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Training courses on the detection of animal proteins in feeds

Project leader: L.W.D. van Raamsdonk

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The organization of training courses for microscopic detection of animal proteins in feeds

Three training courses for microscopists from existing EU member states

L.W.D. van Raamsdonk[1], V.Baeten[2], A.Boix[3], J.S. Jorgenson[4] and Chr. von Holst[3]

1: RIKILT – Institute of Food Safety, Business Unit Safety and Health, Wageningen, the Netherlands
2: Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium
3: Institute for Reference Materials and Measurements (EC-JRC-IRMM), Geel, Belgium
4: Danish Plant Directorate (DPD), Lyngby, Denmark

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RIKILT - Institute of Food Safety
Bornsesteeg 45, 6708 PD Wageningen, the Netherlands
P.O. Box 230, 6700 AE Wageningen, the Netherlands
Tel: +31 0317 475422
Fax: +31 0317 417717
Internet: www.rikilt.wur.nl

MAILING LIST

External:

Directorate – General Health and Consumer Protection (DG-SANCO) (J. Husi-Kallio, Dr. K. de Smet)
Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium (Dr. P. Dardenne)
Institute for Reference Materials and Measurements (EC-JRC-IRMM), Geel, Belgium (Dr. E.Anklam)
Landwirtschaftliche Untersuchungs- und Forschungsanstalt Nord-West (LUFA NORD-WEST), Hameln, Germany (Dr. I. Paradies-Severin)
Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES) Landwirtschaftliche Untersuchungen und Forschung, Vienna, Austria (Dr. F. Wernitznig)
Landesanstalt für Landwirtschaftliche Chemie, Universität Hohenheim, Stuttgart, Germany (Dr. R. Modi)
RIKILT – Institute of Food Safety, Wageningen, the Netherlands (Mr. V. Pinckaers)
Danish Plant Directorate (DPD), Lyngby, Denmark (Mrs. J. Mårtennson, Mrs. A.-M. Råssig Jacobson)
Federal Feed and Food Laboratory (FAVV), Tervuren, Belgium (Mr. J. van Cutsem)
All participants as listed in ANNEX 4.

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SUMMARY

A consortium of five laboratories, under the coordination of RIKILT – Institute of Food Safety, organized three training courses for a total of 25 microscopists for the detection of animal proteins in feeds at three different locations.
A four day program was developed, including general introductions on ring trials, legislation and alternative methods, introduction to microscopy and to the use of the decision support system ARIES, practicing of the sedimentation procedure, extensive practicing on the detection of animal proteins in a range of feed samples, at least two blind tests in order to monitor any progress during the course, and a proper way to evaluate the course and its results.
The first blind series of samples, analyzed by the partners after one and a half day of practicing showed remarkable good results. The third and final test showed comparable results with sensitivity scores generally more than 90%. Exceptions are the samples with a contamination of 0.5% of feather meal, which was not detected in all cases. For several types of contamination an improvement of sensitivity was achieved. The six evaluated aspects of the quality of the contents of the courses and of the educational value all received from the participants a score of 4 or higher at a scale from 1 to 5. The quality of the equipment (microscopes, computers) is the most important aspect of the topic "local organization" and this aspect received the only score below 4 (3.5 overall average). The average score for general organization (4.4) and for usability of the trained topics in own laboratory (4.1) is good. Future training courses should additionally focus on quantification or on the detection of particles other than bones or muscles (feather meal, horn, leather, blood, plasm).
General conclusions and specific recommendations are made in the report. The main conclusion is that the participants have achieved a sufficient level of expertise during the training courses. The optimal number of participants for a training course is eight (8). Each participant should have a research microscope with filter sets for polarization and a set of chemicals and glassware at its possession. The future CRL animal proteins should build up and maintain a collection of research microscopes, stereo microscopes, microscopes for instruction and other equipment necessary for organizing training courses in a regular way. Training courses should be diversified for expertise level (starting level, advanced level). Every official control laboratory should have a license of the support system ARIES. In this way a standardized set of information, documentation and identification tools is available. This will greatly optimize communication for support on specific situations and will facilitate E-learning activities.

1 INTRODUCTION

In 2004 two training courses for the microscopic detection of animal proteins in feeds have been organized on behalf of the Directorate-General Health and Consumer Protection (DG-SANCO) by the Technical Assistance Information Exchange Office (TAIEX; DG-Enlargement) and the Institute for Reference Materials and Measurements (EC-JRC-IRMM). These courses were meant for microscopists from the new member states. In total 16 scientists and technicians have been trained from 9 countries (Malta does not have an active control program). A report is available.
It appeared from recent ring trial studies that training for microscopists from existing member states would be appropriate as well. DG-SANCO decided to grant a project for training of these microscopists and sent a tender to five European laboratories active in this field (CRA-W Gembloux (BE), DPD Lyngby (DK), EC-JRC-IRMM Geel (BE), LUFA NORD-WEST Hameln (DE), RIKILT Wageningen (NL)). These five laboratories decided to present one project proposal under the coordination of RIKILT – Institute of Food Safety for training of 25 microscopists for the detection of animal proteins in feeds at three different locations (RIKILT Wageningen, DPD Lyngby and CRA-W Gembloux). This project proposal was accepted by DG-SANCO.
The organization, results and evaluation of the three training courses is presented in this report.

2 ORGANIZATION

Staff members of DG-SANCO contacted all Chief Veterinary Officers in the 15 existing Member States to propose scientists for attending the courses. This information was forwarded to RIKILT, where the proposed participants were scheduled for the training courses. RIKILT has sent invitations by E-mail and has arranged organizational details with the local organizers and the participants. Luxembourg did not send a name of a proposed participant, since this member state does not perform its own analyses. It appeared to be difficult to arrange the participation of microscopists from some member states. Where necessary this was discussed with the participants during the training course, and contact information has been updated where appropriate. All participants are listed in Annex 4.

The organizers developed a program, based on the experience from the two TAIEX workshops, adjusted to a four day schedule. Topics and requirements were: general introductions should be presented on ring trials, legislation and alternative methods, introduction to microscopy and to the use of the decision support system ARIES are required, sedimentation as part of the procedure should be included, at least two blind tests should be included in the program in order to monitor any progress during the course, and there should be a proper way to evaluate the course and its results. The resulting program is presented in Annex 1.

The training topics were set as follows:

Training 1: goal: to get acquainted with the materials and with ARIES as training tool. Materials: pure meals, labeled.

Additional: feeds collected from practice, blind. This means that some of these feeds are not contaminated and some others are contaminated with fishmeal only. Confusion with vegetable material can be trained. These samples can be used for practicing the sedimentation procedure as well, in order to avoid spilling of valuable material.

Sedimentation: every participant will make sediments of 5 samples selected from practice. Target: 2 samples without and 2-3 samples with fishmeal. One of the samples with fishmeal will be spiked with 0,1 % MBM in advance by the hosting institute.

Training 2a: material: first series of blind samples. Goal: first practice for evaluation of blind feed samples. Results will be reported on a sheet. It is required to do 4 samples in three hours. The evaluation was done individually.

Training 2b: material: second series of blind samples consisting of the material that is sedimented by the participant him/herself. Goal: practice for evaluation of blind feed samples, and checking the efficiency of the individual sedimentation. Results will be reported on a sheet. It is required to do 5 samples in three hours, which means a shorter examination time per sample. Individual evaluation and check of improvement compared to the first blind series, if possible.

- Test: goal: final examination of the performance. Samples are selected from blind series of training 2a.

Final evaluation: in a plenary session the results of both training session 2a and 2b as well as the final test will be evaluated anonymously.

It appeared necessary to accept some modifications in the programs of the local training courses, e.g. for the number of samples per session. These modifications did not have a major influence on the scope of the training.

Additional topics for the Friday morning session were organized depending on the individual possibilities of the local laboratory:

- Wageningen (RIKILT): introduction to the sample preparation room and the sample routing procedures through the laboratories.
- Lyngby (DPD): preparation of oil and fat samples.
- Gembloux (CRA-W): introduction to PCR detection of DNA, and to detection of animal particles by near infrared microscopy and near infrared imaging.

3 RESULTS

The program included three different tests of blind samples in order to test the progress in the knowledge for detection of animal proteins. For a general evaluation of these results the second test is not included, since this test was based on material that was sedimented by the participants. The first and the third (final) test were based on material sedimented by the hosting institute and therefore comparable to each other. The individual results are presented in Annex 2. The pooled results are presented in the Table 1.

The first blind series of samples, analyzed by the partners after one and a half day of practicing showed remarkable good results. The third and final test showed comparable results, except for the samples with an exclusive contamination with animal proteins (i.e. no fishmeal): 0.1% of MBM generally poses no problem, but a contamination with 0.5% of feather meal was not detected in all cases. In recent ring trials scores higher than 90% for the different types of samples have been achieved as well, except for the samples contaminated with both fish meal (5%) and meat and bone meal (terrestrial animal; 0.1%), for which a sensitivity of approx. 76% was achieved in recent ring trials. The results of the current training courses can formally not be compared with official validation studies or proficiency tests, since the participants in a training course received support and training in advance of the tests. Moreover, the tests of the training are not based on officially prepared samples, and the sample sets showed differences between the three training courses. The results of the individual training courses (see Annex 2) are too small for any statistical analysis. Nevertheless, when stating the situation that training just in advance of a test might positively influence the result of that test, it can be concluded that the training courses were successful in general terms.

first blind series, detection of terrestrial animal proteins

composition		results		
fish	MBM	present	absent	percentage correct
none	none	2	23	92 %
5%	none	3	22	88 %
none	0.5% a	3	2	60 %
none	0.1%	33	2	94.3 %
5%	0.1%	32	2	94.1 %

a: 0,5% feather meal

third blind series, final test, detection of terrestrial animal proteins

composition		results		
fish	MBM	present	absent	percentage correct
none	none	1	24	96 %
5%	none	2	33	94.3 %
none	0.5% a	18	7	72 %
none	0.1%	23	2	92 %
5%	0.1%	23	2	92 %

a: 0,5% feather meal

4 EVALUATION

In the evaluation forms a total of 13 items are included, pertaining to several general aspects. The results are fully presented in the tables in Annex 3. These results will be discussed per training course or in general depending on the type of aspect.

Quality of the contents of the courses and of the educational value (program: balance between presentations and workshop activities, quality of the general presentations, quality of introductions to microscopy, time availability for actual sample examination, educational capacity of trainers, applicability and use of ARIES). The six aspects of the training courses all received a score of 4 or higher at a scale from 1 to 5 (see Table 2). For some aspects deviating individual indications have been given for the balance between presentation and workshop activities, and for applicability and use of ARIES. Training on the use of ARIES and instructions for the discrimination between mammal and poultry material were each requested once, although these items were included in the program. The time for the examination of the samples in the blind trials was occasionally indicated as too little (ANNEX 3). It would be advisable to organize future courses at different expertise levels (starting level, advanced level). There were no individual sets of materials available for the participants, which can result in cross-contamination of samples, and which has the consequence that slides regularly can not be prepared at the own work location but centrally. It is remarkable that the training course in Wageningen received the lowest scores over most aspects, although the program, introductions and scope of the training sessions were identical in all three training courses. This apparently more critical view of the participants in Wageningen should be noted in the evaluation of the local organization. The applicability and use of ARIES received a score of 4.0, and the system appeared to be helpful for instruction in a range of specific topics. ARIES was also consulted by participants independently.

Aspect	**Score**
program	4.0
general presentations	4.1
introductions to microscopy	4.0
time availability	4.2
educational capacity of trainers	4.4
applicability and use of ARIES	4.0

Table 2. Scores at a scale from 1 (poor) to 5 (excellent) for six aspects of the organized training courses.

Quality of local organization (quality of the housing facilities, type of equipment, availability of tools and chemicals, quality of food and drinks, feasibility of the hotel). The quality of the equipment (microscopes, computers) is the most important aspect of this topic and received the only score below 4 (3,5 overall average). In Wageningen a set of ten student microscopes from the Wageningen University was used, in Lyngby five different research microscopes were available, and in Gembloux nine different research microscopes were available for ten participants. Photo devices, monitors for instructions, polarization and phase contrast were only occasionally present. These situations were pointed out in several individual comments (see ANNEX 3). Despite the efforts of the organization teams to gather several compound and stereo microscopes at one site, it is clear that this is the most important issue for improvement for future courses. Lyngby scored best for food and drinks due to the excellent lunch buffet regularly available at the institute. The overall score for the quality of food, drinks and hotel is good, but this is not the major topic for the evaluation of the training courses.

General aspects (general organization, usability of the trained topics in own laboratory). The average score for general organization (4,4) and for usability of the trained topics in own laboratory (4,1) is good. It is, however, intriguing that the applicability of the trained skills does not receive a higher value than 4,1. An optimal usability would be the final ambition of future courses. Future training courses should additionally focus on quantification or on the detection of particles other than bones or muscles (feather meal, horn, leather, blood). It is remarkable that one of the participants asked to focus on feather meal, whereas this type of animal protein was deliberately included in the program.

5 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions can be drawn:

- The results show that the participants have achieved a sufficient level of expertise during the training courses. The detection of feather meal remains a problem. The implementation and effect of the new expertise in the own laboratory practice of the participants is not known.
- The participants evaluated the training courses generally at the rating "good". The equipment (e.g. microscopes) was disputed.
- The consortium of organizing laboratories has increased the expertise for organizing training courses.
- Documentation for the national organization of the official control of animal proteins is now available (e.g. one of a few central laboratories versus a network of regional laboratories, involved ministries, etc.), and address lists for most member states.

Based on the experiences of the organizers and the evaluations, the following recommendations can be made:

- The optimal number of participants for a training course is eight (8). Each participant should have a research microscope with filter sets for polarization and a set of chemicals and glassware at its possession. One stereo microscope for every two participants is sufficient.
- The program should be adjusted and optimized, especially for the tests and for the included samples. The quality of the samples should be fixed and each participant should preferably have an own set of material (sample, sediment and flotation) of every sample.
- The future CRL animal proteins should build up and maintain a collection of research microscopes, stereo microscopes, microscopes for instruction and other equipment necessary for organizing training courses in a regular way.
- Training courses should be diversified for expertise level (starting level, advanced level). Additional sessions or courses for detection of specific parts (feather meal, horn, leather, blood), and for quantification, especially in the light of Directive 1292/2005/EC, are recommended.
- Every official control laboratory should have a license of the support system ARIES. In this way a standardized set of information, documentation and identification tools is available. This situation would enhance harmonization of the official control method and the reliability of the detection. The future CRL can refer to ARIES at every occasion that support for NRLs or official control laboratories has to be provided, which will greatly optimize communication and possibilities for E-learning.

6 ACKNOWLEDGEMENTS

The organizers would thank the European Commission for granting the possibility to organize the training courses and the invited trainers for sharing their expertise.

ANNEX 1. Schedule and programme

Table A

		general, microscopy		**Sedimentation**
day 1	13:30 –16:30	introduction		
		lectures: microscopy	v. Raamsdonk	
		legislation	v. Holst/Boix	
		other methods	Baeten	
		results of ring trials	v. Holst/Boix	
		ARIES	v. Raamsdonk	
		demo of sedimentation	lab or media show	
		scope of the training	v. Raamsdonk	
day 2	9:00-12:15	training 1		group 1, 2
	13:15 –16:30	training 1		group 3, 4
day 3	9:00-12:15	training 1, plenary evaluation		group 5
	13:15 –16:30	training 2a, individual evaluation		
day 4	9:00-12:15	training 2b, individual evaluation		
	13:15 –16:15	Test		
day 5	9:00-12:30	extra practice for sedimentation and/or evaluation additional presentation or activities plenary evaluation of training 2a, 2b, test closing session		

Explanation: training 1 and 2a will be based on already prepared sediments
training 2b is based on material sedimented by participants, there is at least one night between sedimentation and use of the material for drying.

Detailed schedule per group of 2 participants (one participant per group in Lyngby)

Table B

group: day:	**1**	**2**	**3**	**4**	**5**
day 2, 9:00-10:30	**sedimentation**	training 1	training 1	training 1	training 1
day 2, 10:45-12:15	training 1	**sedimentation**	training 1	training 1	training 1
day 2, 13:15-14:45	training 1	training 1	**sedimentation**	training 1	training 1
day 2, 15:00-16:30	training 1	training 1	training 1	**sedimentation**	training 1
day 3, 9:00-10:30	training 1	training 1	training 1	training 1	**Sedimentation**
day 3, 11:30-12:15	evaluation	evaluation	evaluation	Evaluation	Evaluation

Materials

Table C

Materials:	Pure					feeds					Practice
Training:	MMBM	MBM	avian	fish	feather	pure	0,1% MBM	5% fish	0,1% MBM + 5 % fish	feather	
training 1:	*2*	*2*	*1*	*4*	*1*						*5*
training 2a:						*1*	*1*	*1*	*1*	*1*	
training 2b:											**5**
test:						*1*	*1*	*1*	*1*		

Samples noted in **bold**: has been sedimented by the participant.
Samples noted in *italics*: has been sedimented by the hosting institute in advance.

ANNEX 2. Results of the blind tests for each training course

Wageningen, May 2005
(10 participants)

first blind series, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	0	10	100 %
5%	none	1	9	90 %
none	0.1%	9	1	90 %
5%	0.1%	18	1	95 %

second blind series, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	0	18	100 %
2%	none	2	16	89 %
none	0.2%	-	-	-
2%	0.2%	10	0	100 %

third blind series, final test, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	0	10	100 %
5%	none	0	10	100 %
none	0.5% a	9	1	90 %
none	0.1%	9	1	90 %
5%	0.1%	9	1	90 %

a: 0.5% feather meal

Copenhagen, September 2005
(5 participants)

first blind series, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	0	5	100 %
5%	none	0	5	100 %
none	0.5% a	3	2	60 %
none	0.1%	4	1	80 %
5%	0.1%	5	0	100 %

a: 0.5% feather meal

second blind series, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	1	16	94 %
2-6%	none	1	18	95 %
none	0.1% a	3	0	100 %
8%	0.1%	3	0	100 %

a: poultry MBM

third blind series, final test, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	0	5	100 %
5%	none	1	4	90 %
none	0.5% a	4	1	80 %
none	0.1%	5	0	100 %
5%	0.1%	5	0	100 %

a: 0.5% feather meal

Gembloux, October 2005
(10 participants)

first blind series, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	2	8	80 %
5%	none	2	8	80 %
none	0.1%	20	0	100 %
5%	0.1%	9	1	90 %

second blind series, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	2 a	17	89 %
5%	none	0	19	100 %
none	0.1%	8	1	89 %
5%	0.1%	-	-	-

a: due to label switching during sedimentation

third blind series, final test, detection of terrestrial animal proteins

Composition		Results		
fish	MBM	present	absent	percentage correct
none	none	1	9	90 %
5%	none	1	19	95 %
none	0.5% a	5	5	50 %
none	0.1%	9	1	90 %
5%	0.1%	9	1	90 %

a: 0.5% feather meal

ANNEX 3. Evaluation of each training course

Wageningen, May 2005
(10 participants)

	excellent (5)	good (4)	acceptable (3)	moderate (2)	*mean*
quality of the housing facilities at RIKILT	5	5			4.5
type of equipment (microscopes, computers)		4	5	1	3.2
availability of tools, chemicals etc.	1	7	1	1	3.8
program: balance between presentations and workshop activities	1	5	3	1	3.6
quality of the general presentations (length, ease of understanding)	1	6	3		3.8
quality of introductions to microscopy		7	3		3.7
time availability for actual sample examination	3	6	1		4.2
educational capacity of trainers		9	1		3.9
applicability and use of ARIES	2	6	1	1	3.9
quality of food and drinks at RIKILT	2	4	2	2	3.6
feasibility of the hotel	3	4	2	1	3.9
general organization	5	3	2		4.3
usability of the trained topics in your own lab.	1	8	1		4.0

topics to be included in the future: *identification of other animal components different to bones*
quantitative analysis (2)
other confirmation tests

Comments: *better equipment: higher tables, chairs, better microscopes (2)*

Copenhagen, September 2005
(5 participants)

	excellent (5)	good (4)	acceptable (3)	moderate (2)	*mean*
quality of the housing facilities at DPD	4	1			4.8
type of equipment (microscopes, computers)	1	3	1		4.0
availability of tools, chemicals etc.	3	1	1		4.4
program: balance between presentations and workshop activities	2	2			4.5
quality of the general presentations (length, ease of understanding)	3	2			4.6
quality of introductions to microscopy	2	2		1	4.0
time availability for actual sample examination	1	2	1	1	3.6
educational capacity of trainers	5				5.0
applicability and use of ARIES	1	4			4.2
quality of food and drinks at DPD	5				5.0
feasibility of the hotel	2	3			4.4
general organization	5				5.0
usability of the trained topics in your own lab.	3	2			4.2

topics to be included in the future: *quantitative analysis (1)*
training on the use of ARIES

Comments: *too many samples or too little time to examine the requested samples according to the Directive; the time pressure forces you to take the easiest way, i.e. to start directly with the sediment*

Gembloux, October 2005
(10 participants)

	excellent (5)	good (4)	acceptable (3)	moderate (2)	*mean*
quality of the housing facilities at CRA-W	2	8			4.2
type of equipment (microscopes, computers)	1	5	3	1	3.6
availability of tools, chemicals etc.	1	7	2		3.9
program: balance between presentations and workshop activities	3	6	1		4.2
quality of the general presentations (length, ease of understanding)	3	6	1		4.2
quality of introductions to microscopy	2	8			4.2
time availability for actual sample examination	6	2	2		4.4
educational capacity of trainers	6	4			4.6
applicability and use of ARIES	1	9			4.1
quality of food and drinks at CRA-W		8	2		3.8
feasibility of the hotel	3	7			4.3
general organization	2	7	1		4.1
usability of the trained topics in your own lab.	3	5	2		4.1

topics to be included in the future: *samples with other prohibited particles, e.g. feather, horn, blood*
quantitative analysis (2)
discrimination between poultry and mammals

Comments: *separate set of samples for each participant, too less polarization units, one set of stereo microscope and compound microscope for each participant (2), good training, good atmosphere*

ANNEX 4. List of organizers, trainers and participants of the three training courses.

Cntr	Organization Name		organizer	Email Address	Address	
NL	RIKILT – Institute of Food Safety	Dr.	L.W.D. van Raamsdonk	leo.vanraamsdonk@wur.nl	Wageningen the Netherlands	
B	DG Joint Research Centre Food Safety & Quality Unit	Dr.	Chr. von Holst	christoph.von-holst@cec.eu.int	Geel Belgium	
B	DG Joint Research Centre Food Safety & Quality Unit	Dr.	A. Boix-Sanfeliu	Ana.BOIX-SANFELIU@cec.eu.int	Geel Belgium	
DK	Ministry of Food, Agriculture and Fisheries, Danish Plant Directorate	Dr.	J.S. Jorgenson	jsj@pdir.dk	Lyngby Denmark	
B	Walloon Agricultural Research Centre	Dr.	V. Baeten	baeten@cra.wallonie.be	Gembloux Belgium	

Cntr	Organization Name		trainer	Email Address	Address	training
NL	RIKILT – Institute of Food Safety	Dr.	L.W.D. van Raamsdonk	leo.vanraamsdonk@wur.nl	Wageningen the Netherlands	all
A	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES) Landwirtschaftliche Untersuchungen und Forschung	Dr.	F. Wernitznig	franz.wernitznig@ages.at	Wien Austria	Wageningen
NL	RIKILT – Institute for Food Safety	Mr.	V. Pinckaers	victor.pinckaers@wur.nl	Wageningen the Netherlands	Wageningen
DK	Ministry of Food, Agriculture and Fisheries, Danish Plant Directorate	Dr.	J.S. Jorgenson	jsj@pdir.dk	Lyngby Denmark	Copenhagen
D	Landesanstalt für Landwirtschaftliche Chemie, Universität Hohenheim (710)	Dr.	R. Modi	modi@lachemie.uni-hohenheim.de	Stuttgart Germany	Gembloux
B	Federal feed and food laboratory	Mr.	J. van Cutsem	jeroen.van.cutsem@favv.be	Tervuren Belgium	Gembloux

Cntr	Organization Name		participant	Email Address	Address	training
A	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES) Landwirtschaftliche Untersuchungen und Forschung	Dr.	R. Weiss	roland.weiss@ages.at	Wien Austria	Gembloux
B	AFSCA, Directorate of Laboratories Federal Laboratory of Feeding	Mr.	R. Scomparin	renaud.scomparin@afsca.be	Liège Belgium	Gembloux

D	LUFA – Augustenberg	Mrs.	B. Allaín Arbe	Betzabe.Allain-Arbe@lufa.bwl.de	Karlsruhe Germany	Wageningen
D	Staatliches Veterinäruntersuchungsamt Krefeld	Mrs.	G. Russ	schulte-sutrum@svua-krefeld.nrw.de	Krefeld Germany	Wageningen
D	LUFA Rostock	Mrs.	M. Dunker	mdunker@lms-beratung.de	Rostock Germany	Copenhagen
D	Futtermittelinstitut Stade	Dr	H. Warnecke	Hinrich.warnecke@laves.niedersachsen.de	Stade Germany	Gembloux
DK	Ministry of Food, Agriculture and Fisheries, Danish Plant Directorate	Mrs.	G. Malm	gum@pdir.dk	Lyngby Denmark	Wageningen
E	Generalitat de Catalunya (GENCAT)	Mr.	A. Abril Rigau	aabril@gencat.net	Cabrils (Barcelona) Spain	Wageningen
E	Laboratorio Agrario Regional	Dr.	F. Lorenzo Martin Moro	marmorfe@jcyl.es	Burgos Spain	Wageningen
E	Laboratorio Agroalimentario de Cordoba	Dr.	C. Gálvez Ramírez	candido.galvez@juntadeandalucia.es	Cordoba España	Wageningen
E	Laboratorio Agroalimentario y de Sanidad Animal Consejeria de Agricultura y Agua Comunidad Autónoma de Murcia	Dr.	F.J. Bleda Fernandez	Fjavier.bleda@carm.es	**El Palmar (Murcia)** **Spain**	Gembloux
E	Laboratorio Arbitral Agroalimentario M.A.P.A.	Mrs.	J.B. de Castro Lopez-V.	Maria.pmillan@mapya.es	Madrid Spain	Gembloux
E	Servicio de Laboratorio y control	Dr.	A. Martinez Arriola	maa9999@gobcantabria.es	**Santander** **Spain**	Gembloux
F	LABORATOIRE DGCCRF de RENNES	Mrs.	I. Taco	ingrid.taco@dgccrf.finances.gouv.fr	Rennes France	Gembloux
FI	Plant Production Inspection Centre (KTTK), Agricultural Chemistry Department	Mrs.	A. Pohto	Arja.pohto@kttk.fi	Vantaa Suomi / Finland	Copenhagen
GR	Ministry of Agricultural development and Food, Feedingstuff Control Laboratory	Mrs.	A. Voudouri	annavoudouri@hotmail.com	Lykovrisi Attikis (Athens) Greece	Gembloux
GR	Aristotle University of Thessaloniki, Faculty of Veterinary Medicine, Laboratory of Anatomy and Histology	Dr.	H. Michaloudi	emichal@vet.auth.gr	Thessaloniki Greece	Gembloux
I	Istituto Zooprofilattico Sperimentale PLV, Sezione di Aosta	Dr.	L. Domenis	lorenzo.domenis@izsto.it	Aosta Italy	Gembloux
I	Istituto Superiore di Sanità, Department of food safety and Veterinary public health	Dr.	G. Vaccari	gabriele.vaccari@iss.it	Rome Italy	Copenhagen

I	Istituto Superiore di Sanità, Department of food safety and Veterinary public health	Dr.	L. Morelli	luisella.morelli@iss.it	Rome Italy	Copenhagen
IE	Official Seed testing Station, Department of Agriculture and Food	Mr.	S. Kearney	seamus.kearney@agriculture.gov.ie	Dublin Ireland	Wageningen
NL	RIKILT – Institute for Food Safety	Mr.	J. Ossenkoppele	jan.ossenkoppele@wur.nl	Wageningen the Netherlands	Wageningen
PT	Laboratório Nacional de Investigação Veterinária	Mr.	M. Santos	Miguel.santos@lniv.min-agricultura.pt	Lisbon Portugal	Wageningen
SWE	SVA, Dept. of Feed Chemistry	Mrs.	A. Berg	Annette.berg@sva.se	Uppsala Sweden	Copenhagen
UK	Veterinary Laboratories Agency	Mrs.	A. Tomlin	a.tomlin@vla.defra.gsi.gov.uk	Warwickshire United Kingdom	Wageningen