REPORT of the VISIT

to the POLISH

NATIONAL REFERENCE LABORATORY

for RESIDUE CONTROL in FOOD

National Veterinary Research Institute

NVRI-PIWet – Puławy – POLAND

29/30 November 2012

Mission carried out by

the European Union Reference Laboratory

of Anses-Lab of Fougeres

E. Verdon

Report edited 14 January 2013

MISSIONS OF THE EUROPEAN UNION REFERENCE LABORATORY

OF THE ANSES-FOUGERES - FRANCE

The ANSES-FOUGERES has been designated as the French National Reference Laboratory for the Control of Veterinary Drug Residue in Food from Animal Origin except for Hormones and Beta-agonists since 1990.

The ANSES-FOUGERES has been appointed as the Community Reference Laboratory for the Control of Antimicrobial Residue in Food from Animal Origin since 1992. It has been comforted in this mission by the Council Directive n° 96/23/EC of 29 April 1996.

The ANSES-FOUGERES is in charge of the chemical residues from the following groups:

- Group A6: nitrofurans, dapsone & chloramphenicol (banned substances: annex IV of regulation no 90/2377/EEC)
- Group B1: antimicrobials, including quinolones and sulfonamides
- Group B2f : other pharmacologically active substances - carbadox & olaquindox
- Group B3e: pharmacologically active dyes - malachite green.

Missions

The functions of EU-RL are:

- to promote and coordinate research into new analytical methods and to inform NRLs of advances in analytical methods and equipment;
- to help NRLs for residues to implement an appropriate quality assurance scheme system based on criteria of the ISO17025:2005;
- to approve validated methods as reference methods, to be integrated into a collection of methods;
- to provide NRLs with the routine analytical methods accepted during the MRL procedures
- to provide NRLs with details of analytical methods and comparative tests to be conducted, and to inform them of the results of the tests;
- to provide NRLs, at their request, with technical advice on the analysis of the substances for which they have been designated the CRL;
- to organize comparative tests for the benefit of the NRLs, the frequency of which shall be determined in agreement with the Commission. Consequently, the CRLs shall distribute blank samples and samples containing known amounts of analytes to be analyzed;
- to identify residues and determine their concentration in cases where the results of an analysis give rise to a disagreement between Member States;
- to conduct initial and further training courses for the benefits of analysts from national laboratories;
- to provide the Commission services, including the SMT programme, with technical and scientific assistance;
- to compile a report on each year's work and transmit it to the Commission;
- to liaise, in the field of analytical methods and equipment, with the NRLs designated by Third Countries in the plans to be submitted in accordance with Article 11 of this Directive (96/23/EC).

POLISH N.R.L.

National Veterinary Research Institute

Veterinary Drug Residue Control in Food

Visited by EU-RL Anses-Fougeres Delegate: Eric Verdon

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NVRI-PIWet – Puławy – 29/30 November 2012

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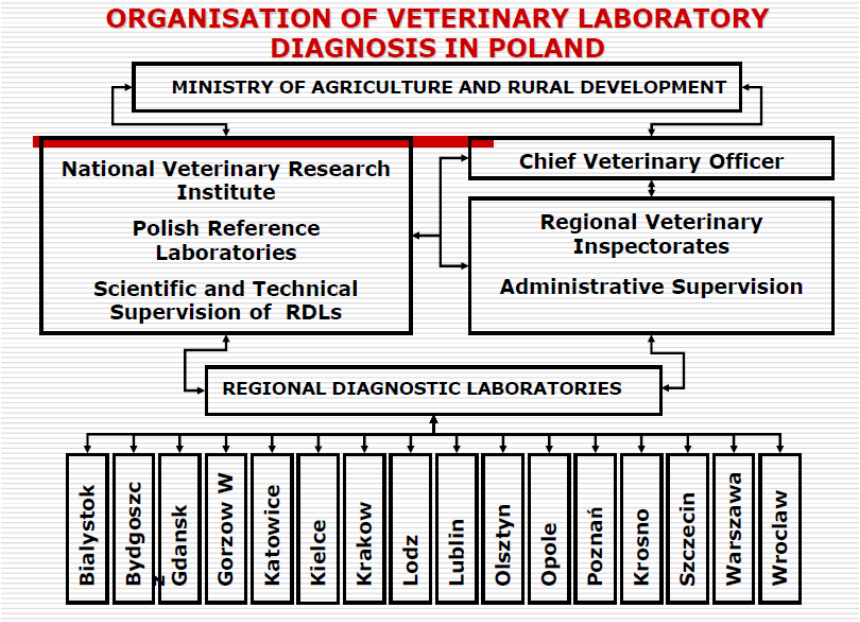
1 ORGANIZATION OF THE SYSTEM OF SURVEILLANCE FOR RESIDUES IN FOOD FROM ANIMAL ORIGIN IN POLAND

The authority in charge of the veterinary drug policy in Poland is the Ministry of Agriculture and Rural Development under which the General Veterinary Inspectorate (GVI) and its services at the regional and district levels are responsible for the development and implementation of the national residue control plan, controls on the distribution and use of veterinary medicinal products and the production, distribution and use of medicated feedingstuffs.



The National Veterinary Research Institute (NVRI-PIWet) hosts the Laboratories acting as the Polish National Reference Laboratories in the different sectors of food safety and food hygiene for the official food control based on Regulation 882/2004/EC and Directives 96/22/EC and 96/23/EC.

The basic fields of activities at NVRI are:

- Statutory research activities,
- Multiannual research programme,
- Reference activities,
- Expertise and service activities,
- Training activities,
- International cooperation.

There is also a network of 8 laboratories acting as routine field laboratories (RFL) located in 8 of the 16 regions of Poland.

The NVRI is designated as NRL for the following residues:

- Anabolic medicines and unauthorized substances in biological fluids, raw materials and food from animal origin (A1, A2, A3, A4, A5, A6 of Dir. 96/23/EC),
- Veterinary medicines in biological fluids, raw materials and food from animal origin (B1, B2a, B2b, B2d, B2e, B3e of Dir. 96/23/EC),
- Environmental contaminants in raw materials and food from animal origin (B2c, B3a, B3b, B3c, B3d of Dir. 96/23/EC),

and thus is the only one laboratory designated in Poland as NRL for all Residues and Contaminants included in the Directive 96/23/EC.

The NVRI collaborates with the national veterinary inspectors in order to carry out the National Residue Control Plan (NRCP). The national veterinary services are in charge of food inspection and of collecting the samples. At the laboratory level, the organization implies the NVRI laboratories to monitor some part of the NRCP for veterinary drug residues in Poland (i.e. about 4,000 routine samples controlled per year extracted from around 29,000 samples to be monitored in Poland according to the 2012 NRCP in place). The NVRI is also in charge of additional quality controls for the agri-food sector and/or at the official request of veterinarian officers.

For collecting the samples on farm and at Border Inspection Posts, the officers of the national veterinary services are organized through the Regional and District Directorates with Veterinary offices located in the 16 Regions and in 12 Border Inspection Posts (4 harbors, 1 airport, 1 railway station and 6 customs roads with non EU countries). The veterinary officers are responsible of the collection and of the transport of the samples up to their final destination at the NVRI facilities.

Border Inspection Posts

2 ORGANIGRAM OF THE NVRI

The NVRI holds a completely new facility funded by the European Union and achieved on 18 November 2008 after 2 years of construction (January 2005 to February 2007). These buildings include 19,000 m^2 of laboratory space and animal facilities meeting the requirements of CL2, CL3 and CL3+ containment levels.

The NVRI is divided in 20 departments of microbiology, virology, radiobiology, pharmacology & toxicology, serological diagnosis, hygiene of food of animal origin and among which 18 departments hold certificates of accreditation from the Polish Accreditation Body (PCA) for their reference control activities.

Two departments are of particular interest from the point of the National Residue Control Plan: one department is the "food hygiene" department (microbiology technology) and the other one is the "pharmacology & toxicology" department (physico-chemistry technology).

The NVRI is currently counting a total staff of 548 people among whom 32 are working in the "microbiological" department with 5 of them interested in antimicrobial testing and 47 are working in the "pharmacology & toxicology" department among whom 9 of them are interested in analytical chemistry for antimicrobial residue testing.

Among the 32 scientific and technical staffs in the "food hygiene" department:

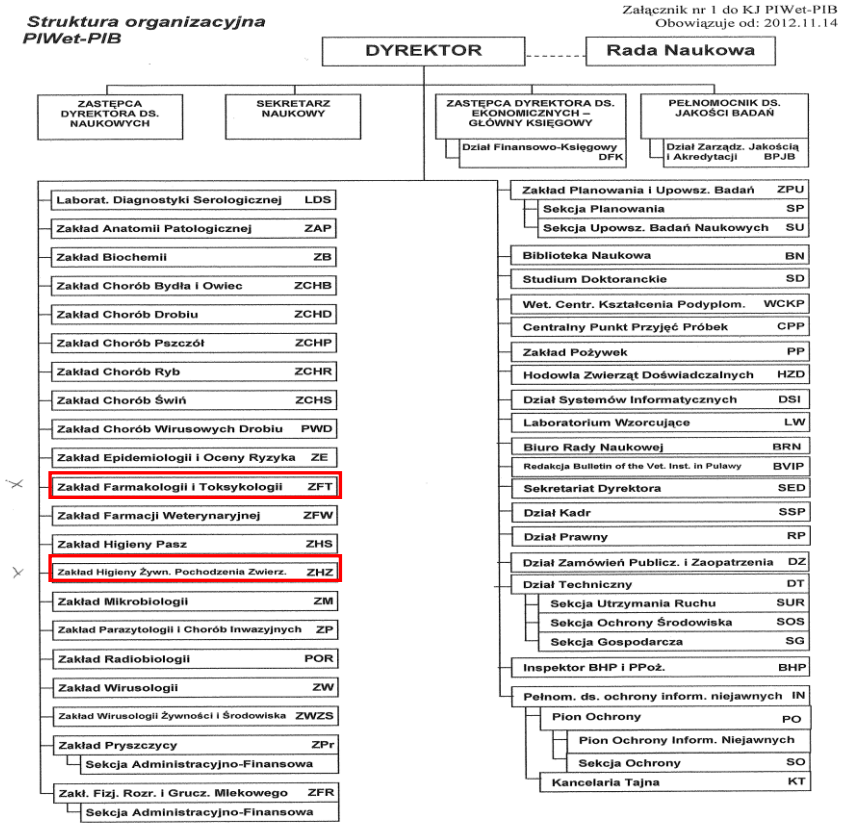
- 6 people have a PhD level from which 1 is Professor;
- 17 people have a Master degree in Science;
- 9 people have a Technical degree in Analytical Science.

Among the 47 scientific and technical staffs in the "pharmacology & toxicology" department:

- 18 people have a PhD level from which 7 are Professors;
- 16 people have a Master degree in Science;
- 13 people have a Technical degree in Analytical Science.

The NVRI is in charge of the control of residues in food from all kind of food-producing animal species (cattle, swine, ovine, caprine, equine, poultry, farmed and wild game, rabbit, and aquaculture) and of all kind of animal products including milk, egg, and honey products.

Organization of the NVRI Departments including 2 of them in charge of the NRCP



3 ORGANIZATION of the ACTIVITY for RESIDUE CONTROL at NVRI:

3.1 Accreditation, reference standard and quality system implemented:

The first accreditation under ISO17025 was obtained at NVRI in 2003. The Polish accreditation body is the PCA (Polish Center for Accreditation). The PCA was accepted as a full member of the European cooperation for Accreditation Organisation (EA) in November 2004.

At NVRI, the scope of accreditation on laboratory testing was first a fixed-term approach based on a list of accredited methods. The flexible scope has also been achieved for several parameters (methods). The list of currently accredited methods is provided in the Annex 1.

3.2 Premises, facilities, staffs:

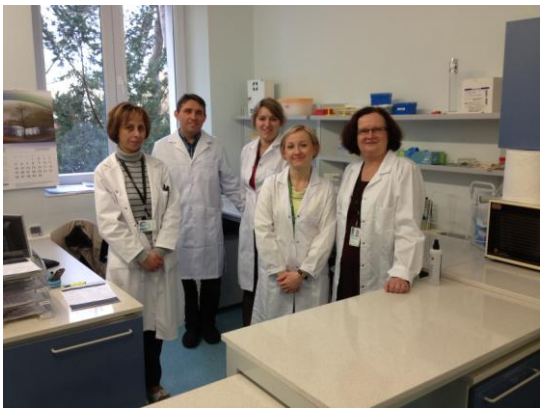
The NVRI is more than half a century old Institute created in 1945. New constructions were recently added in 2008. Located in the Northern entrance of the city of Puławy, the structure is organized as a complex of several blocks/segments with 2 levels, a ground floor and a 1st floor. The 2 Departments in charge of Veterinary Drug Residue Control in Food are located one in the segment E at the first floor for the Department of Pharmacology & Toxicology (see Annex 2) and the other one in the segment C at the first floor for the Department of Food Hygiene (see Annex 3).

Older part of the buildings

Main view of the overal complex
New facilities since 2008

In the Department of Food Hygiene, the residues of veterinary medicinal products are analysed by microbiological methods (Four plate test and NAT Five plate test and also several Rapid Test Kit Methods such as Delvotest SP-NT and Charm Rosa BLTET) in one room for sample preparation and one other room for incubations & readings by 4 analysts.

View of Microbiology analytical rooms and picture of the group of microbiologists



In the Department of Pharmacology & Toxicology, the residues of antimicrobial veterinary medicinal products are analysed by physico-chemical methods in 2 rooms no12 & 13 (sample preparation) and in rooms no14 & 15 (instrumental analyses by HPLC and by LC-MS/MS). They are operated by a group of 9 analysts with sometimes students for training periods.

View of Physico-chemistry analytical rooms and picture of the group of physico-chemists

3.3 Instrumentation:

The NVRI NRL is equipped with all relevant metrological instruments which are controlled by external calibration and by internal calibration. A system of metrological control of the automatic precision pipettes is also in place.
The system of control of the temperatures is operated by sensor checking and reported manually every day for each relevant instrument (storage freezers, fridges, incubators).

At the NVRI NRL, among the overall instrumentation, the physico-chemical instrumentation dedicated to the control of residues and contaminants are distributed as follows (see also Annex 2):

HPLC:	8
GC:	5
LC-MS:	4
GC-MS:	3
AAS:	5
ICP-MS:	1
Hg Analyzer:	2

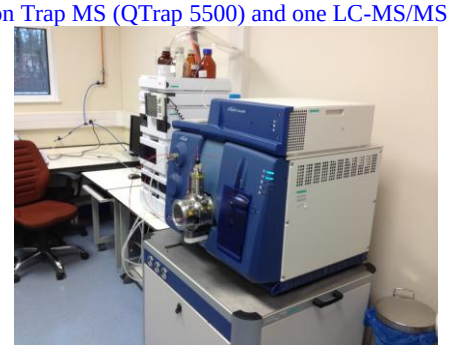
As regard to the instrumentation more specifically dedicated to residues of Veterinary Medicinal Products,

- HPLC-UV-DAD and HPLC-FLD (4 instruments)

. 2 Agilent 1100 system,
. 1 Varian ProSTar system
. 1 Shimadzu LC 10 VT system

- LC-MS(/MS) (4 instruments)

One LC-Ion Trap MS (QTrap 5500) and one LC-MS/MS (API4000) purchased from ABSciex located in room 1.014



One LC-MS Agilent 1200/6130 located in room 1.026 and one LC-MS/MS (API4000) ABSciex located in room 1.021

These instruments are supporting the analyses from the following methods:

- LC-UV-FLD instruments: sulfonamides, lasalocid, nicarbazine, quinolones, dyes, tetracyclines, , zearalenone,

- API 4000s: nitroimidazoles, chloramphenicol, nitrofuran metabolites, dyes confirmation, thyreostatics, beta-agonists, quinolones confirmation, multi-antimicrobial screening, anticoccidials. benzimidazoles, corticosteroids, NSAIDs, stanazolol, steroids,

- QTrap5500: nitroimidazoles, chloramphenicol, nitrofuran metabolites, dyes confirmation, thyreostatics, beta-agonists, quinolones confirmation, multi-antimicrobial screening, anticoccidials. benzimidazoles, corticosteroids, NSAIDs, stanazolol, steroids,

- LC-MS 1200/6130 anticoccidials. benzimidazoles, corticosteroids, , toxicological screening ,

An external maintenance of the LC-MS instruments is performed yearly through specific contract with the instrument company. During this interval, quality control checks (QCs) are routinely operated by the engineers.

3.4 Reception, storage and analysis of samples operated by the NVRI NRL:

The collection of sample is not the responsibility of the NVRI but the one responsibility of the Polish Veterinary Services (National and Regional) under the General Veterinary Inspectorate. Storage disposition and transport conditions are notified by NVRI to the GVI to avoid sample degradation before being delivered at laboratory. The reception of the samples at NVRI is performed by qualified staff dedicated to this task under the Department of Quality Assurance and Registration (ZFR). Sample arrival is acknowledged by one of the 4 qualified head experts / veterinarians at the reception office (at ground floor). Registered into specific LIMS electronic database (in-house NVRI software: MyLIMS PIWet-PIB), the sample is then stored first in special sample storage cabinets under temperature surveillance and control. This storage facility is located in the vicinity of the sample reception office. The NVRI LIMS is not strictly connected to the GVI central database but instructions & reporting results are e-transmitted into the GVI central database through the IT department at NVRI. Through the LIMS, the sample is redirected for analysis to the specific department(s) the expertise is requested from. Further sample preparation for quality checking by qualified analysts is then carried out within the same day before its final storage into the specific department prior to analysis. Generally less than 30 days are spent to achieve screening analyses. If a confirmation is complementary needed (positive result), the accepted delay for release of results is no longer than (2 weeks). For the official samples analyzed directly by confirmatory methods are less than 30 days of spend time or coming from positive screening from the Regional Laboratories, then the accepted delay is 14 Days.

The official delay for analyzing official samples is notified within the Quality Assurance Manual. Less than 1% of samples, per year, is analysed with delay, concerning with equipment troubles. The samples delivered at NVRI represent about 14% of all samples from the Polish NRCP (4000 over 29000 in 2011) and represent also about 50 % of all the samples tested at NVRI for Residues and Contaminants in the 2 dedicated Departments. The remaining 50°% accounts for a quality control service offered to the veterinarians and to several agri-food companies: dairies, meat companies.

In this last case, the samples could be carried out with other prescribed delays; the delay being contracted with each customer from a very urgent matter to a few days/weeks before sending the official result.

Results are registered in each responsible Department and also processed into the LIMS database for final review and confirmation by the Head of the Department before final release to the Reporting Secretariat and for signature by the Director of NVRI or his/her official representative.

3.5 Analytical methods available in the field of the EU-RL competence, Accreditation status:

In regard to the control of antimicrobial veterinary drug residue in food:
- for untargeted monitoring of authorized antimicrobial residues, a microbiological inhibitory plates method is carried out on matrix such as muscle, kidney, liver, egg (a Five Plate Test controlling fluids (meat, kidney, liver juices or egg fluids) is undertaken : the NAT). For milk and milk products Delvotest SP-NT and receptor assays are used. The 7 regional laboratories in charge of screening of antibiotics implement these same test. About 400 samples are screened each year at the NVRI with the NAT method, mainly muscle/kidney or muscle/liver samples. About 100 milk samples are screened using Delvotest SP-NT and Charm MRL BL/TET. In the case of a doubtful/positive result, samples are analyzed once again with the same procedure. If the sample is still screened positive, it is sent for confirmation to the Department of Pharmacology & Toxicology. Further investigation is then conducted to try to identify the group of substances giving rise to a positive inhibition on the microbiological test. In this regard, to fulfil this task in accordance with 657/2002/EC, a multi-antimicrobial confirmatory method has been put in place since 2010.
- for targeted investigations (chloramphenicol, nitrofurans, dyes, antimicrobials in honey,…), the screening is operated by ELISA kit when possible or directly processed by HPLC-UV-FLD or by LC-MS/MS. For example, chloramphenicol is screened by an ELISA kit and if sample is found positive it is then confirmed by LC-MS/MS. Nitrofuran metabolites are screened directly by LC-MS/MS and can be additionally confirmed by the same LC-MS/MS method conducted on a quantitative/confirmatory process. This method may include a solvent washing procedure of the sample prior to extraction when explicit presence of SEM is demonstrated. Malachite green and its leucobase are screened by a HPLC-DAD procedure and can be confirmed by a LC-MS/MS which is able to control also the crystal violet and its leucobase. Discussion was engaged during the EU-RL visit to evaluate the reliability and practicability of the LC-UV-DAD method in regard to the LC-MS/MS method which is more complete and simple to carry out ... Sulfonamides are screened and confirmed in muscle and milk by HPLC-UV and HPLC-FLD procedures respectively. (Fluoro)Quinolones are screened and confirmed in biological materials by HPLC-FLD. Efforts are still on-going to develop a reliable satisfactory strategy for analysis of antimicrobials in honey matrix.
Malachite green and its leucobase is screened by HPLC-DAD and confirmed by LC-MS/MS.

According to the previous updating carried out by the EU-RL in 2009 to evaluate the methods available in the NRLs of the 27 EU-MS for implementation of the antimicrobial residue monitoring in food from animal origin, it was received from the NVRI the following notification updated during the 2012 EU-RL visit:

Biological Screening

Compounds	Matrices	Species		Method	Laboratory	Responsible	Limits (µg/kg)
Antimicrobal substances	M, K, L, E	B, O, P, Py, E, F	MIC	5 plate method (NAT)	Poland NVRI Pulawy	H. Rozanska/J.Osek	
Antimicrobal substances	Mk	B	MIC	Delvotest SP-NT + receptor tests	Poland NVRI Pulawy	H. Rozanska/J.Osek	
Chloramphenicol	M, Mk, E, U, Pl, H, W	B,O,P,Py,F	ENZ	ELISA	Poland NVRI Pulawy	A.Posyniak/J.Zmudzki	0.12-0.20

M: muscle
Mk: milk
L: liver
K: kidney
U: urine
E: eggs
W: water

B: bovine
O: ovine
P: porcine
C: caprine
E: equine
Py: poultry
R: rabbit
H: honey
F: fish

** Letters A & B mean ''under development'' and ''not yet validated" respectively and Letters C, D and E mean different levels of validation according to Decision 657/2002/EC*
(for further details, please refer to document "list of NRLs methods update 2009" included into the EU-RL Website restricted area: http://crl.fougeres.anses.fr)

Confirmatory Methods

Aminoglycosides

Streptomycin	H		LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	
Streptomycin, Dihydrostreptomycin, Gentamicin, Neomycin, Spectinomycin, Kanamycin, Paromomycin	M, Mk	B, P, Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	

Beta-lactams

Amoxycillin, Ampicillin, Penicillin G, Oxacillin, Cloxacillin, Nafcillin, Dicloxacillin, Cefapirin, Cefeperazone, Cefalexin, Cefquinome, Cefalonium, Cefazoline	M, Mk	P, P, Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	13 - 1062 / 16 - 1217
Amoxycillin, Ampicillin, Penicillin G, Oxacillin, Cloxacillin, Nafcillin, Dicloxacillin, Cefapirin, Cefeperazone, Cefalexin, Cefquinome, Cefalonium, Cefazoline	E	Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	27 - 30 / 34 - 42

Macrolides

Tylosin, Tilmicosin, Erythromycin, Spiramycin, Josamycine	M, Mk	B, P, Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	55 - 234 / 66 - 300
Tylosin, Tilmicosin, Erythromycin, Spiramycin, Josamycine	E	Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	25 - 231 / 31 - 275

Nitrofurans

AOZ, AMOZ, AHD, SEM	M,Mk, E,U,Pl ,H	B,O,P,Py,F	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	0.10-0.57 / 0.14-0.71
Furazolidone, Nitrofurantoin, Nitrofurazone, Furaltadone	W		LC-DAD-LC/MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	0.57-0.70 / 0.66-0.88

Amphenicol compounds

Chloramphenicol	M,Mk,E,U, Pl,H,W	B,O,P, Py,F	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	0.09-0.15 / 0.13-0.25
Florfenicol	M	F	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	54.4 / 57.4

Quinolones

Ciprofloxacin, Enrofloxacin, Difloxacin, Danofloxacin, Marbofloxacin, Norfloxacin, Sarafloxacin, Oxolinic acid, Flumequine, Nalidixc acid	M, Mk	B, P,Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	35 - 471 / 142 - 576
Ciprofloxacin, Enrofloxacin, Difloxacin, Danofloxacin, Marbofloxacin, Norfloxacin, Sarafloxacin, Oxolinic acid, Flumequine, Nalidixc acid	E	Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	25 28 / 31 - 38
Ciprofloxacin, Enrofloxacin	M	B, P,Py, F	HPLC-FLD	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	116 - 120 / 133 - 146

Sulfonamides & Diaminopyrimidine derivatives

S-methazine, S-merazine, S-dimetoxin, S-diazine, S-methoxazole, S-metoxypyridazine, S-guanidine, S-tiazole, S-monometoxine, S-doxine, S-chinoxaline	M, Mk	B,P,Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	103 - 114 / 118 -137
S-methazine, S-merazine, S-dimetoxin, S-diazine, S-methoxazole, S-metoxypyridazine, S-guanidine, S-tiazole, S-monometoxine, S-doxine, S-chinoxaline	E	Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	25 - 29 / 32 - 39
S-methazine, S-cetamide, S-dimetoxin, S-thiazole, S-methoxazole, S-metoxypyridazine, S-merizne	H		HPLC-FLD	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	27 - 35 / 42 - 47
S-methazine, S-dimetoxin, S-diazine, S-methoxazole, S-metoxypyridazine, S-merizne	M, Mk	B, P, Py, O, F	HPLC-Uv	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	117 124 / 132 - 151

Tetracyclines

Tetracycline, Oxytetracycline, Doxycycline, Chlortetracycline	M, Mk	B,P, Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	110 - 115 / 125 - 133
Tetracycline, Oxytetracycline, Doxycycline, Chlortetracycline	E	Py	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	28 - 238 / 39 - 285
Tetracycline, Oxytetracycline, Doxycycline, Chlortetracycline	M	B,P,Py	LC-UV	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	109 - 120 / 125 - 138

Tetracycline, Oxytetracycline, Doxycycline, Chlortetracycline	H		LC-UV	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	29 - 33 / 36 - 44
Dyes for Aquaculture animals							
Malachite green, Leucomalachite green	M	F	HPLC-UV/FLD	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	0.13-0.15 / 0.32-0.37
Malachite green, Leucomalachite green, Crystal Violet, Leuco Crystal violet, Brilliant green	M	F	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	0.03-0.09 / 0.07-0.23
Carbadox - _Olaquindox							
QCA, DCBX	M	P	LC-MS	Poland NVRI Pulawy	A. Posyniak / J. Zmudzki	E	1.04 - 1.96 / 1.46 - 2.74

Considering the validation part of the activity for method development, the methods implemented at NVRI (or transferred from EU-RLs) are all considered validated under the scope of the Commission Decision no 657/2002/EC and most of them are now accredited under the flexible scope according to the PCA accreditation (last audit : 19-20/11/12). The accreditation status of the NVRI methods for monitoring antimicrobials and other veterinary drugs in food is addressed in the Annex 1.
Overall, this is a set of 39 methods that is currently registered under the NVRI list dedicated to control of Residues & Contaminants in food and including the 4 methods from the Department of Food Hygiene (antimicrobial large scope inhibitory tests and rapid kits). From these 39 methods, 28 of them are implemented through a flexible scope of accreditation.
Concerning the microbiological inhibitory methods, the Four Plate Test was the previous screening method in place at NVRI for antimicrobial residues in meat tissues. More recently, the NAT five plate test protocol has been replacing it since 2011.
Considering the entire list of 39 registered methods, it is stressed a lot of work has been engaged in this laboratory to cover the large field of veterinary drug residues in food from animal origin.
Although 14 physico-chemical methods registered in the NVRI list of methods did not pass through a validation process as of the 2012 list; only 2 of these 14 methods are in the field of antibacterial residue control and recently introduced:

- a multi-antibacterial residues LC-MS/MS method in biological materials,
- a tetracycline HPLC confirmatory method in biological materials.

Finally, as already mentioned above, the physico-chemical confirmation of positive screening of antibacterial inhibitory activity by the five plate test method is now also strengthened by the implementation of the new multi-antibacterial LC-MS/MS method.

3.6 Analytical development currently operating at NVRI or in project for 2013-2015 period

The NVRI current R&D in the field of antimicrobial residue control in food contains:

- The validation of the multi-antibacterial residues LC-MS/MS method in biological materials.
- The extension of the multi-antibacterial residues LC-MS/MS method to difficult matrices such as honeys or eggs.

In line with this particular need, a hands-on training session is scheduled at mid-December 2012 at Anses-Fougeres laboratory for 2 junior scientists of the NVRI-PiWet Pulawy laboratory.
This activity is in strong connection with the EU-RL effort to transfer more technical knowledge and practical training on LC-MS/MS analysis of antimicrobial multi-residue screening and confirmatory methods in honey as of its 3 last programmes of 2011, 2012 and 2013.

The NRL is also developing a certain number of projects in collaboration with other departments of the NVRI and also with other polish Institutes.
It can be cited as recent projects:

- Collaboration with the Department of Honey Bee Diseases at NVRI to determine the occurrence of sulphonamides in bee colonies and their depletion over two years after treatment with Polisulfamid®.
- Collaboration with the Department of Poultry Diseases at NVRI to evaluate the contamination of poultry after treatment with antibacterial compounds.

- Collaboration with the Department of Virology/Bacteriology of Fishes at NVRI to evaluate the presence of dyes (malachite green) after treatment of farmed fishes.

In the near future, are intended the following developments/researches at NVRI for improving the veterinary drug residue control:

- Development of a new confirmatory procedure for beta-lactams and aminoglicosides,
- Development of a new analytical procedure for chloramphenicol with QuEchERS sample preparation,
- Development of multi-residue screening for antibacterials in honey,
- Development of multi-residue procedure for eggs.

3.7 NRL activity - Organization of training and proficiency testing for regional field laboratories:

The NVRI holds an important part of its work in scientifically and technically coordinating a network of routine laboratories located in the different regions of Poland. A programme for the network is prepared each year with at least a meeting workshop organized at Pulawy laboratory 3 to 4 times a year including sometimes hands-on training sessions for the experts from the concerned regional laboratories. Several proficiency testing studies are also scheduled on a year basis.
The food hygiene department provided several proficiency tests (PT) since 2008 to the attention of the 7 regional laboratories for determining their proficiency to control antibacterial residues by means of the plate test inhibitory methods: 4 plate test method until 2010 and now the NAT 5 plate test method. This NRL team also provides advices concerning rapid biomethods available in Poland for the control of inhibitory residues in milk at farm tanks and at dairies production level. The team is also requested from the GVI to proficiency test the field laboratories (private and public labs) dedicated to the autocontrol of milk production.
As a follow-up of its transfer of analytical methods to regional laboratories, the pharmacology & toxicology department's team provides a set of proficiency testing studies to evaluate to the network. See the annex 4

for presentation of the 2012 NRL programme of activities organized by the 2 NVRI Departments for the network of regional laboratories.

3.8 NRCP samples:

Considering the 2012 NRCP, it is about nearly 5,000 analyses that have to be operated at the NRL NVRI level among the 29,000 samples forecast in the NRCP. At the NRL, about 300 analyses have to be addressed for screening by the NAT five plate test method, and about 110 (milk) for Delvotest SP-NT and receptor tests. About a 100 analyses have to be performed for screening by ELISA test kits. By LC-MS/MS are programmed 300 analyses and 100 analyses by HPLC-DAD/FLD (antibacterials).
A quick overview of the samples analyzed at NVRI up to the end November for the year 2012 in antimicrobial & dye residue control is as follows:

Antibacterial Substances	Food commodities	Methods	Number of required analyses for 2012 NRCP	Number of analyses tested *	Analyses found non compliant *
		TOTAL :	≈ 29,000	≈ 5,000	
antimicrobials	tissues, eggs	NAT Five plate test	5110	299	0
antimicrobials	milk	Delvotest SP-NT and receptor kits	1756	109	0
antimicrobials	all foods from AO	LC-MS/MS screening	620	49	0
streptomycins	honey	LC-MS/MS	10	10	0
aminoglycosides	meat and milk	LC-MS/MS	620	49	0
beta-lactams	meat and milk	LC-MS/MS	620	49	0
beta-lactams	eggs	LC-MS/MS	160	11	0
macrolides	meat and milk	LC-MS/MS	620	49	0
macrolides	eggs	LC-MS/MS	160	11	0
nitrofuran metabolites	all foods from AO	LC-MS/MS	603	37	0
nitrofurans	drinking water	HPLC	142	10	0
chloramphenicol	all foods from AO	ELISA/LC-MS/MS	2160	110	0
florfenicol	aquaculture products	LC-MS/MS	30	30	0
quinolones	all foods from AO	LC-MS/MS	620	49	0
quinolones	eggs	LC-MS/MS	160	11	0
enro/ciprofloxacin	meat and fish	HPLC-FLD	245	10	0
sulfonamides	meat & milk	LC-MS/MS	620	49	0
sulfonamides	eggs	LC-MS/MS	160	11	0
sulfonamides	honey	LC-FLD	140	16	3
sulfonamides	meat and milk	HPLC-UV	620	33	0
tetracyclines	muscle and milk	LC-MS/MS	620	49	0
tetracyclines	eggs	LC-MS/MS	160	11	0
tetracyclines	meat	HPLC-UV	460	26	0
tetracyclines	honey	HPLC-UV	10	10	0
dyes (MG & CV)	aquaculture products	LC-MS/MS	16	16	2
malachite green	aquaculture products	LC-UV/FLD	150	16	0
lincomycin	meat	LC-MS/MS	620	49	0
lincomycin	eggs	LC-MS/MS	160	11	0

** Here are of concern those analyses only performed at the NVRI laboratories and not including the samples analysed in the regional laboratories.*

Considering the number of samples found to be already tested and the period of the year of the EU-RL visit, it is worth to be noted that 87 % of the samples forecast for physico-chemical analyses within the 2012 NRCP have been fully completed for the part of the NRCP dedicated to veterinary drug residue control.

4 PARTICIPATION OF THE NRL TO INTERLABORATORY PROFICIENCY TESTING STUDIES:

In the field of analysis of veterinary drug residues, the NVRI participated to quite all of the EU proficiency testing studies organized by the EU-RL of Fougères and also participated to some other PT provided by other providers (FAPAS, EU-RL-BVL, EU-RL-Rikilt).

In regard to antimicrobial and dye residues, hereafter are the last 6 years of participations to EU-RL Anses-Fougeres PTS and the overall evaluation of the results obtained:

Organizer (year)	Object	Number of samples	Results (as Z-score values)
CRL-AFSSA (2006)	Carbadox-Olaquindox in Pig Tissues (Confirmation)	4	No Participation
CRL-AFSSA (2006)	Antimicrobials in whole Eggs (Screening and Confirmation)	5	WW-AQ: Satisfactory Results confirmed with a good precision of measurement but wrong interpretation of compliance in regard to limits in eggs
CRL-AFSSA (2007)	Chloramphenicol in Milk and in Honey (Confirmation)	6	XI-BF: Satisfactory Results in milk and in honey except for 1 inaccurate result in honey
CRL-AFSSA (2007)	Penicillins and Cephalosporins in Milk (screening step)	6	XV-MD: Satisfactory Results in screening with no-false negative/positive results
CRL-AFSSA (2007)	Penicillins and Cephalosporins in Milk (Confirmation step)	6	XV-MD: Satisfactory Results in confirmatory step identifying all 4 beta-lactams with only 1 inaccurate result for cloxacillin measurement
CRL-AFSSA (2008)	Nitrofuran metabolites in Meat (Screening step)	6	YE-EA : Satisfactory Results in screening with no-false negative/positive results excepting the AHD (false negative).
CRL-AFSSA (2008)	Nitrofuran metabolites in Meat (Confirmation step)	6	YE-EA: Satisfactory Results in confirmatory step identifying all metabolites excepting the AHD (false negative).
CRL-AFSSA (2008)	Tetracyclines in Pork muscle (confirmation step)	4	YS-BF : Satisfactory Results for material 1 (blank), for material 2 (OTC) and for material 3 (Doxy) and for material 4 (TTC).
CRL-AFSSA (2009)	Multi-Antibiotics in Milk (screening step)	5	ZU-TZ : Screening by Delvotest SP-NT is satisfactory for the 4 beta-lactam analytes but not satisfactory for the 2 tetracyclines at 1 MRL / 2 MRL levels
CRL-AFSSA (2009)	Multi-Antibiotics in Milk (confirmation step)	5	ZU-TZ : Satisfactory Results in confirmatory step for cloxacillin and oxytetracycline but not satisfactory for penicillin-G (false negative), cefquinome (questionable quantification) and cephalonium (unsatisfactory quantification)
CRL-AFSSA (2009)	Dyes in Aquaculture Fish (confirmation step)	3	AE-NT : Satisfactory Results in overall compartments of the confirmatory step (identification and quantification)
EU-RL-AFSSA (2010)	Chloramphenicol in aquaculture products (confirmation step)	3	BI-DQ : Satisfactory Results in overall compartments of the confirmatory step (identification and quantification)
EU-RL-ANSES (2010)	Multi-Antibiotics in Muscle (screening step)	4	BQ-LF : Satisfactory Results in overall compartments of the screening step by a multi-plate test (no false negative / no false positive)
EU-RL-ANSES (2010)	Multi-Antibiotics in Muscle (confirmation step)	4	BQ-LF : Satisfactory Results in overall compartments of the confirmatory step (identification and quantification)

EU-RL-ANSES (2011)	**Nitrofuran metabolites in honey (confirmation step)**	**3**	**DA-VE : Satisfactory Results in overall compartments of the confirmatory step (identification and quantification)**
EU-RL-ANSES (2011)	**Quinolones in aquaculture Products (confirmation step)**	**3**	**DM-RW : Satisfactory Results in overall compartments of the confirmatory step (identification and quantification)**
EU-RL-ANSES (2012)	**Tetracyclines in Pork muscle (confirmation step)**	**4**	**EQ- On-going PT at time of this report**

5 RELATIONS WITH THE EU-RL ANSES-FOUGERES:

The number of participations to the Proficiency Testing Studies and also to the Workshops and/or Training sessions organized by the EU-RL towards the EU-NRLs network is considered to be very satisfactory over the past 6 years. Also, the results obtained in the Proficiency Testing Studies organized by the EU-RL are at a very acceptable level.

Cooperation between EU-RL Anses-Fougeres and the NVRI has been organized several times in the past (2005 in Pulawy, 2008 in Fougères, 2012 in Fougères). In the future there might be some common interest in the field of VMP residues in bee products and also in building a larger scope "multi-antibiotic LC-MS screening approach" or an even more strategic "multi-VMP classes LC-MS screening approach".

6 CONCLUSION AND GENERAL COMMENTS:

This visit allowed the EU-RL delegate to discover and appreciate the activity of the National Reference Laboratory for the Control of Veterinary Drug Residue in Food as it is implemented for Poland at the NVRI NRL level in Puławy. The EU-RL delegate particularly appreciates the context of opened discussions with the different responsibles of veterinary drug residue activities.

The various activities of routine control carried out at NVRI are conducted according to a policy of systematically accredited methods.

Regarding the facilities and the instrumentation, the laboratory is very well equipped with up-to-date equipment.

Regarding the total staff engaged in the residue monitoring in food, the number and the quality of the recruited staff with quite a lot of junior scientists is pretty well organized both for the routine control and for the R&D activities.

The control of authorized antimicrobial substances in meat and milk is monitored by means of a screening microbiological method (NAT Five Plate Test, Delvotest SP-NT and receptor tests). A correct set of confirmatory methods is developed, validated and accredited to confirm by means of both HPLC and LC-MS/MS technology. But still work has to be implemented to issue adequate methods for some families of antibiotics such as aminoglycosides and macrolides and to extend to other food commodities such as honey and egg matrices.

The full implementation of the NRCP is achieved quite satisfactorily.

In regard to the Proficiency Testing participation to antibiotic PTS provided by the EU-RL, the results are now fully satisfactory for the last 3 years indicating a lot of work engaged to fulfill this requirement in the recent past years and corrective actions done in regard to the previous deviations encountered.

Staff (with quite a lot of junior analysts) receives a correct number of specific trainings to enhance their skills and especially in the physico-chemistry part. This fact triggers some speed up into the development of strategic methods.

The NVRI do not neglect its functions of NRL acting with a network of routine laboratories. It covers satisfactorily the tasks dedicated to the activities of a Reference Laboratory for Poland and particularly the yearly rounds of Proficiency Testings after the transfer of the analytical methods.

7 REPORT OF THE MISSION RELEASED TO THE COMMISSION:

A draft report was sent to the NVRI responsibles of "Food Hygiene" and "Pharmacology & Toxicology" Departments (Dr Hanna Rosanska & Dr Jan Zmudzki/Dr Andrzej Posyniak) within 4 weeks after the mission was completed in order to receive in return their comments and adjustments of the draft at the EU-RL delegate level within the 4 following weeks.

As soon as read, commented and agreed by the NVRI responsibles, this report in its final format was made available to the European Commission (DGSANCO) strictly, through the EU-RL ANSES-Fougeres website on the DG-Sanco part (http://crl.fougeres.anses.fr). A copy of the final report was also transmitted in January 2013 by email to the Director General of the NVRI : Dr. Krzysztof Niemczuk and to the responsible of the 2 Departments at NVRI.

ANNEXS

DOCUMENTS and INFORMATIONS EXCHANGED DURING THE VISIT:

Received from NVRI:

1. List of accredited (*) and non accredited methods at NVRI
2. Building facilities at NVRI Segment E: Department of Pharmacology & Toxicology
3. Building facilities at NVRI Segment C : Department of Food Hygiene
4. Programme of activities of the NRL toward the network of regional laboratories for 2012

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ANNEX 1

List of accredited (*) and non accredited methods at NVRI for Veterinary Drug Residue Control (Antimicrobial & dye substances) in the Department of Hygiene of Food of Animal Origin and in the Department of Pharmacology & Toxicology

Scope of accreditation of the Department of Hygiene of Food of Animal Origin NVRI Puławy.

Status: 2012.11.30

1. **Microbiological, diffusion method of the detection of residues of inhibitory substances – „4-plate". ZHZ/PB-01, ed. 3, valid from 2010.01.25**	*
2. **Detection of the residues of β-lactams and tetracyclines in milk using CHARM ROSA MRL BL/TET test. ZHZ/PB-04, ed. 2, valid from 2012.02.13**	*
3. **Microbiological method of the detection of antibacterial substances – Delvotest SP-NT. ZHZ/PB-17, ed. 2, valid from 2012.02.13**	*
4. **Microbiological screening method of the detection of antibacterial substances (5-plate). ZHZ/PB-24, ed. 2, valid from 2012.02.13**	*

Scope of accreditation of the Department of Pharmacology & Toxicology (1)

List of accredited procedures, Department of Pharmacol. and Toxicol.		Page 1 from 3
No.	Symbol	Title
1.	ZFT/PB/01-01* ed.4;2012-05-18	Determination of concentrations of organochlorine pesticide and polychlorinated biphenyl in food of animal origin and feed by gas chromatography
2.	ZFT/PB/01-02* ed.2;2011-08-22	Determination of residues of organophosphate pesticides in food of animal origin by gas chromatography
3.	ZFT/PB/01-03* ed.3;2012-08-10	Determination of pyrethroid residues in food of animal origin by gas chromatography
4.	ZFT/PB/01-09 ed.2;2012-08-10	Determination of Polichlorinated Aromated Hydrocarbons in food of animal origin by gas chromatography-mass spectrometry
5.	ZFT/PB/02-01* ed.4;2011-08-31	Determination of sulfonamide residues in the biological material by liquid chromatography with UV detection
6.	ZFT/PB/02-06* ed.2;2011-09-12	Determination of sulfonamide residues in the biological material by liquid chromatography with FL detection
7.	ZFT/PB/02-07* ed.2;2011-07-25	Determination of fluoroquinolone residues in the biological material by liquid chromatography
8.	ZFT/PB/02-11* ed.4;2012-07-12	Determination of chloramphenicol residues in the biological material by liquid chromatography coupled with mass spectrometry
9.	ZFT/PB/02-12* ed.3;2011-08-31	Determination of nitrofurane metabolite residues in the biological material by liquid chromatography coupled with mass spectrometry
10.	ZFT/PB/02-17* ed.3;2012-10-15	Determination of nitroimidazole residues in the biological material by liquid chromatography coupled with mass spectrometry
11.	ZFT/PB/02-20 ed.2; 2011-09-12	Determination of antibacterial compounds residues in the biological material by liquid chromatography coupled with mass spectrometry
12.	ZFT/PB/03-08* ed.4; 2011-08-31	Determination of β-agonists residues in the biological material by liquid chromatography coupled with mass spectrometry
13.	ZFT/PB/04-01* ed.4;2011-08-31	Determination of lasalocid residues in tissues by liquid chromatography
14.	ZFT/PB/04-15* ed.2;2011-08-31	Determination of macrocyclic lactones residues in animal tissues and milk using liquid chromatography

Scope of accreditation of the Department of Pharmacology & Toxicology (2 & 3)

List of accredited procedures, Department of Pharmacol. and Toxicol.	Page 2 from 3

No.	Symbol	Title
15.	ZFT/PB/04-17* ed.2;2011-08-31	Determination of benzimidasoles in animal tissues by liquid chromatography
16.	ZFT/PB/04-18* ed.4;2012-09-06	Determination of coccidiostats residues in tissues and eggs by liquid chromatography coupled with mass spectrometry
17.	ZFT/PB/04-20* ed.4;2011-08-31	Determination of nicarbazine in feed and premix by liquid chromatography
18.	ZFT/PB/04-21* ed.3;2011-08-31	Determination of benzimidazoles in milk by liquid chromatography coupled with mass spectrometry
19.	ZFT/PB/05-01* ed.5;2011-07-22	Determination of lead and cadmium in biological material by graphite furnace atomic absorption spectrometry method
20.	ZFT/PB/05-02* ed.5;2011-07-22	Determination of total mercury in biological material by atomic absorption spectrometry method
21.	ZFT/PB/05-03* ed.5;2011-07-22	Determination of arsenic in biological material by atomic absorption spectrometry method
22.	ZFT/PB/06-01 ed.4;2011-09-09	Determination of ochratoxin A in feed by liquid chromatography
23.	ZFT/PB/06-02 ed.4;2011-09-09	Determination of ochratoxin A in animal tissues by liquid chromatography
24.	ZFT/PB/06-09 ed.1;2007-04-16	Determination of deoxynivalenol in feed by liquid chromatography
25.	ZFT/PB/06-10 ed.1;2007-04-16	Determination of zearalenone in feed by liquid chromatography
26.	ZFT/PB/07-05* ed.3;2011-08-31	Determination of corticosteroids residues in animal tissues using liquid chromatography - mass spectrometry
27.	ZFT/PB/07-06* ed.3;2011-08-31	Determination of non-steroidal anti-inflammatory drugs residues in animal tissues by liquid chromatography coupled with mass spectrometry
28.	ZFT/PB/08-01* ed.2; 2011-07-29	Determination of dye residues in fish by liquid chromatography
29.	ZFT/PB/08-02* ed.2; 2011.07.29	Determination of dye residues in fish by liquid chromatography coupled with mass spectrometry

List of accredited procedures, Department of Pharmacol. and Toxicol.	Page 3 from 3

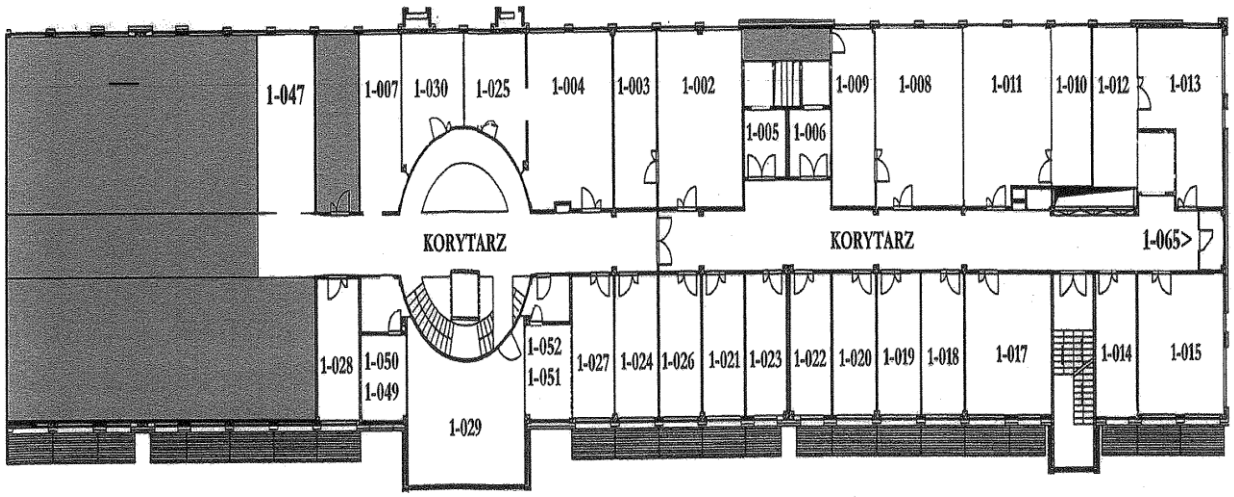
No.	Symbol	Title
30.	ZFT/PB/10-01 ed.3; 2011.08.18	Determination of residues of trenbolone in biological samples with gas chromatography - mass spectrometry
31.	ZFT/PB/10-02 ed.3; 2011-08-18	Determination of gestagenes in fat tissue of slaughter animals with gas chromatography - mass spectrometry
32.	ZFT/PB/10-05 ed.3;2011-08-18	Determination of 17 β-estradiol and testosterone in serum with gas chromatography - mass spectrometry
33.	ZFT/PB/10-06 ed.3;2011-08-18	Determination of zeranol and metabolites in biological samples with gas chromatography - mass spectrometry
34.	ZFT/PB/10-12* ed.6;2012-09-10	Determination of residues of anabolic hormone in urine and muscle tissues of slaughter animals with gas chromatography - mass spectrometry
35.	ZFT/PB/10-21 ed.4;2011-08-21	Determination of thyreostatic drugs in biological samples by liquid chromatography tandem mass spectrometry
36.	ZFT/PB/10-20 ed.4;2012-09-10	Determination of residues of stanozolol and 16β-hydroxystanozolol in biological samples by liquid chromatography – mass spectrometry
37.	ZFT/PB/10-22 ed.2,2011-11-21 zgłoszona do akredytacji	Determination of residues of steroid hormons in biological samples by liquid chromatography – mass spectrometry
38.	ZFT/PB/11-01* ed.5;2011-08-31	Determination of melamine in biological samples by in biological samples by liquid chromatography – mass spectrometry
39.	ZFT/PB/02-13 Prepared for accreditation 2013	Determination of tetracyclines in biological samples by liquid chromatography

Flexible accreditation *

ANNEX 2

Building premises at NVRI for NRL activities
Segment E: Department of Pharmacology & Toxicology

Budynek laboratoryjny, Segment E - 1 piętro

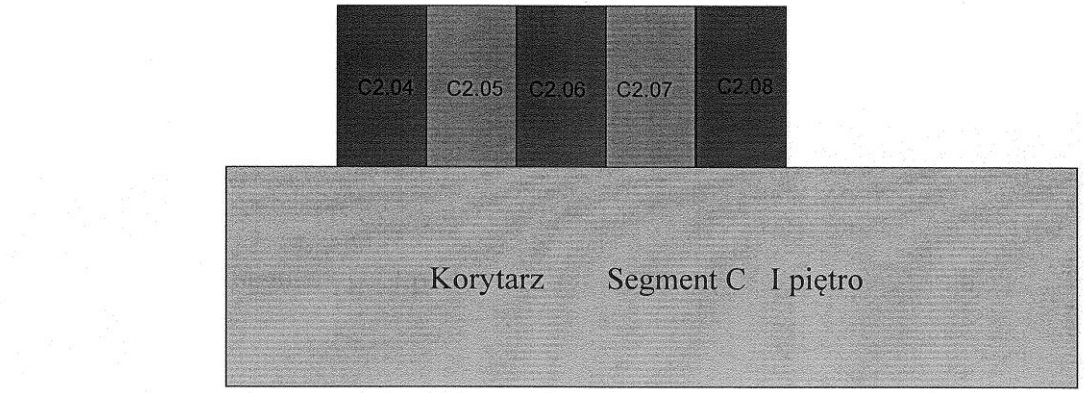


1-047 : Room for feed sample preparation
1-007 : Room for feed sample preparation
1-030 : Room for chemistry glassware washing
1-025 : Sample reception at Department + Quality Assurance Office of the Department
1-004 : Sample preparation room for environmental contaminants (mycotoxins)
1-028 : Secretary room
1-049/1-050 : Toilets
1-029 : Social room
1-051/1-052 : Toilets
1-027 : Balance room
1-024 : Sample preparation room for toxicological diagnosis
1-026 : Analytical room for general toxicology and mycotoxins (HPLC)
1-021 : Analytical room for group B2 and A1-A4 groups ((LC-MS)
1-023 : Analytical room for pesticides (GC & GC-MS)
1-022 : Analytical room for pesticides (GC)
1-020 : Analytical room for group B2(HPLC)
1-018 : Analytical room for heavy metals (AAS)
1-019 : Analytical room for heavy metals (IPS-MS)
1--017 : sample preparation room for heavy metals (Pb, Hg, Cd)
1-009 : Sample preparation room for pesticides (organochlorides, organophosphorus, pirethroids)
1-008 : Sample preparation room for pesticides (organochlorides, organophosphorus, pirethroids)
1-011 : Sample preparation room for group B2 substances (antiparasitics, anticoccidials, …)
1-010 : Sample preparation room for group B2 substances (antiparasitics, anticoccidials, …)
1-012 : Sample preparation room for antimicrobial and dye substances ()
1-013 : Sample preparation room for antimicrobial and dye substances
1-014 : Analytical room for antimicrobial and dye substances (LC-MS)
1-015 : Sample preparation room for forbidden compounds (chloramphenicol, nitrofuranes, melamine, β-agonists)

ANNEX 3
Building premises at NVRI for NRL activities
Segment C: Department of Food Hygiene

Załącznik nr 6 do KJZ ZHZ
Obowiązuje od: 2012.09.03

Schemat pomieszczeń laboratoryjnych Zakładu Higieny Żywności Pochodzenia Zwierzęcego



- Pracownia pozostałości substancji przeciwbakteryjnych - antimicrobials
- Pomieszczenie wspólne – Pomieszczenie aparaturowe
- Korytarz
- Pomieszczenia ZB i ZCHS

3/3

C2.05 : Sample preparation room for antimicrobial residue screening by inhibitory tests

C2.07 : Analytical room for readings

ANNEX 4

Programme of activities toward the network of regional laboratories
For 2012

Programme of PT for Inhibitory Microbiological Methods

REGULAMIN UCZESTNICTWA W PROGRAMIE MIĘDZYLABORATORYJNYCH BADAŃ BIEGŁOŚCI W ZAKRESIE POZOSTAŁOŚCI SUBSTANCJI PRZECIWBAKTERYJNYCH W ŻYWNOŚCI POCHODZENIA ZWIERZĘCEGO W 2012 ROKU

§ 1. Cel programu

1. Celem programu międzylaboratoryjnych badań biegłości w zakresie pozostałości substancji przeciwbakteryjnych jest stworzenie możliwości potwierdzenia kompetencji laboratoriów stosujących skriningowe metody wykrywania tych substancji w surowcach i produktach zwierzęcego pochodzenia
2. Program jest organizowany i realizowany w oparciu o normę PN-EN ISO/IEC 17043:2011 „Ocena zgodności. Ogólne wymagania dotyczące badań biegłości"

§ 2. Organizator programu

1. Organizatorem programu jest Państwowy Instytut Weterynaryjny-Państwowy Instytut Badawczy, Krajowe Laboratorium Referencyjne Zakładu Higieny Żywności Pochodzenia Zwierzęcego, Al. Partyzantów 57, 24-100 Puławy, NIP 716-00-10-761, KRS 0000118357
2. Badania biegłości organizowane są w oparciu o Art. 25 Ustawy z dnia 29 stycznia 2004 r. o Inspekcji Weterynaryjnej (Dz. U. Nr 33 poz. 287 z późn. zm.).
3. Dane teleadresowe Organizatora: Państwowy Instytut Weterynaryjny – Państwowy Instytut Badawczy, Zakład Higieny Żywności Pochodzenia Zwierzęcego, Al. Partyzantów 57, 24-100 Puławy, tel.: 81 889 30 00, faks: 81 886 25 95
4. Osoba do kontaktów z Uczestnikami programu:
dr Hanna Różańska - tel.: 81 889 33 59, e-mail: PT.sh@piwet.pulawy.pl
5. Pod adres e-mail zawarty w pkt. 4 należy kierować wszelką korespondencję dotyczącą badań biegłości, w tym zgłoszenia udziału w badaniach i karty wyników.

§ 3. Adresaci programu

1. Program badań adresowany jest do laboratoriów wykonujących badania w kierunku pozostałości substancji przeciwbakteryjnych w surowcach i produktach zwierzęcego pochodzenia, zwłaszcza do laboratoriów urzędowych w rozumieniu art. 25 ustawy o Inspekcji Weterynaryjnej (Dz. U. Nr 33, poz. 287 z 2 marca 2004 r. z późn. zm.).

§ 4. Uczestnictwo w programie

1. Zgłoszenie uczestnictwa w programie następuje poprzez **poprawne wypełnienie i odesłanie Formularza Zgłoszeniowego na właściwy adres e-mailowy Organizatora** przed upływem terminu podanego w § 6.
2. Po otrzymaniu zgłoszenia Organizator nadaje Uczestnikowi indywidualny numer identyfikacyjny (kod Uczestnika), pod którym przedstawione zostaną wyniki badań.
3. W terminie podanym w § 6 Uczestnik otrzyma od Organizatora e-mail z potwierdzeniem przyjęcia zgłoszenia i nadanym kodem Uczestnika.

§ 5. Zakres badań oferowanych w 2012 roku

1. Wykrywanie substancji przeciwbakteryjnych w mleku – runda I/2012
2. Wykrywanie substancji przeciwbakteryjnych w mięsie mielonym – runda II/2012
3. Uczestnik może wybrać dowolną rundę badań.
4. Organizator zastrzega sobie prawo do odwołania organizacji danego kierunku badań biegłości w przypadku zgłoszenia się niewystarczającej liczby Uczestników. O fakcie odwołania organizacji takiego kierunku badań zainteresowani Uczestnicy zostaną powiadomieni drogą elektroniczną w terminie przesyłania potwierdzeń przyjęcia zgłoszeń.

§ 6. Harmonogram badań

W 2012 r. przeprowadzone będą następujące rundy badań biegłości:

Etap	Runda I/2012	Runda II/2012
Nadsyłanie zgłoszeń	do 15.03.2012	do 3.09.2012
Potwierdzenie przyjęcia zgłoszenia i nadanie kodu Uczestnikowi*	do 20.03.2012	do 14.09.2012
Wysyłanie próbek do Uczestnika	27.03.2012	25.09.2012
Dostarczenie próbek do Uczestnika**	28.03.2012 do godz. 15.00	26.09.2012 do godz. 15.00
Rozpoczęcie badań przez Uczestnika	w ciągu 24 godzin od otrzymania próbek	w ciągu 24 godzin od otrzymania próbek
Termin nadsyłania wyników badań	do 13.04.2012	do 12.10.2012
Potwierdzenie otrzymania wyników badań	do 16.04.2012	do 16.10.2012
Termin wysyłania sprawozdania z badań	do 30.04.2012	do 31.10.2012

* potwierdzenie przyjęcia zgłoszenia wraz z nadanym kodem zostanie przesłanie pod adres e-mail Uczestnika wskazany w Formularzu Zgłoszeniowym
** termin dostarczenia próbek do Uczestnika jest uzależniony od firmy kurierskiej, zwykle próbki dostarczane są do godz. 15:00. W sytuacjach niezależnych od Organizatora, próbki mogą być dostarczone po godz. 15:00

§ 7. Dostarczenie próbek do badań

1. Transport próbek do Uczestników badań odbywa się za pośrednictwem firmy kurierskiej.
2. W trakcie transportu próbki znajdują się w warunkach chłodniczych.
3. Uczestnik powinien otrzymać próbki w terminie wskazanym w § 6.
4. Jeżeli Uczestnik nie otrzyma przesyłki w oznaczonym terminie należy niezwłocznie zgłosić ten fakt Organizatorowi.
5. Jeżeli próbki zostaną dostarczone do Uczestnika po terminie wskazanym w § 6 Uczestnik może przystąpić do badań lub zrezygnować z badań danej rundy.
6. Rezygnację z badań należy niezwłocznie zgłosić Organizatorowi wysyłając e-mail z tytułem „Rezygnacja z badań biegłości" z podaniem przyczyny rezygnacji, na adres podany w § 2 pkt. 4.
7. Przystąpienie przez Uczestnika do badań na warunkach określonych w pkt. 5 niniejszego paragrafu i ewentualne uzyskanie przez Uczestnika wyników niezadowalających nie może stanowić podstawy roszczeń wobec Organizatora o nie dokonanie lub zwrot zapłaty za udział w badaniach, o odszkodowanie lub inną formę zadośćuczynienia.

§ 8. Zawartość przesyłki

1. W skład przesyłki wysyłanej do Uczestnika wchodzą:
   - Próbki do badań odpowiadające zakresowi oznaczeń w danej rundzie, oznaczone napisem „PRÓBKA ..."

- Wkłady chłodzące

2. Ewentualne uszkodzenie pojemnika z próbkami do badań lub brak któregoś z elementów wymienionych w pkt. 1 należy niezwłocznie zgłosić Organizatorowi.
3. **Instrukcja postępowania z próbkami oraz inne dokumenty związane z organizacją badań biegłości, w tym Formularz Zgłoszeniowy, Karty Wyników i Regulamin uczestnictwa w Programie, dostępne są na stronie internetowej: www.piwet.pulawy.pl w zakładce Badania Biegłości, Zakład Higieny Żywności Pochodzenia Zwierzęcego PIWet-PIB.**

§ 9. Wykonanie badań

1. Przed przystąpieniem do badań Uczestnik zobowiązany jest zapoznać się z Instrukcją postępowania z próbkami i przestrzegać jej w trakcie trwania badań.
2. Do badań należy przystąpić w ciągu 24 godzin od otrzymania próbek.
3. **W Kartach Wyników należy podać metody badawcze, jakimi zostało wykonane badanie.**

§ 10. Przekazanie wyników Organizatorowi

1. Do przekazania wyników służą Karty Wyników (dostępne na stronie internetowej www.piwet.pulawy.pl), które po wypełnieniu należy **odesłać na właściwy adres e-mailowy Organizatora, podany w § 2 pkt. 4 przed upływem terminu wskazanego w § 6. Do dnia podanego w § 6 Organizator prześle Uczestnikom potwierdzenie otrzymania ich wyników badań. Nieotrzymanie potwierdzenia wyników badań we wskazanym terminie należy niezwłocznie zgłosić Organizatorowi drogą elektroniczną.**
2. Wyniki nadesłane do Organizatora po terminie wskazanym w § 6 nie będą uwzględniane w sprawozdaniu z badań.

§ 11. Opracowanie wyników

1. Opracowanie wyników stanowi porównanie wyników uzyskanych przez Uczestnika z wynikiem przypisanym.
2. Każdy Uczestnik badań w terminie wskazanym w § 6 otrzyma sprawozdanie z badań, które będzie przesyłane wyłącznie drogą elektroniczną na adres podany w Formularzu Zgłoszeniowym.
3. W przypadku laboratoriów urzędowych, informacje o przeprowadzonych badaniach oraz uzyskanych wynikach zostaną przekazane Głównemu Lekarzowi Weterynarii oraz Departamentowi Bezpieczeństwa Żywności i Weterynarii Ministerstwa Rolnictwa i Rozwoju Wsi.
4. **Laboratoria urzędowe, uzyskujące wyniki niezgodne zobowiązane są do przesłania Organizatorowi informacji o przyczynach powstałych niezgodności oraz o zaplanowanych działaniach korygujących i zapobiegawczych (w terminie do 14 dni od otrzymania sprawozdania z badań danej rundy), a następnie o zrealizowaniu takich działań (w terminie do 14 dni od zrealizowania zaplanowanych działań korygujących i zapobiegawczych). Informacje te należy przesyłać na adres e-mailowy Organizatora wskazany w § 2 pkt. 4.**

§ 12. Reklamacje

1. Każdy Uczestnik ma prawo do złożenia reklamacji na adres e-mail Organizatora wskazany w § 2 pkt. 4, w której zawarte zostaną zastrzeżenia i uwagi w stosunku do sposobu przeprowadzenia danej rundy międzylaboratoryjnych badań biegłości, w tym do oceny wyników otrzymanych przez Uczestnika.
2. Termin złożenia reklamacji upływa z 14 dniem od wysłania do Uczestnika sprawozdania z badań.
3. Organizator rozpatrzy reklamację w ciągu 30 dni i jej wynik prześle Uczestnikowi.
4. W przypadku uznania reklamacji za zasadną Uczestnikowi przysługuje zwrot opłaty wniesionej Organizatorowi za badania objęte reklamacją.
5. W przypadku nie zaakceptowania wyniku reklamacji Uczestnikowi przysługuje prawo do odwołania się do Dyrektora PIWet-PIB.

§ 13. Koszt uczestnictwa w badaniach

1. Uczestnictwo w międzylaboratoryjnych badaniach biegłości dla Laboratoriów Inspekcji Weterynaryjnej jest bezpłatne.
2. Pozostałe Laboratoria wnoszą opłatę w wysokości 253 zł **(kwota brutto)**:
3. Termin wysłania faktury za uczestnictwo w badaniach biegłości wynosi 7 dni od daty wysyłki próbek do Uczestników. Termin płatności wynosi 14 dni od daty otrzymania faktury. Organizator programu ma prawo wstrzymania wysyłki sprawozdania z badań do Uczestnika, który nie wniósł opłaty w stosownej wysokości w ustalonym terminie.

§ 14. Rezygnacja z uczestnictwa w badaniach

1. Każdy Uczestnik może zrezygnować z udziału w badaniach wysyłając zawiadomienie na adres mailowy Organizatora podany w § 2 pkt. 4 w terminie do 14 dni przed dniem wysyłania próbek do Uczestników wymienionym § 6.
2. Rezygnacja nie zostanie uwzględniona, jeżeli nastąpi w terminie krótszym niż 14 dni od daty wysyłki próbek do Uczestników, co skutkuje wysłaniem próbek do badań a następnie wystawieniem faktury za uczestnictwo w badaniach. Pkt. 2 nie ma zastosowania w przypadku rezygnacji na zasadach opisanych w § 7.

Programme of activities toward the network of regional laboratories
For 2012

Programme of PT for Physico-chemical Methods

PROGRAM PT for 2012.

- Opracowanie wieloskładnikowej metody oznaczania mikotoksyn w paszach z zastosowaniem techniki chromatografii cieczowej sprzężonej z tandemową spektrometrią mas

Planuje się opracowanie ilościowej metody do jednoczesnego oznaczania aflatoksyn, ochratoksyny A, deoksyniwalenolu, zearalenonu, toksyn T-2 i HT-2 w paszach
Do oczyszczania próbek zostaną zastosowane dwie metody:

- oczyszczanie na kolumienkach powinowactwa immunologicznego VICAM Myco 6 in 1™ LC-MS/MS;
- oczyszczanie zgodnie z metodą otrzymaną z Europejskiego Laboratorium Referencyjnego ds. mikotoksyn w Geel w Belgii.

Po wprowadzeniu własnych modyfikacji, do rutynowego oznaczania próbek pasz zostanie wybrana jedna z powyższych metod. Mikotoksyny będą oznaczane ilościowo z wykorzystaniem chromatografii cieczowej sprzężonej z tandemową spektrometrią mas z trybem jonizacji przez elektrorozproszenie LC/ESI – MS/MS

Zorganizowane zostaną również 2 kontrole (oceny) w zakresie badań pozostałości w Zakładach Higieny Weterynaryjnej we Wrocławiu i w Katowicach. Po wizytach przygotowane zostaną raporty.

Przeprowadzona zostanie również 1 wizyta kontrolna w laboratorium biorącym udział w Planie Urzędowej Kontroli Pasz.

3. Organizacja badań porównawczych pomiędzy krajowymi laboratoriami urzędowymi oraz zapewnienie odpowiedniego późniejszego zastosowania takich badań porównawczych

Laboratorium referencyjne ZFT planuje zorganizowanie ok. 25 badań biegłości (PT) dla oceny metod stosowanych w laboratoriach regionalnych ZHW w ramach urzędowej kontroli żywności i pasz. W każdym z organizowanych badań biegłości uczestniczy od 4 do 10 laboratoriów ZHW.

Proponowany zakres badań biegłości w roku 2012 będzie obejmował metody oznaczania:

I kwartał 2012

- hormony steroidowe: metylotestosteron, etynyloestradiolu, boldenon, metyloboldenon w moczu
- chloramfenikol w mięśniach
- makrocykliczne laktony w mleku

6/9

II kwartał 2012

- zeranol i metabolity w moczu zwierząt rzeźnych
- antybiotyki w mięśniach (III kwartał)
- benzoimidazole w wątrobie level tat
- pestycydy chloroorganiczne w tłuszczu zwierzęcym
- kongenery polichlorowanych bifenyli (PCB) w tłuszczu zwierzęcym
- pestycydy fosforoorganiczne w tłuszczu zwierzęcym
- kongenery polichlorowanych bifenyli (PCB) w paszy
- ołów w rybach
- kadm w rybach
- rtęć w rybach
- arsen w rybach

} Nie prosiły laboratoria ZHW przygotowano próbki jaj i mleka, badanie mięśni ryb zostanie przeprowadzone w IV kw. br.

- ochratoksyna A w paszy

III kwartał 2012

- 17β-estradiol i testosteron w surowicy bydlęcej
- beta-agoniści w moczu II kwartał (na prośbę ZHW - wątroba + nerka)
- kokcydiostatyki w jajach kurzych – wątroba
- ołów w mięśniach
- kadm w mięśniach
- rtęć w mięśniach

} IV kwartał - mięśnie ryb

- arsen w mięśniach
- ołów w paszy
- kadm w paszy
- rtęć w paszy
- arsen w paszy

IV kwartał 2012

- stanozolol i 16β-OH-stanozolol w moczu bydlęcym
- fluorochinolony w mięśniach muscle.
- kokcydiostatyki jonoforowe w paszach

Że wyniki na zeranol – potrzebne powtórzenie będzie
zeranol – mocz
na prośbę ZHW powtórzone zostanie w IV kw.
badania (Pb i Cd) w mleku.