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# Veterinary drugs and growth promoting agents in animal products

Annual report 2010 of the National Reference Laboratory

RIKILT Report 2011.014

A.A.M. Stolker and S.S. Sterk

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**Report 2011.014** **October 2011**

Project number: 72.036.01
BAS-code: WOT-02-003-025
Project title: National Reference Laboratory 96/23/EC

Project leader: A.A.M. Stolker, S.S. Sterk

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This research was (partly) funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation (WOT programme Food Safety, Theme Veterinary drugs).

**Distribution list:**

- Food and Consumer Product Authority (nVWA-Oost), (J. A. van Rhijn, E. van de Made, H. Keukens)
- Food and Consumer Product Safety Authority (nVWA), The Hague (A. Lam)

# Summary

This report of the National Reference Laboratory (NRL) for residues of veterinary drugs in products of animal origin according to 96/23/EC describes the activities employed in 2010. The main tasks of the NRL are the communication with Routine Field Laboratories (RFL), the preparation of quality control samples and the advisory function for the competent authority and RFL. Furthermore the NRL organizes comparative tests, ensures that the RFL observes the residue limits, disseminates information obtained from the European Reference Laboratories (EURLs) and participates in trainings organized by the Commission or by the EURLs

Communication and advices

In the period 2010 the NRL organized 6 official meetings with RFL and several informal meetings by telephone and e-mails. The competent authority was advised on National Plan monitoring activities through the official 'Werkgroep National Plan' meetings. Furthermore the competent authority was advised on the use of hair as sample material, the natural occurrence of Chloramphenicol and its isomers, the occurrence of nortestosterone in porcine animals and on the pro's and contra's of commercial screening-kits for antibiotics.

The RFL asked for GC-MS/MS confirmation analysis for anabolic steroids in urine (187 samples). The samples were 'suspected' after screening by GC-MS..

The NRL advised 3 times National Food control Laboratories outside the Netherlands (Brasil, South Korea, India) - on their request - about different methods of analysis for the determination of veterinary drugs.

Participation in workshops and proficiency tests

The NRL participated in workshops (3 times) and proficiency tests (4 times) organized by EURLs. The NRL participated also in 5 proficiency tests organized by other organizations like FAPAS and Progetto. The results of the proficiency tests were all acceptable (Z-score <2).

RIKILT is accredited (according to ISO 17043 (previously known as ILAC G13:2000)) for the organization of proficiency tests. In 2010 - like in 2009- one international proficiency test for screening and confirmation of antibiotics in bovine muscle was organized by RIKILT. The laboratories were asked to first carry out a screening analysis followed by a quantitative confirmatory analysis for the compounds found suspected. Thirty-six laboratories subscribed for participation in the proficiency study. Within the timeframe of the study 35 laboratories submitted results: 34 laboratories submitted results for the screening analysis and 27 for the quantitative confirmatory part. Three laboratories did not detect any antibiotics using their screening methodology. Seventeen laboratories characterized all three samples correctly (compliant or suspect) based on the screening analysis and of these fourteen laboratories indicated the correct compound groups for all samples. If each method is considered separately, the false negative rate for the microbiological methods is 38%, for biochemical methods this is 25%, both caused

by the Charm II test, and for instrumental analysis this is 23% all caused by missing sulfachloropyridazine.

The proficiency test of 2009 organised by RIKILT included macrolides, quinolones and aminoglycosides in bovine muscle the test of 2010 included tetracyclines, sulfonamides and dapson. The test of 2009 showed a false positive rate of 7%, in 2010 this is 15%.

In the coming period collaboration and communication of the NRL and RFLs will be continued. The NRL website will be optimized. Special attention will be paid to the implementation at the RFL of a GC-MS/MS method for the confirmation of anabolic steroids in urine, LC-MS/MS method for the analysis of coccidiostats in meat and an universal LC system for veterinary drug analysis.

# Contents

# 1 Introduction

The European Commission is committed to protecting consumers from intolerable health hazard, which may be associated with residues of veterinary drugs or even of non-licensed or forbidden substances in animal products intended for human consumption. For this purpose legislation on veterinary drug residue control has been established as the indispensable basis of the consumer protection within the EU. The European residue legislation commits the Member States to establish an annual National Residue Control Plan (NRCP) and provides for the establishment of a hierarchically structured system of European Reference Laboratories (EURLs), National Reference Laboratory (NRLs) and Routine Field Laboratories (RFLs).

The responsibilities of the NRL are described in 96/23/EC and included the following items:

- coordinating the work of the other RFLs responsible for residue analysis, in particular by coordinating the standards and methods of analysis for each residue or residue group concerned,
- assisting the competent authority in organizing the plan for monitoring residues,
- periodically organizing comparative tests for each residue or reside group assigned to them,
- ensuring that national laboratories obey the limits laid down,
- disseminating information supplied by the EURLs,
- ensuring that their staff members are able to take part in further training courses organized by the Commission or by EURLs.

This report described the activities of 2010 of the NRL for veterinary drugs and growth promoting substances according to EU document 96/23/ EC. It covers the groups of compounds assigned to RIKILT-NRL regarding veterinary health viz. nitrofurans, dapson, nitroimidazoles, chloroform, antibiotics (including sulphonamides, quinolones, tetracyclines), anthelmintics, coccidiostats and non-steroidal antiinflammatory drugs (NSAIDs). These groups belong to Group A6, Group B1, B2 (a, b, e) compounds as described in EU document 96/23/EC.

Since 2010 the groups of compounds assigned to RIVM-NRL have been merged with the RIKILT-NRL groups. That means that stilbenes, stilbene derivatives, and their salts and esters, antithyroid agents, steroids, resorcyclic acid lactones (including zeranol) and beta-agonists are added. These groups belong to Group A1, A2, A3, A4 and A5 compounds as described in EU document 96/23/EC.

# 2 Methods

One of the tasks of the NRL is to communicate with the Competent Authority, RFLs and other NRL on issues regarding the control of residues of veterinary drugs. Sometimes the communication is on a regular base and sometimes ad hoc. The same is applicable to the advices given by the NRL. Sometimes advice is requested and sometimes advice is given. The communication and advice activities employed by the NRL in 2010 are described below.

## 2.1 Communications

### 2.1.1 With the Competent Authority

On a regular base there are meetings between the competent authority The Ministry of Economic Affairs, Agriculture and Innovation (EL&I) on the content of the National Monitoring Plans.

In 2010 RIKILT participated in the working group setting up the plan for monitoring residues.

During 2010 there were 3 meetings of this working group. Minutes are available.

### 2.1.2 With Routine Field Laboratory (Food and Consumer Product Safety Authority, Laboratory Region East)

On a regular base the management of RIKILT communicate with the management of the Food and Consumer Product Safety Authority, Laboratory Region East (in this report referred to as nVWA).

On a regular base the analytical technicians of RIKILT communicate with the technicians of nVWA. See also item 3.1.

The NRL had 3 official management meetings with RFL and 6 technical meetings and several times unofficial meetings by telephone and e-mails. Minutes are available of the official meetings.

### 2.1.3 With the National Reference Laboratory

On a regular base the management of the NRL in the field of veterinary drug residues meets the management of other laboratories in the field of veterinary drugs analysis so called Q3 (Quality-assurance, Quality-control, Quartet) meetings. In the past four laboratories viz. RIKILT, Ducares, VWA and RIVM were involved nowadays due to the merger of RIKILT and RIVM laboratories three laboratories are involved in the Q3 meetings.

In 2010 the Q3 group had two official meeting at RIKILT on March 2nd and September 23th in which nVWA, RIKILT and Ducares participated. From these meetings minutes are available.

## 2.2 Advices

### 2.2.1 To the RFL regarding new EU guidelines and regulations

The RFL was informed about the proficiency test and workshops organized by EURLs in Berlin, Fougeres and Wageningen. Three employees of the RFL participated in the workshop organized by the EURL in Wageningen regarding the analyses of anabolic steroids.

### 2.2.2 To the director of EURL-Berlin regarding at NRL available screening and confirmatory analytical test method

The NRL was asked by the EURL-BVL (Berlin) to provide a list of all available screening and confirmatory methods available at the NRL for the analysis of substances belonging to groups A5, B2a, B2b and B2e of Council Directive 96/23/EC. The request letter is attached (Annex 1). The list describing the available screening and confirmatory methods of the NRL was sent to the EURL.

### 2.2.3 To the Competent Authority (nVWA-ministry of EL&I)

The Competente Authority (nVWA-EL&I) was advised by the NRL regarding three different items:

- Problems observed by the use of commercial available antibiotic screening kits. From the results of the NRL proficiency test it was concluded that the commercial available screening test are not always used in a proper way resulting in false negative results. This problem has to be brought to the attention of the EU. Therefore the NRL adviced the compentent authority to initiate this discussion in a Residue Working Meeting in Brussels.
- The use of hair as a sample material for the detection of illegal compounds especially steroid esters.
- The natural occurrence of chloramphenicol and the item of the possible different stereoisomers available from chloroamphenicol.
- The natural occurrence of nortestosterone in porcine animals.

# 3 Coordinating activities

## 3.1 Preparation of quality control samples

The activities employed for the RFL regarding the preparation of quality control samples are described in a separate project (WOT programme on Food Safety - Part 3 Veterinary Drugs; project 7203701 Analytical Chemical Quality Control project for Laboratory nVWA). In 2010 430 quality control samples for 37 different analyses were transferred. The details regarding the prepared samples, the analytical results obtained and the discussion/meetings between nVWA and RIKILT are described in RIKILT Annual report 2010 'De Chemische Borging van Laboratorium nVWA door RIKILT' by B.J.A. Berendsen, report 2011 (draft).

## 3.2 Providing analytical methods and analytical services

On request RIKILT will provide the RFLs with methods of analysis and reference materials. Primary this responsibility is focused on the Dutch laboratory (nVWA). In 2010 the analytical method for coccidiostats in food was optimized (RIKILT RSV A1103) to include the new maximum levels (MLs see EU 2009/8/EG). The additional validations of the extended scopes was performed under a separate project (see Project no. 7261302 Expertise Onderhoud Chemie).

The method for thyreostats in urine, RIKILT RSV A1118, was transferred to nVWA. Training and sample exchange and comparative analysis were part of the transfer.

A set of 187 urine samples were analysed by GC-MS/MS. The samples were found suspected for anabolic steroids (sometimes more steroids in one sample) during GC-MS screening analysis. At the NRL the samples were reanalyzed by using GC-MS/MS. A total number of 197 analyses were performed 23 results were non-compliant (total number of 9, 17$\beta$- and 10, 17$\alpha$-Nortestosterone and 4, 17$\beta$-Boldenon observations). The confirmatory analysis were administered under a separate project (see Project no. 7271501).

## 3.3 Providing information

The following documents were communicated with the RFL:

- Guidelines for the validation of screening methods for residues of veterinary medicines (initial validation and transfer) European Reference Laboratories Residues (EURLs) 20/01/2010.
- COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
- COMMISION REGULATION (EU) No 759/2010 of 24 August 2010 amending the Annex to Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance tildipirosine.
- COMMISSION REGULATION (EU) No 875/2010 of 5 October 2010 concerning the authorisation for 10 years of an additive in feedingstuffs.

- COMMISSION REGULATION (EU) No 885/2010 of 7 October 2010 concerning the authorisation of the preparation of narasin and nicarbazin as a feed additive for chickens for fattening (holder of authorisation Eli Lilly and Company Ltd) and amending Regulation (EC) No 2430/1999.
- COMMISSION REGULATION (EU) No 914/2010 of 12 October 2010 amending the Annex to Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance sodium salicylate.
- All the new maximal residue limits have been included in the RIKILT-NRL MRL database. The RFL will be informed by e-mail each time the database is mutated.
- Next to the national RFLs several national food laboratories from other countries were asking for trainings and advices.
- The Food and Veterinary office (FVO) of the European Commission was supported by S.S. Sterk of the NRL for their mission to Poland 21-29 March 2010.
- L. Stolker was invited to become an FAO/IAEA agreement holder within the Programme Development of rapid screenings method for the detection of antibiotics and anthelmintics (programme no. CRP D5.20.36). For this project the National Veterinary Research & Quarantine Service in Seoul and Busan (Republic of Korea) were visited.
- L. Stolker is a member of the Codex CCRVDF EWG (=electronic working group) chaired by J. Kay. A first draft of a discussion paper became ready but additional work has to be done before there is a final version of an official paper on the ‘Validation of multi-methods’.

# 4 Participating in EURL workshops

In 2010 three employees of RIKILT participated to the following workshops:

- EURL-Workshop from ANSES in Fougeres (March 16-17; 2010) was dedicated to Biological screening methods. M. Pikkemaat a molecular microbiologist from the NRL participated in this workshop and presented a paper with the title: 'Results of a proficiency test on antibiotics in bovine muscle: are screening approaches sufficiently adequate'?
- EURL-Workshop Technical, Analytical and Statistical Issues, May 5-8, BVL Berlin. The workshop consists of two parts. Part 1 Practical session on the analysis of non-steroidal antiinflammatory drugs (NSAIDs). Part 2 Theoretical session about new legislation and the use of multi-methods. L. Stolker from the NRL participated in the theoretical session and presented a paper with the title: Experiences with ToF for the identification of 'unknowns'.
- EURL-Workshop regarding hormones at RIKILT Wageningen from 1-5 November. The workshop focused on the theoretical and practical part of analysis of natural hormones in urine. Several NRL staff contributed and participated to the workshop.

# 5 Participating in proficiency test

Due to the scope of the NRL task assigned, RIKILT participated in proficiency tests organized by the EURLs, Progetto and other international organizations:

In 2010

- Antibiotics in porcine meat (ANSES, screening and confirmation) not yet reported
- NSAIDs in milk (BVL): not yet reported
- Nitrofurans in porcine meat (Progetto): z-score 0.43
- Sulfonamides in bovine meat (Progetto): z-score -0.86
- Phenylbutazon in bovine meat (FAPAS): z-score 0.5
- Antibiotics in bovine meat (RIKILT): z-scores -0.49; 0.20; 0.26 en -1.48
- Chloroamphenicol in fish (ANSES): z-scores 0.75 en 0,08
- Nitrofurans in shrimps (FAPAS): z-score 0.3
- Dyes in fish (ANSES): z-score -0.49

All z-scores reported so far were good; <2.

# 6 Presentations, publications and posters

## 6.1 NRL - Presentations

Presentation were given on the following occasions:

- Delegation from Brasil (Mecosur) visited RIKILT October 29th and B. Berendsen and S.S. Sterk informed the visitors about the EURL-NRL-RFL network and about National Monitoring Programmes and Proficiency tests.
- A lecture with the title 'Official control of veterinary drugs in food; EU perspectives' was presented during the 'KSEA's 30th Anniversary International Symposium' in Busan, Republic of Korea (July 8-9; 2010) by L. Stolker.
- During the 6th Int. Symposium on Hormone and Veterinary Drug Residue Analysis (June 1-4, 2010 Ghent, Belgium) the following lectures were given
- L. Stolker: Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass.
- M. Nielen: Can we defend our data? Experiences from court cases in the Netherlands.
- R. Peters: Identification of anabolic steroids in herbal- and sports supplement preparations using bioassay-guided fractionation, UPLC/TOFMS analysis and accurate mass database searching.

## 6.2 Publications, reports and posters

### 6.2.1 Reports

The following RIKILT reports have been published:

- Title: Veterinary drugs in animal products; Annual report 2009 of the National Reference Laboratory; Author: A.A.M. Stolker, RIKILT report no. 2010.008.
- Title: Proficiency test for antibiotics in bovine meat; Authors: I.J.W. Elbers, B.J.A. Berendsen, M. Pikkemaat and A.A.M. Stolker, RIKILT report no. 2010.010.

### 6.2.2 Products of interest for the NRL function (not on the expenses of the NRL project)

Publications

- Stolker AAM, Peters RJB, Zuiderent R, Bussolo JD, Martins C. Fully automated screening of veterinary drugs in milk by turbulent flow chromatography and tandem mass spectrometry. Anal Bioanal Chem. 2010;397:2841-2849.
- Holthoon van F, Mulder PPJ, Bennekom van EO, Heskamp H, Zuidema T, Rhijn van JA. Quantitative analysis of penicillines in porcine tissues, milk and animal feed using derivatisation with piperidine and stable isotope dilution liquid chromatography tandem mass spectrometry. Anal. Bioanal. Chem. 2010; 396:3027-3040.
- Berendsen B, Stolker L, de Jong J, Nielen M, Tserendory E, Ruuragchas S, Cannavan A, Ellliott Ch. Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. Anal. Bionanal. Chem. 2010; 397;1955-1963.

- Berendsen BJA, Zuidema T, de Jong J, Stolker AAM, Nielen MWF. Discrimination of eight chloramphenciol isomers by liquid chromatography tandem mass spectrometry in order to investigate the natural occurrence of chloramphenicol Anal. Chim. Acta. 2011; DOI 10.1016/j.aca.2010.11.009 in press.
- Berendsen BJA, Pikkemaat MG, Stolker AAM. Are antibiotic screening approaches sufficiently adequate? A proficiency test. Anal. Chim. Acta. 2011; 685; 170-175.

Book chapter contributions

- Book title: Chemical Analysis of Antibiotic Residues in Food;
  Editors: Jian Wang, James MacNeil and Jack Kay (in press).

- Chapter 4: Sample preparation; extraction and clean-up; AAM Stolker, M Danaher.
- Chapter 7: Single Residue Quantitative and Confirmatory Methods; JA Tarbin, AAM Stolker, BJA Berendsen, RA Potter.

Posters

During the 6th Int. Symposium on Hormone and Veterinary Drug Residue Analysis (June 1-4, 2010 Ghent, Belgium) the following posters were presented:

- Title: A statistical approach to detect abuse of natural hormones in cattle;
  Authors: Blokland et al.
- Title: The use of a non-targeted LC-ToF-MS multi residue method for the detection of growth promoters in meat and hair; Authors: Blokland et al.
- Title: Application of robust very high pressure nano liquid chromatography time of flight mass spectrometry for veterinary drugs; Authors: Gerssen et al.
- Title: Proficiency test for thyreostats in samples of porcine urine; Authors: Herbold et al.
- Title: The search for robust biomarkers in bovine urine of dehydroepiandrosterone (DHEA) administration by metabolomic profiling: extending the control population;
  Authors: Mulder et al.
- Title: Time-saving sample treatments for the screening of steroid esters in hair of bovine calves; Authors: Nijrolder et al.
- Title: Screening methods for detection of antibiotic residues in slaughter animals: comparison of the EU-Four plate method, Nouws antibiotic test and Premi test (applied to muscle and kidney); Authors: Pikkemaat et al.
- Title: A flow cytometric immunoassay for the detection of antibodies specific for (R) BST in serum of dairy cows; Authors: Smits et al.
- Title: Analysis of chloramphenicol enantiomers in feed using chiral liquid chromatography; Authors: Stolker et al.
- Title: Fully automated screening of veterinary drugs in milk by turbulent flow chromatography and tandem mass spectrometry (TFC-MS/MS); Authors: Stolker et al.
- Title: A method for the determination of antiviral drug residues in egg and poultry meat by LC-MS/MS; Authors: Weigel et al.
- Title: Development of a method for the analysis of coccidiostats in egg and milk to monitor maximum levels [EC/124/2009]; Authors: Zuidema et al.

Other NRL activities

## 6.3 Organization of proficiency tests

In 2006 RIKILT has obtained accreditation for organizing proficiency tests focusing on veterinary drugs.

The following proficiency tests were organized by RIKILT:

- Proficiency test for antibiotics in bovine meat - screening and confirmation. Results of this proficiency study are described in RIKILT report 2010.010 of December 2010 'Proficiency test for antibiotics in bovine meat - screening and confirmation' by I. Elbers, B.J.A. Berendsen, M. Pikkemaat and A.A.M. Stolker. The summary is presented in Annex II.

## 6.4 Plan for NRL activities 2011

The quality assurance program for the RFL will be continued as well as the regular meetings between the NRL, RFL and the NRLs within the Netherlands (technical meetings and Q3 meeting).

Furthermore the employees of the NRL will participate in the workshops organized by the EURLs and in the organized proficiency tests (for the relevant groups of compounds).

In the coming period collaboration and communication of the NRL and RFLs will be continued. The NRL website will be optimized. Special attention will be paid to the implementation at the RFL of a GC-MS/MS method for the confirmation of anabolic steroids in urine, a method for natural hormones in serum with GC-MS/MS, LC-MS/MS method for the analysis of coccidiostats in meat and an universal LC system for veterinary drug analysis.

# 7 Conclusions and recommendations

From the activities described in this annual report it is concluded that the NRL for veterinary drugs in animal products is properly implemented. The NRL communicates with, and gives advices to the (inter)national authority and the RFL. The NRL coordinates the work of the RFL and participates in EU workshops and proficiency tests. Furthermore the NRL organizes each year a proficiency tests (PT) which is open for (inter)national participation. From the results of this PT (screening and confirmation of antibiotics in beef) it was concluded that the microbiological screening techniques used nowadays for national monitoring programmes show a significant number of false negative results. It is recommended to start within the EU an Expert Working Group to evaluate these results and to advice about the use of microbiological screening tests.

Finally the NRL recommended also to start a discussion regarding the revision (for example initiated by the EURLs) of EU 2002/657/EC (Guidelines for method validation) to include criteria for the use of new analytical approaches (full scan MS, accurate masses etc).

# References

Council Directive 96/23/EC, Official Journal L 125 , 23/05/1996 P. 0010 - 0032.

RIKILT report 2010.010, Elbers, Berendsen, Pikkemaat and Stolker, Proficiency test for antibiotics in bovine meat - screening and confirmation.

RIKILT report 2011.xxx. status in preparation, B.J.A. Berendsen, De chemische borging van laboratorium VWA door RIKILT jaarrapportage 2010.

# Annex I
# Letter of request

E-mail from EURL- Berlin

Dear colleagues,

Our publication entitled “List of screening and confirmatory analytical test methods used in the NRLs of the European Union for substance groups A5, B2a, B2b, B2e and in the German Routine Field Laboratories for all substance groups of Annex I, Council Directive 96/23/EC" (2008) is to be updated.

Please return the completed tables to us (by e-mail to: NRL-TAM@bvl.bund.de) by 31 March 2010 at the latest.

We kindly ask you to fill in the tables carefully - for help in filling them in correctly please refer to the instructions given in the file NRL_instruction_2010, the provided examples and the inst. no. If you are not in charge of certain substance groups subject to this survey, please forward the tables to the colleague(s) in charge.

The number of your institute can be found in the title of the Excel file: NRL-xxx-Results_2008.

Thank you very much for your help.

Yours sincerely

on behalf

Dr. Petra Gowik

(Head CRL Berlin)

# Annex II
# Summary report 2010.010: Proficiency test for antibiotics in bovine meat - screening and confirmation

The proficiency test for antibiotics in bovine muscle was organized by Rikilt - Institute of Food Safety and

in accordance with ISO/IEC Guide 43-1 and 43-2 and ILAC-G13. The quantitative and

confirmatory part was carried out under accreditation (Dutch Accreditation Board, ILAC-G13).

For this proficiency study, three test materials were prepared:

- a blank bovine muscle material;
- a bovine muscle material containing oxytetracycline aimed at 120 µg/kg;
- a bovine muscle material containing sulfachlorpyridazine aimed at 90 µg/kg, sulfadimidine aimed at 120 µg/kg and dapson aimed at 5 µg/kg.

The materials containing antibiotics were all prepared by spiking blank bovine muscle materials followed by cryogenic homogenization. During homogeneity testing, all materials proved to be sufficiently homogenous for proficiency testing. The stability test demonstrated that no statistically significant loss of oxytetracycline and sulfadimidine occurred during the timescale of the proficiency test. For sulfachloropyridazine and dapson a minor loss occurred during the thaw-freeze cycle that was included in the stability test.

The participating laboratories were first asked to carry out a screening analysis. After reporting the screening results they were asked to carry out a quantitative confirmatory analysis for the compounds found suspect and at least for tetracyclines and sulfonamides including dapsone. Thirthy-six laboratories subscribed for participation in the proficiency study but for one of them it was not possible to get the samples through customs. Within the timeframe of the study 35 laboratories submitted results: 34 laboratories submitted results for the screening analysis and 27 for the quantitative confirmatory part. Three laboratories (labs 2, 19, 26) did not detect any antibiotics using their screening methodology. Seventeen laboratories (labs 3, 4, 5, 9, 11, 12, 15, 16, 18, 21, 23, 25, 28, 30, 34, 35 and 37)characterized all three samples correctly (compliant or suspect) based on the screening analysis and of these fourteen laboratories (3, 4, 5, 11, 12, 15, 16, 18, 21, 25, 28, 30, 35 and 37) indicated the correct compound groups for all samples.

The false positive and false negative rate were determined for all the individual laboratories and for all individual methods applied. A result is considered to be a false negative result if an antibiotic group/compound present in the sample is not detected. When evaluating the results for the individual labs (that in some cases carried out several different methods) fifteen false positive results out of 102 results occurred and twenty-one false negative results out of 64 results occurred.

After evaluating the results for all individual methods applied it became clear that the majority of false negative results was caused by using microbiological methods and the failure to detect

sulfachloropyridazine in targeted instrumental screening methods. An overview of the screening analysis results evaluated on basis of all individual methods applied is presented in Table 1. Dapson was left out of the calculations, because it was not found in any of the screening analyses.

If each method is considered separately, the false negative rate for the microbiological methods is 38%, for biochemical methods this is 25%, both caused by the Charm II test, and for instrumental analysis this is 23% all caused by missing sulfachloropyridazine. The proficiency test of 2009 organised by RIKILT included macrolides, quinolones and aminoglycosides in bovine muscle. The test of 2009 organised by RIKILT showed a false positive rate of 7%, in 2010 this is 15%.

Regarding the applied methods it is concluded that:

- many combinations of screening tests are used to cover the broad range of antibiotic groups;
- many false negative results are obtained, especially for microbiological screening methods.
- all false negative results obtained by instrumental methods can be explained by not including sulfachloropyridazine in the method.

*Table 1: Overview of correct, false negative and false positive results for microbiological, biochemical and instrumental screening methods.*

| Material | A | B | C | |
|---|---|---|---|---|
| False positives | 7 | 4 | 7 | |
| Microbiology methods | 7 | 3 | 4 | |
| Biochemical methods | 0 | 1 | 0 | |
| Instrumental methods | 0 | 0 | 3 | |
| | | Oxytetracycline | Sulfadimidine | Sulfachloropyridazine |
| No. of methods applied for the compound groups included* | | 38 | 37 | 37 |
| Correct results | 41 | 29 | 30 | 23 |
| Microbiology methods | | 14 | 9 | 9 |
| Biochemical methods | | 3 | 3 | 3 |
| Instrumental methods | | 12 | 18 | 11 |
| False negatives | | 9 | 7 | 14 |
| Microbiology methods | | 8 | 6 | 6 |
| Biochemical methods | | 1 | 1 | 1 |
| Instrumental methods | | 0 | 0 | 7 |

**: Because some laboratories applied several different methods and some laboratories do not have all compounds relevant for this proficiency test included in their method, this number is different from the number of laboratories.*

Twenty-five laboratories carried out a quantitative and confirmatory analysis for tetracyclines and twenty-seven for sulfonamides including dapsone. Twenty-seven labs included sulfadimidine in their quantitative/confirmatory method, 19 labs included sulfachloropyridazine and 16 labs included dapson.

False negatives occurred during the confirmatory analysis due to the absence of sulfachloropyridazine RIKILT Report 2010.010 5 and/or dapson in the method. One laboratory detected 63 µg/kg sulfaclozin which is considered as a false positive result.

For the quantitive analysis of oxytetracycline 20 out of 25 laboratories (80%) obtained satisfactory results. For sulfadimidine this was 26 out of 27 laboratories (96%), for sulfachloropyridazine 17 out of 18 (94%) and for dapsone 12 out of 13 (92%).

Based on the results of this proficiency test it is concluded that:

- considering the high percentage of false negative results, effort is needed to improve the effectiveness for the screening of veterinary drugs in muscle samples;
- microbiological screening methods relatively often cause false positive results;
- for effectively applying targeted instrumental screening methods (LC-MS/MS or LC-UV);
- effort is needed to include a wider range of compounds;
- the quantification of especially oxytetracycline is not satisfactory for some laboratories.

RIKILT - Institute of Food Safety is part of the international knowledge organisation Wageningen UR (University & Research centre). RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

RIKILT advises national and international governments on establishing standards and methods of analysis. RIKILT is available 24 hours a day and seven days a week in cases of incidents and food crises.

The research institute in Wageningen is the National Reference Laboratory (NRL) for milk, genetically modified organisms, and nearly all chemical substances, and is also the European Union Reference Laboratory (EU-RL) for substances with hormonal effects.

RIKILT is a member of various national and international expertise centres and networks. Most of our work is commissioned by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and the new Dutch Food and Consumer Product Safety Authority. Other parties commissioning our work include the European Union, the European Food Safety Authority (EFSA), foreign governments, social organisations, and businesses.

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