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# Veterinary drugs and growth promoting agents in animal products

Annual report 2011 of the National Reference Laboratory

RIKILT Report 2012.008

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





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**Report 2012.008**

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# Summary

This report of the National Reference Laboratory (NRL) for residues of veterinary drugs and growth promoting agents in products of animal origin according to Council Directive 96/23/EC describes the activities employed in 2011. 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 Union Reference Laboratories (EURLs) and participates in trainings organized by the Commission or by the EURLs

## Communication and advices

In 2011 the NRL advised the Competent Authority on National Plan monitoring activities through the official 'Werkgroep National Plan' meetings. Furthermore, the Competent Authority was advised on the analysis of enantiomers of chloramphenicol in urine, the effects of the proposed revision of Council Directive 96/23/EC and the contamination of 'sulfamethoxazole feed top-dressings' with dapsone. The Competent Authority was also advised about the consequences of the ban of medicated feed by the Nevedi (The Netherlands Feed Industry Association).

The NRL advised 3 times National Food Control Laboratories outside the Netherlands (Sri Lanka, South Korea and China) - on their request - about different methods of analysis for the determination of veterinary drugs including aminoglycoside analysis.

## 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 3 proficiency tests organized by other organizations like FAPAS and IRMM. The results of the proficiency tests were all acceptable (Z-score <2) with one exception metamizol in milk which was due to miscalculation.

RIKILT is accredited (according to ISO 17043) for the organization of proficiency tests.

In 2011 - as in 2009 and 2010 - 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 submitted results: 30 laboratories submitted results for the screening analysis and 33 for the quantitative confirmatory part. Ten laboratories characterized all three samples correctly (compliant or suspect) based on the screening analysis and indicated the correct compound groups for all samples. When evaluating the results for the individual labs (that in some cases carried out several different methods) 14 false positive results and 52 false negative results out of 160 results occurred. The majority of false negative results was caused by the failure to detect neomycin, oxytetracycline or nafcillin in the microbiological methods.

The results are in line with the results obtained in previous proficiency tests in 2009 and 2010. The relative high percentage of false negatives makes it necessary that laboratories use more than one analytical method for reliable screening.

#### Other activities in 2011

- Special attention was paid to the introduction of the GC-MS/MS technique at the RFL.
- The NRL published in a peer reviewed journal the stability results of antibiotic reference standards. This information supports/facilitates the RFL in their laboratory accreditation process.
- A team of the Food and Veterinary Office visited the Netherlands and during this visit the NRL was audited.
- Collaborations regarding research on method development/optimisation etc. with QLIP laboratory and the FAVV laboratories in Belgium were initiated.

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# 1 Introduction

The European Commission is committed to protecting consumers from intolerable health hazards, 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 Union Reference Laboratories (EURLs), National Reference Laboratory (NRLs) and Routine Field Laboratories (RFLs).

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

- Coordinating the work of the 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 residue 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 describes the activities in 2011 of the NRL for veterinary drugs and growth promoting substances according to Council Directive 96/23/EC. It covers the groups of compounds assigned to RIKILT-NRL regarding veterinary drugs viz. nitrofurans, dapsone, 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 Council Directive 96/23/EC. Furthermore the RIKILT-NRL includes stilbenes, stilbene derivatives, and their salts and esters, antithyroid agents, steroids, resorcylic acid lactones (including zeranol) and beta-agonists. These groups belong to Group A1, A2, A3, A4 and A5 compounds as described in Council Directive 96/23/EC.

## 2 Methods

One of the tasks of the NRL is to communicate with the Competent Authority, RFLs and other NRLs 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 pro-active. The communication and advice activities employed by the NRL in 2011 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 Residue Monitoring Plans.

In 2011 RIKILT participated in the working group setting up the plan for monitoring residues.

During 2011 there were 3 meetings of this working group. Minutes are available.

#### 2.1.2 With Routine Field Laboratory (Dutch Food and Consumer Product Safety Authority)

On a regular base the management of RIKILT communicates with the management of the Foodsafety Laboratory of the Dutch Food and Consumer Product Safety Authority (in this report referred to as NVWA-labVV).

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

The NRL had one official management meeting with the RFL and at least 6 technical meetings and several unofficial contacts by telephone and e-mail. Minutes are available of the official meeting.

#### 2.1.3 With other National Laboratories

On a regular base the management of the NRL in the field of veterinary drugs and growth promoting agents meets the management of other laboratories in this field, the so called Q3 (Quality-assurance, Quality-control, Quartet) meetings. RIKILT, NVWA and TNO-Ducares laboratories are involved in the Q3 meetings.

In 2011 the Q3 group had two official meetings, one at TNO-Ducares, Utrecht on March 3rd and one on September 8th at RIKILT, Wageningen. In both meetings NVWA, RIKILT and TNO-Ducares participated. From these meetings minutes are available.

The NRL arranged meetings with QLIP regarding the analysis of veterinary drugs in milk. The NRL prepared some quality assurance samples which are analysed by RIKILT and QLIP the results are described in a separate confidential report regarding 'Borging QLIP door RIKILT'; 'Jaarrapport NRL melk- en melkproducten 2011, Inclusief niveauvergelijking RIKILT-NRLs in de zuivelsector' authors M. Alewijn et al. report no.: draft (Projectnummer: 121.71.313.03).

## 2.2 Advices

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

The RFL was informed about the proficiency tests and workshops organized by EURLs in Berlin, Fougères and Wageningen. One employee of the RFL participated in the workshop organized by the EURL in Wageningen regarding the analyses of anabolic steroids.

### 2.2.2 To the Competent Authority (NVWA-Ministry of EL&I)

The Competent Authority (NVWA-EL&I) was advised by the NRL regarding four different items:

- Analytical possibilities to separate chloramphenicol and its enantiomer dexamphenicol.
- The consequences of the revision of Council Directive 96/23/EC.
- The consequences of the 'ban' of medicated feed in the Netherlands. The Nevedi (The Netherlands Feed Industry Association) decided to stop the production of (antibiotic) medicated feed in the Netherlands. The main argument to stop the production is the unavoidable carry-over and as a consequence the possible contribution to the antimicrobial resistance problem. However possible alternatives like water medication also have specific problems.
- The contamination of sulfamethoxazole (animal feed) top-dressings with the prohibited antibiotic dapsone.

In the past there were some doubts about the use of microbiological screenings assays used throughout the EU. The results of the proficiency tests organised by RIKILT regarding this item demonstrated a relative high percentage of false negative results. This observation was communicated with the EU reference laboratory in Fougères during the EU Workshop in 2009. It appeared that laboratories do not properly validate the screening method. In other words, sometimes it was(is) not completely clear which antibiotic at which concentration level is detected by the assay. In 2010 the EURL's came up with guidelines to validate the screening assays (including microbiological based screening assays). Laboratories that implement these guidelines correctly get a clear view on the scope of the method they use.

Furthermore the EURL for antibiotics promotes the use of LC-MS for screening purposes to overcome the limitations of microbiological screening techniques. However, also this approach, until now, is not applicable for all antibiotics of interest.

- A team of the Food and Veterinary Office visited the Netherlands and visited/audited the NRL. The NRL informed the FVO regarding the NRL activities.

## 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 separate projects (WOT program on Food Safety - Part 3 Veterinary Drugs; project 7203701 Analytical Chemical Quality Control project for Laboratory NVWA and project 7201901 Borging antibioticascreening NVWA Laboratorium). For the analytical chemical quality control in 2011 422 quality control samples for approximately 37 different analyses were transferred. The details regarding the prepared samples, the analytical results obtained and the discussion/meetings between NVWA-labVV and RIKILT are described in RIKILT Annual report 2011 'De Chemische Borging van Laboratorium NVWA door RIKILT ' by B.J.A. Berendsen, report 2012 (in preparation).

For the quality control of the microbial antibiotic screening methods, in 2011 502 quality control samples for four different tests (NNNT, NAT, Poultry scan and Egg scan) were transferred. The details regarding the samples and results and the discussion/meetings between NVWA-labVV and RIKILT are described in RIKILT Annual report 2011 'Niveaucontrole NVWA, microbiologische antibiotica screening' by S. Oostra-van Dijk and M.G. Pikkemaat, report 2012 (in preparation).

At present screening of antibiotic residues in 'suspected' slaughter animals is carried out by NVWA using the NNNT (Nieuwe Nederlandse Niertest) supplemented with a plate test for tetracyclins to avoid a blind spot for the latter class of compounds.

Although to date no significant deviations were noticed in the quality control results, it has to be mentioned that, considering the state-of-the-art of analytical methods, the current analytical approach is becoming rather obsolete. Therefore from a scientific point of view, the NRL strongly recommends to replace the NNNT by a more contemporary analytical approach for the screening. To this end the NRL recommends implementing the NAT system for screening of antibiotic residues. Application of the NAT ensures the detection at MRL level of the current array of antibiotics but also enables widening the array of antibiotics covered in the screening.

The quality control samples prepared for the QLIP laboratory are:

- 5 samples milk containing beta-lactams
- 5 samples milk containing anthelmintics

The results of QLIP were evaluated against the RIKILT reference values taking into account the method uncertainties. Next to the quality assurance samples the NRL performed a study on the stability of anthelmintics in milk samples.

The evaluation of the results obtained for the quality control samples and the results of the stability tests are communicated with QLIP and are described in a separate confidential report regarding 'Borging QLIP door RIKILT' (project no. 121.71.313.03); see also section 2.1.3.

## 3.2 Providing analytical methods and analytical services

On request RIKILT will provide the RF's with methods of analysis and reference materials. In 2011 the following specific services were provided:

- the analytical method for coccidiostats in meat at Maximum Levels (as described in EU 2009/8/EG) is transferred to the RFL. The additional validations of the extended scopes was performed under a separate project (see Project no. 7261302 Expertise Onderhoud Chemie).
- the more generic LC system for the analysis of antibiotics was transferred and implemented at the RFL. The method development and validation was done in 2010.
- the NRL published in an official peer reviewed journal the results of the stability study of antibiotic reference standards. This document helps RFL's by (extension of) the laboratory accreditation.
- the RFL was advised regarding the analysis of thyreostats and natural hormones.
- the RFL was assisted by the implementation of the GC-MS/MS for multi-residue analysis and the analysis of natural hormones in serum.
- the RFL was advised regarding the use of blank reference urines for the analysis of steroids.
- the RFL was advised regarding the hydrolysis step for the analysis of boldenone and alfa-trenbolone.

## 3.3 Providing information

### 3.3.1 The following documents were communicated with the RFL

- 'Questionnaire concerning the dissemination of external information by the EURL Berlin'; NRL and RFL were invited to give their opinion on the activities of the EURL in Berlin.
- Information regarding the new beta-agonist phenylethanolamine A.
- Information/documentation concerning EURL workshops and proficiency tests.

All the new Maximum Residue Limits have been included in the RIKILT-NRL MRL database. The RFL was informed by e-mail each time the database was mutated.

### 3.3.2 Other activities regarding providing/exchanging information

- L. Stolker joined as the FAO/IAEA Agreement Holder the second annual progress meeting of projects included in the Programme CRP D5.20.36 of FAO/IAEA (Programme Development of rapid screenings method for the detection of antibiotics and anthelmintics). The project progress meeting was in Kandy, Sri Lanka (14-18 March). During this meeting information regarding EU legislation and NRL activities was given.
- L. Stolker is a member of the Codex CCRVDF EWG (=electronic working group) cochaired by Canada and the UK (contact J. Kay). A second 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'.
- The NRL informed the FVO audit team about the NRL activities during the inspection of the Dutch laboratories (24-28 October).
- S. Sterk participated in the FVO team during an audit in Switzerland (16-21 of January, 2011). (This activity was not on the expenses of the project).

### **3.3.3 Activities with other laboratories**

Next to the national RFLs several national food laboratories from other countries asked for trainings and advice. These activities which are relevant for the NRL function are additionally financed by the trainees.

- A team of Brazilian scientists (LANAGRO) was trained in the use of the ToF MS technique for veterinary drug analysis (9-13 May).
- A team of Korean scientists from the Animal, Plant and Fisheries Quarantine and Inspection Agency, Ministry for Food, Agriculture, Forestry and Fisheries (QIA) from the Republic of Korea was trained for 2 weeks in the use of LC-MS for veterinary drugs and monitoring plans in the EU in general (22 Augustus - 3 September).
- An employee of the University of Dunarea de Jos Galati, Romania, was trained - in combination with an internship - for 9 months for the multi-analyte analysis of drug residues in water (1 January - 1 September).
- A team of Russian scientists was trained in the analysis of hormones in meat and fish (5-12 December).
- Collaboration regarding research on method development/optimalisation etc with QLIP and the FAVV laboratories in Belgium were initiated.

## 4 Participating in EURL workshops

In 2011 employees of RIKILT participated to the following workshops:

- EURL-Workshop from ANSES in Fougères (June 9-10; 2011) which was dedicated to the analysis of honey, discussion on the use of multi-analyte methods using HRMS, the need for new confirmation criteria for new analytical techniques, stability studies of antibiotics and proficiency testing. L. Stolker from the NRL participated in this workshop and presented a paper with the title: Identification of 'unknowns' in feed by microbiological screening and LC-ToF-MS.
- EURL-Workshop Technical, Analytical and Statistical Issues, May 3-6, BVL Berlin. The workshop consisted of two parts. Part 1 Practical session on the analysis of beta-agonists in hair. Part 2 Theoretical session about new legislation and the use of multi-methods for e.g. anthelmintics. Th. Meijer from the NRL participated in this workshop and presented a paper with the title: Isoxsuprine in veal calves - how does it get there?
- 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 to and participated in the workshop.

Approximately, 27 experts from the NRL's - of the 27 EU member states - participated in these workshops.

## 5 Participating in proficiency tests

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

In 2011

- Antibiotics in porcine meat (ANSES) : z-scores -0.02; 0.18; 0.01; 1.2
- NSAIDs in milk (BVL): z-scores >2 (for all metamizol results); 0.33; 0.12; 0.3; 0.03
- Antibiotics in bovine meat (RIKILT): z-scores -1.99; 0.91; 1.25; -0.02
- Steroid esters in hair (EURL RIKILT): z-scores 0.24; 1.11
- Tetracyclines in bovine kidney (FAPAS): z-score -0.2
- Tetracyclines in fish (FAPAS): z-score -1.6
- Nitrofurans in honey (ANSES) : z-scores 0.73; 1.68; 0.49; 0.21
- Stilbenes in bovine urine (IRMM): z-score 0.8; 1.54; 1.55

The methods involved are LC-MS/MS confirmatory methods except the method for stilbenes in bovine urine which is an GC-MS/MS method. All z-scores were good (< 2) except the results for metamizol in milk. The high z-scores obtained for this compound were due to a mistake in concentration levels used for the calibration line calculations (only for this set of samples); after recalculations all the z-scores were <2.



## 6 Presentations, publications and posters

### 6.1 NRL – Presentations

Presentations were given on the following occasions:

- During the SaskVal meeting (meeting on harmonisation of validation systems) in Saskatoon, Canada an employee of the NRL (L. Stolker) presented a paper with the title: Validation of screening methods; the EU approach (20-24 June).
- During the annual meeting of the AOAC in New Orleans an employee of the NRL (L. Stolker) presented during a workshop on validation, a paper on validation systems within the EU; Validation approach for veterinary drug analysis versus pesticide analysis (18-22 September).
- During the WOT symposium (Utrecht, November 22) "Dierbehandelingsmiddelen: samenwerking, trends en innovatie" several employees of the NRL presented lectures. For the symposium programme see Annex I.

### 6.2 Publications, reports and posters

#### 6.2.1 Reports and publications

The following RIKILT reports have been published:

- Title: Annual report 2010 of the National Reference Laboratory; Veterinary drugs and growth promoting agents in animal products, A.A.M. Stolker and S.S. Sterk, report no. 2011.014.
- 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. 2011.521.

Peer reviewed publications

- Berendsen BJA, Elbers IJW, Stolker AAM. Determination of the stability of antibiotics in matrix and reference solutions using a straightforward procedure applying mass spectrometric detection. Food Add. Contam. DOI 10.1080/19440049.2011.604045.
- Berendsen BJA, Pikkemaat MG, Stolker AAM. Are antibiotic screening approaches sufficiently adequate? A proficiency test. Anal. Chim. Acta. 2011; 685; 170-175.

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

Publications

- Berendsen BJA, Zuidema T, de Jong J, Stolker AAM, Nielen MWF. Discrimination of eight chloramphenicol isomers by liquid chromatography tandem mass spectrometry in order to investigate the natural occurrence of chloramphenicol; Anal. Chim. Acta. 700 (2011) 78-85.2011.

- Berendsen BJA, Essers ML, Stolker AAM, Nielen MWF. Quantitative trace analysis of eight chloramphenicol isomers in urine by chiral liquid-chromatography coupled to tandem mass spectrometry; *J Chromatogr. A* 1218 (2011) 7331-7340.
- Berendsen BJA, Wegh R, Essers ML, Stolker AAM, Weigel S. Quantitative trace analysis of a broad range of antiviral drugs in poultry muscle using column-switch liquid chromatography coupled to tandem mass spectrometry. *Anal. Bioanal. Chem.* DOI 10.1007/s00216-011-5581-3.
- Berendsen BJA, Stolker AAM, Nielen MWF. Assessment of liquid chromatography-tandem mass spectrometry approaches for the analysis of ceftiofur metabolites in poultry muscle. *Food Addit. Contam.* DOI 10.1080/19440049.2011.635348.

#### Book chapter contributions

Book title: Chemical Analysis of Antibiotic Residues in Food. Editor Jian Wang, James MacNeil and Jack Kay (published Wiley Series on Mass Spectrometry; ISBN 978-0-470-49042-6).

- Chapter 4 Sample preparation; extraction and clean-up; AAM Stolker, M Danaher, p125-152.
- Chapter 7 Single Residue Quantitative and Confirmatory Methods; JA Tarbin, AAM Stolker, B.J.A. Berendsen, RA Potter, p227-262.

Book title: Methods in Molecular Biology 747 (2011) 219 – 232 Chapter 9. Hormone analysis in food products, Mass Spectrometry in Food Safety - Methods and Protocols; M.H. Blokland, S.S. Sterk.

#### Posters

During different International conferences:

RAFA (Recent Advances in Food Analysis) 1-4 November, 2011 Praag, Czech Republic

- Oosterink E, Driessen W, Zuidema T, Pikkemaat M, Stolker L. Identification of microbial growth inhibitors in animal feed by LC-ToF-MS with accurate mass database searching.
- Chitescu CL, Oosterink E, de Jong J, Stolker AAM, Accurate mass screening of pharmaceuticals and fungicides in water by UHPLC-Exactive Orbitrap MS.

Symposium on MS in Food and Feed organized by the KVCV Food Division, June 9th, Merelbeke, Belgium.

- Berendsen BJA, Stolker AAM, Nielen MWF. Quantitative trace analysis of eight chloramphenicol isomers in urine by chiral liquid chromatography coupled to tandem mass spectrometry.

### 6.3 Organisation 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 2011.521 of September 2011 '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 2012

- 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).
- 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).
- Collaboration and communication of the NRL and RFLs will be continued, including a technical meeting with participation of QLIP.
- Special attention will be paid to the implementation of a more generic extraction method for antibiotics at the RFL.
- In 2011 the NRL started with collecting/publishing the available data regarding the stability of standard reference materials. This activity will be continued, information available within all laboratories of the Q3 working group will be collected and will be made assessable to each other. In order to facilitate the extension of the laboratory accreditation.

## 7 Conclusions and recommendations

From the activities described in this annual report it is concluded that the NRL for veterinary drugs and growth promoting agents in animal products has implemented all aspects as described in Council Directive 96/23/EC. The NRL communicates with, and gives advices to the (inter)national authorities and the RFLs, coordinates the work of the RFLs 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. However, based on the outcome of this PT, the NRL is of the opinion that further harmonization of screening for antibiotic compounds should be a priority for the EU. The NRL will continue with its efforts to contribute to this through its dialogue with other NRLs and the responsible EURL.

## References

- Council Directive 96/23/EC, Official Journal L 125 , 23/05/1996 P. 0010 - 0032.
- RIKILT report 2011.521, Elbers, Berendsen, Pikkemaat and Stolker, Proficiency test for antibiotics in bovine meat - screening and confirmation.
- RIKILT report 2012. in preparation, B.J.A. Berendsen, De chemische borging van laboratorium VWA door RIKILT jaarrapportage 2011.

# Annex I

## Aankondiging

De Adviescommissie van het WOT-Voedselveiligheid Thema Dierbehandelingsmiddelen nodigt u uit voor een Symposium

“Dierbehandelingsmiddelen: samenwerking, trends en innovatie”

Datum: Dinsdagmiddag 22 november 2011

Plaats: NWWA, Catharijnesingel 59, Utrecht

### Voorlopig programma

13.00-13.30	Ontvangst
13.30-13.45	Woord van welkom Albert Lam (NWWA), voorzitter adviescommissie
13.45-14.15	Integratie van het wettelijke dierbehandelingsmiddelenonderzoek in Nederland Michel Nielen (RIKILT), themaleider
14.15-14.45	Trends in de opsporing van antibiotica in de voedselketen Linda Stolker (RIKILT), NRL antibiotica residuen
14.45-15.15	Pauze
15.15-15.35	Naar een controlesystematiek voor natuurlijke hormonen in runderurine Marco Blokland (RIKILT), projectleider
15.35-15.55	Naar een controlesystematiek voor eiwitgroeihormonen in melkkoeien Susann Ludwig (RIKILT), projectmedewerker / Michel Nielen, themaleider
15.55-16.30	Afsluiting, informele discussie, en borrel

## Annex II

# Summary report 2011.521: 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 43-1, 43-2 and 17043. The quantitative and confirmatory part was carried out under accreditation (Dutch Accreditation Board, ISO/IEC 17043).

For this proficiency test, three test materials were prepared:

- bovine muscle containing neomycin aimed at 550 µg/kg and oxytetracycline aimed at 120 µg/kg.
- incurred oxytetracycline bovine muscle aimed at 120 µg/kg.
- bovine muscle containing nafcillin aimed at 350 µg/kg.

The fortified materials were all prepared by spiking blank bovine muscle materials followed by cryogenic homogenization. The incurred material was prepared by mixing the incurred material with blank bovine muscle 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 occurred during the timescale of the proficiency test. For neomycin and nafcillin a 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 aminoglycosides, tetracyclines and β-lactams. Within the timeframe of the study 36 laboratories submitted results: 30 laboratories submitted results for the screening analysis and 33 for the quantitative confirmatory part.

Ten labs indicated the correct compound groups for all samples in the screening part (labs 8 (only for LC-MS/MS analysis, not for microbiological analysis), 12, 16, 17, 22, 24, 29, 30, 33 and 35). The false positive and false negative rates 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) 14 false positive results and 52 false negative results out of 160 results occurred.

After evaluating the results for all individual methods applied it became clear that the majority of the false negative results was caused by microbiological methods and the failure to detect neomycin, oxytetracycline or nafcillin in targeted instrumental screening methods. An overview of the screening analysis results evaluated based on every individual applied method is presented in Table 1.

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

Material	total	A		B	C
		NEO	OTC	OTC	NAF
No. of labs submitted results		26	33	33	32
No. of methods applied	160	34	43	43	40
<b>False positives</b>					
Microbiological methods	11	0		2	9
Biochemical methods	3	1		1	1
Instrumental methods	0	0		0	0
<b>False negatives</b>					
Microbiological methods	42	16	10	10	6
Biochemical methods	1	1	0	0	0
Instrumental methods	9	2*	1	1**	5

\* 1 false negative result was reported by not detecting NEO, although it was included in method

\*\* false negative result was reported by not detecting OTC, although it was included in method

Fourteen laboratories carried out a quantitative and confirmatory analysis for aminoglycosides of which one laboratory did not include NEO in the method and one laboratory did not detect NEO. Ten laboratories obtained satisfactory z-scores ( $z < |2|$ ). Thirty laboratories carried out a quantitative and confirmatory analysis for tetracyclines in material A of which 27 obtained satisfactory z-scores. Twenty-nine laboratories carried out a quantitative and confirmatory analysis for tetracyclines in material B of which 25 obtained satisfactory z-scores. Twenty-four laboratories carried out a quantitative and confirmatory analysis for  $\beta$ -lactams of which five did not include NAF in the method. Seventeen laboratories obtained satisfactory z-scores.

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

- Like previous years, the screening part of the test demonstrates the drawbacks in the analytical approach for the analysis of antibiotics in muscle samples;
- 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 detect microbiologically active compounds (broad range), but relatively often cause false positive results, because of the inability to distinguish (correctly) between antibiotic groups;
- Instrumental methods result in less false negative results, but effort is needed to include a wider range of compounds;
- Fortified OTC material is representative for incurred OTC material;
- The quantification of NEO and NAF in bovine muscle needs attention;
- Materials to be analysed for the presence of  $\beta$ -lactams should be stored at a temperature below  $-70^{\circ}\text{C}$  and/or should undergo a stabilization procedure.





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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