)
3	26.9 (28.4; 25.7)	26.8 (27.8; 25.9)	26.9 (28.1; 25.8)	POS (POS; POS)
4	26 (29; 17)	27 (42; 17)	26.5 (35.5; 17)	POS (POS; NEG)
5	24.6 (27.9; 24)	24.4 (28.2; 22)	24.5 (28.1; 23)	POS (POS; POS)
6	44.1 (20.3; 45.4)	44.1 (20.9; 44.5)	44.1 (20.6; 44.95)	POS (POS; POS)
7	19.2* (20.1; 25.66)	19.8* (20.4; 26.30)	20 (20.2; 25.98)	POS (POS; POS)
8	21.3 (26.4; 23.02)	22.6 (24.9; 22.50)	22.0 (25.7; 22.76)	POS (POS; POS)
9	23.8 (25.7; 24)	23.0 (/, /)	23.4 (25.7; 24)	POS (POS; POS)
10	58.00 (0; 33.17)	64.00 (/, 34.25)	61.00 (0; 33.71)	POS (NEG; POS)
11	28.30 (32.20; 42.10)	27.00 (31.00; 46.10)	27.65 (31.60; 44.10)	POS (POS; POS)
13	**14** (43 ; 48)	**14** (43 ; 50)	**14** (43; 49)	**NEG** (POS; POS)

Note: diameter of paper disk = 12.7 mm; results of the previous ring tests in between brackets: *: difficult to measure the inhibition zone (no clear lining).

Labs 1, 2 and 13 reported a negative result for disk D; the other labs reported a positive result for disk D. Hence, labs 1, 2 and 13 obtained **false negative results**.

Remark that the individual inhibition zones obtained by lab 7 are smaller than these obtained by lab 1, but that lab 7 interpreted the result as positive (due to numeric rounding) whereas lab 1 interpreted the result as negative.

Note that labs 6 and 10, both using Niertest Agar Base, reported extremely large inhibition zones for disk D and that lab 13, using the same test medium, obtained no inhibition zone. The same detection pattern was observed for lincomycin (disk B) but for this substance larger inhibition zones were obtained by lab 13.

6.5 Disk E

Table 7. Results of disk E, spiked with 0.5 µg amoxicillin.

LAB	Inhibition zone for DISK E (mm)			Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	1	2	Average	
ILVO	25.4	25.8	25.6	POS
1	25.63	26.31	25.97	POS
2	26.0	24.4	25.2	POS
3	27.4	27.0	27.2	POS
4	24	24	24	POS
5	31.3	31.7	31.5	POS
6	26.2	25.1	25.7	POS
7	31.1	29.8	30	POS
8	25.4	25.6	25.5	POS
9	24.1	24.1	24.1	POS
10	25.76	27.77	25.27	POS
11	24.50	24.40	24.45	POS
13	24	22	23	POS

Note: diameter of paper disk = 12.7 mm.

All labs reported a positive result for disk E. Hence, no false negative results were obtained for disk E.

6.6 Disk F

Table 8. Results of disk F, spiked with 1 µg sulfadiazine.

LAB	Inhibition zone (mm)				Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	CONTROL DISK sulfadimidine 1µg	DISK F			
		1	2	Average	
ILVO	20.8 (23.9; 23.5)	27.2 (30.0; 29.5)	27.4 (30.9; 29.4)	27.3 (30.5; 29.5)	POS (POS; POS)
1	22.79 (19.81; 22.8)	27.10 (28.95; 33.6)	26.42 (28.25; 32.8)	26.76 (28.6; 33.2)	POS (POS; POS)
2	21.1 (20.4; 23.2)	26.4 (26.6; 27.4)	26.6 (26.0; 27.0)	26.5 (26.3; 27.2)	POS (POS; POS)
3	18.8 (20.4; 17.6)	22.9 (29.0; <15)	23.1 (28.6; <15)	23.0 (28.8; <15)	POS (POS; NEG)
4	19.0 (20.5; NEG)	24 (23; /)	25 (25; 15)	24.5 (24; 15)	POS (POS; NEG)
5	21.2 (19.6; 17)	27.6 (25.3; 18)	26.7 (25.7; 18)	27.2 (25.5; 18)	POS (POS; NEG)
6	20.6 (20.9; 20.65)	25.6 (26.1; 29.5)	25.6 (29.0; 29.5)	25.6 (27.6; 29.5)	POS (POS; POS)
7	22 (20.0; 21.63)	27.8 (26.8; 26.11)	28.1 (27.7; 26.06)	28 (27.2; 26.09)	POS (POS; POS)
8	22.9 (22.0; 24.99)	25.3 (25.1; 33.09)	25.0 (26.3; 32.58)	25.2 (25.7; 32.84)	POS (POS; POS)
9	22.4 (21.7; not used)	24.7 (27.9; 13)	24.7 (/; /)	24.7 (/; 13)	POS (POS; NEG)
10	28.63 (21.13; 26.15)	28.87 (26.26 ; 33.69)	30.04 (/; 34.65)	29.46 (26.26; 34.17)	POS (POS; POS)
11	18.20 (16.60; 12.70)	23.90 (27.65; 12.70)	23.10 (26.50; 12.70)	23.50 (27.08; 12.70)	POS (POS; NEG)
13	17 (18; 25)	**19** (21; 33)	**18** (21; 33)	**18.5** (21; 33)	**NEG** (POS; POS)

Note: diameter of paper disk = 12.7 mm; results of the previous ring tests in between brackets.

Lab 13, that reported a borderline positive result (17 mm) for their control disk spiked with 1 µg sulfadimidine, reported a negative result for disk F. Hence, lab 13 obtained a false negative result for disk F and is not screening sensitively the group of sulphonamides with their plates. All other labs reported a positive result for disk F. Once again, the results obtained by lab 13 are not in line with the results obtained by labs 6 and 10, despite they are using the same test medium.

Remark also that labs 3, 4 and 11 that obtained smaller inhibition zones for their control disk spiked with 1 µg sulfadimidine, also obtained smaller inhibition zones for disk F.

6.7 Disk G

Table 9. Results of disk G, spiked with 0,1 µg enrofloxacin.

LAB	Inhibition zone for DISK G (mm)			Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	1	2	Average	
ILVO	24.6	24.4	24.5	POS
1	22.42	22.44	22.43	POS
2	25.9	25.7	25.8	POS
3	25.2	25.4	25.3	POS
4	27	27	27	POS
5	28.4	28.5	28.5	POS
6	25.0	25.0	25.0	POS
7	28.1	28.9	29	POS
8	24.4	24.5	24.5	POS
9	24.0	24.0	24.1	POS
10	21.25	21.85	21.55	POS
11	30.70	27.25	29.00	POS
13	28	28	28	POS

Note: diameter of paper disk = 12.7 mm.

All labs reported a positive result for disk G. Hence, no false negative results were obtained for disk G.

6.8 Disk H

Table 10. Results of disk H, a blank disk.

LAB	Inhibition zone for DISK H (mm)			Result according to the national legislation: positive (POS, ≥20 mm) or negative (NEG, <20 mm)
	1	2	Average	
ILVO	12.7 (12.7; 12.7)	12.7 (12.7; 12.7)	12.7 (12.7; 12.7)	NEG (NEG; NEG)
1	13 (13; 13)	13 (13; 13)	13 (13; 13)	NEG (NEG; NEG)
2	13 (13; <13)	13 (13; <13)	13 (13; <13)	NEG (NEG; NEG)
3	<15 (<15; <15)	<15 (< 15; <15)	<15 (< 15; <15)	NEG (NEG; NEG)
4	/ (12; /)	/ (12; /)	/ (12; /)	NEG (NEG; NEG)
5	12.7 (12.7; 12)	12.7 (12.7; 12)	12.7 (12.7; 12)	NEG (NEG; NEG)
6	13.0 (12.7; 12.7)	13.0 (12.7; 12.7)	13.0 (12.7; 12.7)	NEG (NEG; NEG)
7	<13 (<13; <13)	<13 (< 13; <13)	<13 (< 13; <13)	NEG (NEG; NEG)
8	13 (13; 13)	13 (13; 13)	13 (13; 13)	NEG (NEG; NEG)
9	13 (13; 13)	13 (/; /)	13 (13; 13)	NEG (NEG; NEG)
10	0 (0; <1)	0 (/; <1)	0 (0: <1)	NEG (NEG; NEG)
11	- (12.90; 12.70)	- (12.90; 12.70)	- (12.90; 12.70)	NEG (NEG; NEG)
13	13 (13; 13)	13 (13; 13)	13 (13; 13)	NEG (NEG; NEG)

Note: diameter of paper disk = 12.7 mm; results of the previous ring tests in between brackets.

Disk H was a blank disk free from antimicrobial substances.

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

The national legislation [3] imposes to measure the diameter of the inhibition zones, including the paper disk (with a diameter of 12.7 mm).

Note that labs 4, 10 and 11 did not take into account the diameter of their paper disks in their result.

Remark also that lab 3 reported inhibition zones <15 mm as described in the former Ministerial Decree of 18 December 1973 [4].

7. CONCLUSIONS

Table 11 gives an overview of the results obtained by the 12 laboratories.

Table 11. Overview of the results per lab and per sample (12 labs, 8 samples).

LAB	Number of correct results	Number of false positive results	Number of false negative results
1	7 (7; 8)	0 (0; 0)	1 (1; 0)
2	7 (7; 8)	0 (0; 0)	1 (1; 0)
3	8 (6; 5)	0 (0; 0)	0 (2; 3)
4	8 (8; 5)	0 (0; 0)	0 (0; 3)
5	8 (8; 6)	0 (0; 0)	0 (0; 2)
6	7 (7; 7)	0 (0; 0)	1 (1; 1)
7	**8 (8; 8)**	**0 (0; 0)**	**0 (0; 0)**
8	8 (8; 7)	0 (0; 0)	0 (0; 1)
9	8 (8; 3)	0 (0; 0)	0 (0; 5)
10	7 (6; 7)	0 (0; 0)	1 (2; 1)
11	8 (7; 6)	0 (0; 0)	0 (1; 2)
13	5 (8; 8)	0 (0; 0)	3 (0; 0)

Note: results of previous ring tests in between brackets.

DISK	Compound	Number of correct results
A	sulfadimethoxine	**11/12**
B	lincomycin	**10/12** (7/13)
C	oxytetracycline	**12/12**
D	ceftiofur	**9/12** (11/14; 12/13)
E	amoxicillin	**12/12**
F	sulfadiazine	**11/12** (13/14; 8/13)
G	enrofloxacin	**12/12**
H	-	**12/12** (14/14; 13/13)

Again, a large variation is observed in the results. In reality, even a larger variation can be expected since in this ring test no manipulation in the handling of kidneys is included. It is clear that still not all laboratories detect antimicrobial substances at the same residue level.

In this ring test, no false positive results were obtained and no false negative results were obtained by labs 3, 4, 5, 7, 8, 9 and 11.

While the two previous ring tests showed 8 and 18 false negative results, respectively, 7 false negative results were obtained in this ring test. So, it can be concluded that the ring test results again slightly improved for most of the laboratories.

Only lab 13 obtained worse results than in the previous ring tests, especially due to the missing of the two sulphonamides, a class of veterinary drugs relevant for animals husbandry. It is not reflected in the results that lab 13 is using the same test medium as labs 6 and 10.

No false negative results were obtained for 0.1 µg oxytetracyline, 0.5 µg amoxicillin and 0.1 µg enrofloxacin; 0.5 µg sulfamethoxine, 1 µg lincomycin, 2 µg ceftiofur and 1 µg sulfadiazine were considered as negative by 1, 2, 3 and 1 out of 12 laboratories, respectively.

Generally, the results of this ring test are again slightly better than the results of the previous ring tests with the exception of lab 13, obtaining 3 false negative results in this ring test.

Note that only lab 7 obtained excellent results in the three ring tests.

Laboratories should strictly follow the prescribed protocol (volume of agar in petri dish, incubation temperature, duplicate analyses, interpretation of results, ...) . This also implies the compulsory use of control disks, and subsequently, the disqualification of plates not fulfilling the criteria (i.e. plates shown not to be fit for purpose, are not be used).

8. REFERENCES

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