Community Reference Laboratory for Animal Proteins in feedingstuffs

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CRL-AP Proficiency Test 2007
Final report

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Summary

On behalf of DG Sanco, the Community Reference Laboratory for animal proteins in feedingstuffs (CRL-AP) organized in 2007 the present proficiency test for all National Reference Laboratories (NRLs) having in charge the analyses of animal proteins in feedingstuffs in each of the Member States of the European Union. The goal of the study was to evaluate the performance of the NRLs to apply the microscopic method for qualitative detection of animal constituents in feedingstuffs as stated by Commission Directive 2003/126/EC[†].

The number of participants was 27 (26 NRLs and 1 foreign participant). All participants received 4 blind samples of materials adulterated by mammalian meat and bone meal and/or fish meal. Blank materials were also sent in order to check for possible cross-contamination. The homogeneity of the materials was tested by the CRL-AP and considered as sufficient.

This proficiency test indicates that the individual global performance is excellent (= full set of correct answers) for 68% of the participants (compared to the 55% observed from the CRL-AP Interlaboratory Study 2006). Three participants still had good performances, with a single false positive for MBM in the 5% Fish or for fish in the blank. Five participants were nevertheless underperforming and require improvement of proficiency.

Results from the study also emphasize the importance of having to count a minimum number of animal particles before establishing a final decision related to the presence or absence of animal constituents in a feed. This should avoid possible false positive results for samples with a contamination level below the limit of detection. Such an improvement will help to guarantee a good reproducibility in case of counter analysis.

Keywords :

Meat and bone meals – Processed animal proteins – Microscopy – Qualitative analysis

[†] Referred through the document as EC 126/2003 directive

1. Foreword and aim of the study

Community Reference Laboratories (CRL) were created in order to ensure a high level of quality and a uniformity of the results provided by European control laboratories. On 29 April 2004, the European Parliament and the Council have adopted the Regulation (EC) No 882/2004, improving the effectiveness of the official food and feed controls while redefining the obligations of the relevant authorities and their obligations in the organization of these controls.

On 23 May 2006, the Commission Regulation (EC) No 776/2006, has nominated the Walloon Agricultural Research Centre as Community Reference Laboratory for animal proteins in feedingstuffs (CRL-AP, http://crl.cra.wallonie.be) for the 2006-2011 period. The new Community Reference Laboratory has to develop the following priority axes:

(i) To provide National Reference Laboratories (NRLs) with detailed analytical methods, including reference methods for the network of Member State NRLs;

(ii) To coordinate application by NRLs of the methods by organizing interlaboratory studies;

(iii) To develop new analytical methods for the detection of animal proteins in feedingstuffs (classical microscopy, near infrared microscopy, polymerase chain reaction, immunology …);

(iv) To conduct training courses for the benefit of NRL staffs from Member States and future Member States;

(v) To provide scientific and technical assistance to the European Commission, especially in cases of disputed results between Member States.

It is in this framework that the CRL-AP has organized in 2007 a proficiency test aiming to evaluate the performance of the NRLs to detect the presence of processed animal proteins by the microscopic method as stated in the EC 126/2003 directive [1].

The present final report is based on a preliminary version submitted as a working document on 15 October 2007 to the NRL network for approval. This final version was thus modified taking into account the NRL's comments received on 30 October 2007.

2. Introduction

Mid July 2007, 4 blind samples for the CRL-AP Proficiency Test 2007 have been sent by express shipment to the 26 NRLs and to one laboratory outside this network, namely the Canadian Food Inspection Agency which introduced a demand to participate to a proficiency test organized by the European Union. The detailed list of the 27 participating labs is included in Annex 1.

The following instructions have been given to each participating lab:

- Analysis of the 4 blind samples by applying the protocol described in EC 126/2003 directive [1].
- Mention has been done that each participating laboratory was sole responsible to reach appropriate homogeneity of the sample sub-portions that had to be taken from the whole sample vial for analysis. Therefore the CRL-AP strongly recommended them to take appropriate mixing and/or grinding procedure for achieving this sub-sample homogeneity.
- Qualitative analyses have been requested for each of the 4 samples. Participants were asked to provide additional data such as the number of slides observed, whether or not they observed the whole slides, the sample and sediment weights, the number of particles they had detected for their diagnostics and to further specify the exact nature of the particles when their number were less or equals to 5.
- The results had to be encoded by way of an Excel report form -downloadable from the CRL-AP intranet (Annex 2). Participants were asked to carefully read the instructions on how to fill in the result form and to testify they did it prior to encoding their results. No other support for communicating the results was accepted.
- A summarized results sheet was automatically generated without the need for the participant of re-encoding the data. Participants were asked to sign the summarized results sheet and to send it by fax to the CRL-AP. Results were taken into consideration only when both the Excel file and the fax were received.
- The results had to be sent at once to the CRL-AP by 21 August 2007. Notification has been done that this date was a deadline and that results arriving later would not be accepted.

On the 27 participating laboratories, only results from 26 labs were accepted after exclusion of one participant, lab number 2, because the latter one did not report its results.

According to the aim of the present study and to the fact that they are not from a member state NRL, results from the Canadian Food Inspection Agency, although being faultless, were not included in the survey computation. Thus results from this report only originate from 25 participants.

3. Material and methods

3.1. Material

3.1.1. Description of the samples

Four different samples containing typical compound feed and processed animal proteins (PAPs) from various animals at different concentration levels have been prepared as shown in table 1.

The composition was established taking into account the following features:

- Target concentration of mammalian meat and bone meal (referred as MBM through the text) that was selected is 0.1 %, reflecting what is generally considered for the time being as the adulteration level that a method should be able to detect
- Presence of fishmeal at 5 % that could interfere with the detection of constituents from terrestrial animals when using classical microscopy [2] (often so-called "masking effect").

Each participating lab received about 55g of 4 blind samples to which a unique random number was assigned. Details of the samples are indicated in table 1.

Table 1: Composition of blind samples set used in the CRL-AP Proficiency Test 2007.

Sample	Material	Nr of replicates
A	Blank	1
B	"Challenger"	1
C	0.1% MBM + 5% Fish	1
D	5%Fish	1
Total		**4**

3.1.2. Materials used in the preparation of the samples

The **base of the test material** (Sample A or blank feed matrix) was a classical compound feed produced under strict controlled conditions in a pilot plant. The matrix was composed of wheat, corn, soya bean meal, and rapeseed, fat of plant origin, limestone, salts, minerals and vitamins. Sediment content of the compound feed was about 2.22% (STD 0.09%). Prior to use, the material was tested by classical microscopy and Polymerase Chain Reaction (PCR) in order to confirm the absence of any interfering substances from animal origin.

Another classical compound feed from a feed producer (Sample B or "challenger" feed matrix) was also used. This material was almost similar in composition to the blank and had a sediment content of 1.20% (STD 0.13%). Preliminary analysis by classical microscopy revealed sparse traces of MBM (far below the limit of detection of 0.1%). Nevertheless results from PCR did not allow confirming this contamination. The intention of using this "challenger" was to investigate on the possibility of the participants to detect this type of extremely low supposed contamination and to compare the results obtained with that from a material contaminated at the actual limit of detection *i.e.* 0.1% — and to analyse the results based on the number of animal particles found.

Different **processed animal proteins** were included in the study:

- The **MBM** used in the study was a mix of an 80% bone meal with a pure meat meal, both of bovine origin, in order to achieve a bone content of about 20%. MBM was treated at 133°C and 3 bars for 20 min.
- The **fish meal** used in the study was a mix of two fish meals used in DG-Sanco 2006 study: 1/3 Fish I and 2/3 Fish III. The fishbone content was of about 11%. Purity of the fish meals was investigated in the DG-Sanco 2006 study [5].

3.1.3. Description of the mixing procedures

Prior to their use in mixes, the feed matrixes and the processed animal proteins (MBM and fish meal) were sieved in order to keep only particles from the 0 – 1000 µm fractions.

The **stepwise dilution procedure** developed by CRA-W and JRC-IRMM was used to produce the materials 0.1% MBM + 5% Fish and 5% Fish. This procedure has been successfully used in four former European interlaboratory studies (DG-Sanco 2003 [2], DG-Sanco 2004 [3], STRATFEED [4] studies, DG-Sanco 2006 [5]) aiming to evaluate different classical microscopy protocols.

3.2. Qualitative analysis

Qualitative analysis concerned the presence or absence of terrestrial (MBM) and/or fish material for 3 of the blind samples (the "challenger" sample was not concerned for proficiency purposes as the contamination level of this material is far below the actual limit of detection). These binary results were analysed by classical statistics: accuracy, sensitivity and specificity. All those statistics were expressed as fractions.

Accuracy is the fraction of correct positive and negative results, was calculated by the following equation:

$$\text{Accuracy } AC = \frac{PA + NA}{PA + ND + PD + NA}$$

Where *PA* is the number of correct positive results (Positive Agreements), *NA* the number of correct negative results (Negative Agreements), *ND* the number of false negative results (Negative Deviations) and *PD* the number of false positive results (Positive Deviations).

Sensitivity is the ability of classifying positive results as positive, was calculated as follows:

$$\text{Sensitivity } SE = \frac{PA}{PA + ND}$$

Specificity is the ability of classifying negative results as negative, was calculated as follows:

$$\text{Specificity } SP = \frac{NA}{PD + NA}$$

The *AC*, *SE* and *SP* were calculated separately for each laboratory and for each requested parameter (terrestrial material and fish material) for the estimation of its proficiency. A consolidated *AC* over both parameters was used to rank each participant. Finally a global *AC* was also calculated for each material (except for "challenger") in order to estimate the performance of the method.

The 95% confidence intervals were calculated from the Binomial distribution (with lower limit $p = 0.025$ and upper limit $p = 0.975$).

4. Results

Gross results from all participants are to be found in Annex 3.

4.1. Homogeneity study

Homogeneity study has been realised for all materials used. The following table synthesises the results.

Table 2: Homogeneity – Detection of terrestrial or fish particles by classical microscopy

Material	Sediment	Terrestrial	Fish
Blank	2.27%	-	-
	2.26%	-	-
	2.27%	-	-
	2.20%	-	-
	2.36%	-	-
	2.08%	-	-
	2.16%	-	-
	2.21%	-	-
	2.08%	-	-
	2.32%	-	-
« Challenger »	1.13%	-	-
	1.17%	-	-
	1.12%	+ (1)	+ (1)
	1.13%	-	-
	1.11%	+ (1)	-
	1.08%	+ (1)	-
	1.26%	-	-
	1.54%	-	-
	1.20%	-	-
	1.24%	-	-
0.1% MBM + 5% Fish	3.40%	+	+
	3.43%	+	+
	3.45%	+	+
	3.47%	+	+
	3.42%	+	+
5% Fish	3.33%	-	+
	3.40%	-	+
	3.58%	-	+
	3.40%	-	+
	3.27%	-	+

Legend: + = present, - = not present, in brackets the number of particles detected in the "challenger".

In the “challenger” we found 3 terrestrial particles and 1 particle looking like fishbone over the ten sediments analysed (total of 27 slides).

In addition, PCR analyses have been performed on the blank and on the “challenger” and these tests could not detect any constituent of animal origin (analysed targets: cattle, pig, chicken, sheep and fish).

4.2. Qualitative analysis

4.2.1. Preliminary remarks

On the respect of the EC 126/2003 Directive:

- Labs 1, 3, 9, 18 and 20 rounded the sediment weight at 0.01g and not at 0.001 g as stated in directive EC 126/2003.
- Labs 11 and 21 did not respect the EC/126/2003 Directive instructions which indicate that when fish is detected at least 3 slides have to be observed: “… at least two additional slides […] shall be examined.”
- Labs 26 and 30 reported respectively one and two results for blanks based on 2 slides.
- Lab 9 reported one result for a blank based on a single slide observation.

On the respect of the present test instructions:

- Labs 18, 19 and 25 did not report the number of slides used as it was requested.
- Lab 31 did not report the number of slides observed but mentioned to have observed the whole sediment. It did not report the requested detailed data because it mentioned having a problem of understanding the instructions. However that lab did not contact the organiser in order to solve this problem although this possibility was clearly recommended in the instructions.
- Lab 18 did not report the detailed data at all.

4.2.2. Overview of results and performance of the method

Table 5 summarizes the results submitted by the 25 laboratories for the 3 types of materials submitted to qualitative analysis.

Table 5: Global results expressed as accuracy (AC) for the three samples

Sample	Material	N	AC	
			Terrestrial	Fish
A	blank	25	0.960 (1)	0.960 (1)
C	0,1% MBM + 5% Fish	25	0.840 (4)	1.000
D	5% Fish	25	0.840 (4)	1.000

Accuracy means sensitivity in case of ND and specificity in case of PD. In brackets the number of ND or PD. (Legend: n = number of observations).

It must be highlighted that there is no case report of “no results” although it was one of the possible result options in case of inconclusive results on the presence or the absence of relevant or in case of inability of the laboratory to provide a result. This means that participants were confident enough in their observations.

The overall results, expressed in terms of accuracy, indicate a satisfying global performance for the method.

Two cases of false positive results were reported for the blank, one for presence of MBM and one for presence of fish (4% or 1/25 for each). Four false positive results (16% or 4/25) about MBM presence were reported for the 5% Fish sample.

For the sample containing 0.1% MBM + 5% Fish I also some false negative results were reported (16% or 4/25).

4.2.3. Detailed review of results for each sample material

Blank :

Lab 15 reported 4-5 “snails” particles on a total of 5 slides, and classified those as fish particles (we believed that lab having counted foraminifers).

Lab 32 reported 3 terrestrial bones and cartilage fragments on a total of 3 slides.

Lab 3 detected 2 fish particles, but classified it as free from fish material because of a possible cross-contamination.

0.1% MBM + 5% Fish:

Labs 7, 20 and 21 were unable to detect the presence of MBM in the sample. Lab 7 established its decision of absence of terrestrial animal constituents on the observation of 3 slides, lab 20 on 5 slides and lab 21 on only 2 slides.

Lab 5 detected only 1 terrestrial bone particle from the sediment on a total of 6 slides, but decided to classify the sample as free from terrestrial particles.

Of note the fact that lab 11 established the presence of MBM on the sole observation of 1 bone particle on a total of 2 slides.

5% Fish:

Four labs detected terrestrial particles in this sample:

Labs 17 and 32 reported respectively 3 and 4-5 terrestrial bones on a total of 20 and 4 slides.

Lab 20 detected 6-10 terrestrial particles on a total of 7 slides, and lab 10 reported 11-50 terrestrial particles on a total of 3 slides.

4.2.4. Performances of individual labs in qualitative analysis

Individual performances were assessed for each participant by calculation of the accuracy, sensitivity and specificity over the blind samples. This was realized separately for both the detection of terrestrial material and fish material. A ranking of the labs was prepared based on the accuracy.

Results are to be found in tables 6 and 7 (next page).

Concerning the ability to detect terrestrial animal constituents, 7 labs provided incorrect results according to the following details :

- ND for MBM in the 0.1% MBM + 5% Fish : labs 5, 7, 21 and 20
- PD for MBM in the 5% Fish : labs 10, 17, 20 and 32
- PD for MBM in the blank : lab 32

Concerning the ability to detect fish material only one lab (15) encountered problems: a PD for fish in the blank.

Tables 6 (left) and 7 (right): Lab proficiencies regarding the detection of terrestrial and fish material. Ranking follows AC values.

Terrestrial			
lab code	AC	SE	SP
1	1.000	1.000	1.000
3	1.000	1.000	1.000
4	1.000	1.000	1.000
9	1.000	1.000	1.000
11	1.000	1.000	1.000
15	1.000	1.000	1.000
16	1.000	1.000	1.000
18	1.000	1.000	1.000
19	1.000	1.000	1.000
23	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
27	1.000	1.000	1.000
29	1.000	1.000	1.000
30	1.000	1.000	1.000
31	1.000	1.000	1.000
34	1.000	1.000	1.000
10	0.667	1.000	0.500
17	0.667	1.000	0.500
5	0.667	0.000	1.000
7	0.667	0.000	1.000
21	0.667	0.000	1.000
32	0.333	1.000	0.000
20	0.333	0.000	0.500

Fish			
lab code	AC	SE	SP
1	1.000	1.000	1.000
3	1.000	1.000	1.000
4	1.000	1.000	1.000
5	1.000	1.000	1.000
7	1.000	1.000	1.000
9	1.000	1.000	1.000
10	1.000	1.000	1.000
11	1.000	1.000	1.000
16	1.000	1.000	1.000
17	1.000	1.000	1.000
18	1.000	1.000	1.000
19	1.000	1.000	1.000
20	1.000	1.000	1.000
21	1.000	1.000	1.000
23	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
27	1.000	1.000	1.000
29	1.000	1.000	1.000
30	1.000	1.000	1.000
31	1.000	1.000	1.000
32	1.000	1.000	1.000
34	1.000	1.000	1.000
15	0.667	1.000	0.000

A general ranking of the participants was realized on a consolidated evaluation, including their proficiency in detecting both terrestrial and fish materials through the 3 blind samples (table 8 next page):

The individual global performance is excellent (= full set of correct answers) for 17 labs out of 25 NRLs or in other words for 68% of the participants which is higher than what was observed from the CRL-AP Interlaboratory Study 2006 (55%). Three participants (cells in blue italics in table 8) had still acceptable performances, with a single false positive detection of MBM in the 5% Fish or for fish detection in the blank.

Five participants were underperforming (cells in red through the whole line in table 8) and require improvement of proficiency. This represents 20% of less performing labs compared to 23% from the CRL-AP Interlaboratory Study 2006.

Table 8: General lab proficiency regarding the detection of terrestrial and fish material. Ranking follows AC values as primary key and SE as second key.

Consolidated			
lab code	AC	SE	SP
1	1.000	1.000	1.000
3	1.000	1.000	1.000
4	1.000	1.000	1.000
9	1.000	1.000	1.000
11	1.000	1.000	1.000
16	1.000	1.000	1.000
18	1.000	1.000	1.000
19	1.000	1.000	1.000
23	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
27	1.000	1.000	1.000
29	1.000	1.000	1.000
30	1.000	1.000	1.000
31	1.000	1.000	1.000
34	1.000	1.000	1.000
10	0.833	1.000	0.667
15	0.833	1.000	0.667
17	0.833	1.000	0.667
5	0.833	0.667	1.000
7	0.833	0.667	1.000
21	0.833	0.667	1.000
32	0.667	1.000	0.333
20	0.667	0.667	0.667

Another element with reference to the CRL-AP Interlaboratory Study 2006 has to be added to this analysis. The 5 NRLs that appeared underperforming in 2006 and that followed either training organised by the CRL-AP or recommendations, were performing excellently for the present study. Besides a single exception, the same statement can be made for the NRLs from the new member states that joined the Union at the beginning of 2007, and from one NRL that had to follow training because it did not participate at the CRL-AP Interlaboratory Study 2006.

From the 5 underperforming NRLs of this study, 4 are NRLs that were performing satisfactory in 2006. This proves that continuous formation and knowledge refreshment is absolutely required for keeping the NRL network at a high level of proficiency.

4.2.5. On the limit of detection and the number of particles observed for a diagnostic:

From the first CRL-AP annual workshop, held in Gembloux in April 2007, some NRLs highlighted the problem of taking a decision on the presence or absence of constituents of animal origin based on the detection of a very low number of animal particles. The EC 126/2003 directive mentions that the method should allow detecting very small amount of constituents of animal origin (< 0.1%). This is subject to interpretation, namely how to make a correct qualitative interpretation of results when the contamination level is inferior to the limit of quantification or the limit of detection. This is a key issue especially in cases of discussion on the values of counter analysis results when such low contamination would inhibit reproducibility.

Through the use of sample "challenger" (for reminder this sample was discovered, during the test preparation, to contain minute amounts of animal particles far below the commonly accepted 0.1% limit of detection) we tried by comparing the results on this sample with those on sample 0.1% MBM + 5% Fish to translate this concern in terms of minimal quantity of animal particles having to be observed before being able to declare a sample as positive with a sufficient confidence.

0.1% MBM + 5% Fish:

Results were analysed only for those participants who provided the requested additional information about the number of particles detected and the number of slides analysed, thus excluding labs 18 and 31.

Nb of labs	Nb of terrestrial particles	Nb of slides
3	0	10
2	1	8
3	3	9
3	4 to 5	13
6	6 to 10	19
6	11 to 50	61

av. min nb of particles/slide	7
av. max nb of particles/slide	28

"Challenger":

On the 25 participants, only three labs detected particles from animal origin:

Lab 10 and 21 counted both 11-50 terrestrial particles respectively on a total of 3 and 2 slides.

Lab 16 counted 6-10 fish particles on a total of 15 slides

Nb of labs	Nb of animal particles	Nb of slides
22	0	84
1	6 to 10	15
2	11 to 50	5

av. min nb of particles per slide	1
av. max nb of particles per slide	4

From the present study it appears clearly that the contamination level of the "challenger" sample is below the detection limit if one considers the number of slides to be analysed currently for an analysis. Taking into

account that a 0.1% contamination is considered to be the limit of detection of the technique, we suggest discussing on the possibility of introducing an interpretation rule for a sample with scarce particles of suspect origin. In such situation, a sample could be declared positive either for terrestrial or for fish material if and only if from the analysis of a minimum number of slides a minimal total number of particles of the same nature (terrestrial or fish) are counted. This would prevent false positive results from possible lab cross-contamination which can never be totally excluded and it will guarantee a better reproducibility of results in case of counter analysis. Nevertheless further analyses and simulations still have to be realised to make an interpretation rule proposal concrete; further discussions with all NRL network partners on this issue are also planned during the 2nd CRL-AP Workshop in 2008.

5. Conclusions

Results are summarized in the following table (table 9):

Table 9: Synthesis of performances and comparison with those from CRL-AP Interlaboratory Study 2006.

	Present CRL-AP study (% of 25 NRLs)	***Last CRL-AP study (% of 22 NRLs)***
Able to detect terrestrial material	84%	*82%*
Able to detect fish material	100%	*100%*
False detection of fish material	4%	*23%*
False detection of terrestrial material	16%	*18%*
Faultless answering	68%	*55%*

The individual global performance is excellent (= full set of correct answers) for 68% of the participants (compared to the 55% observed from the CRL-AP Interlaboratory Study 2006). Three participants had fair performances with a single false positive, for MBM in the 5% Fish or for fish in the blank. Five participants were nevertheless underperforming and require improvement of proficiency.

From the organiser side, another information has to be put forward as a conclusion of this proficiency test. From the 8 NRLs that followed the trainings organised by the CRL-AP during 2007 or followed recommendations for improvements (i.e. the 5 NRLs that were underperforming during the previous interlaboratory study, NRLs from the new member states that joined the Union beginning of 2007, and from one NRL that had to follow training because it did not participate at the CRL-AP Interlaboratory Study 2006), besides a sole exception, all those have now achieved a good proficiency level and are performing well. From the 5 underperforming NRLs of this study, 4 are NRLs that were performing satisfactory in 2006 (some bad results here might be explained to possible reporting errors but are nevertheless errors). This proves that continuous formation and knowledge refreshment (or eventually implementing procedures to avoid reporting errors) are absolutely required for keeping the NRL network at a high level of proficiency.

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6. References

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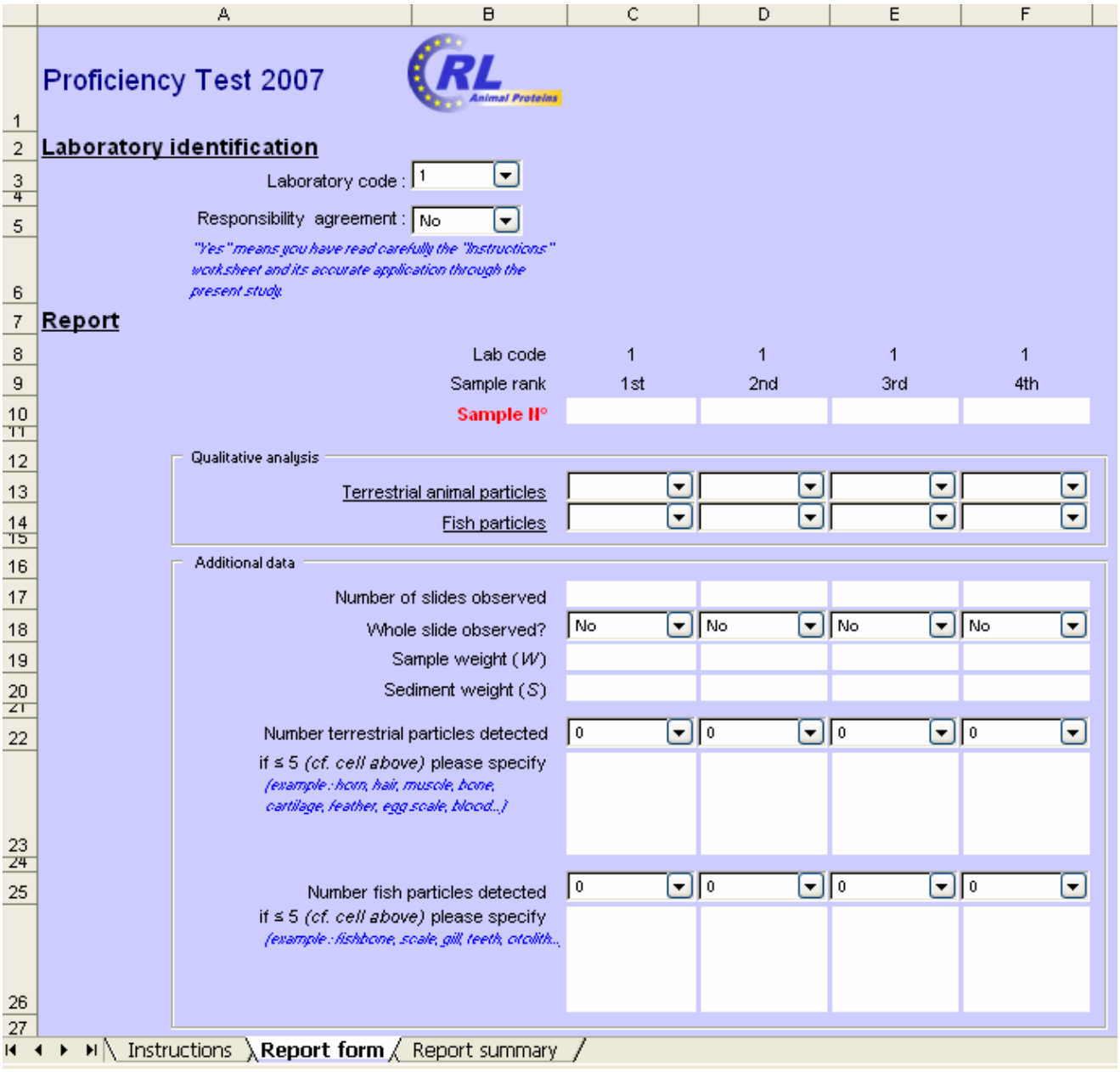
Annex 1

List of participants

Country	Institute Name
Austria	Austrian Agency for Health and Food Safety
Belgium	Federal Agency for the Safety of the Food Chain
Bulgaria	National Diagnostic Research Veterinary Medical Institute
Canada	Canadian Food Inspection Agency
Cyprus	Cyprus Veterinary Services
Czech republic	Central Institute of sampling and testing in Agriculture
Denmark	The Danish Plant Directorate
Estonia	Veterinary and Food Laboratory
Finland	Finnish Food Safety Authority
France	DG for Fair Trading, Consumer Affairs and Fraud Control-Laboratory Directorate Rennes
Germany	Federal Institute for Risk Assessment
Greece	Feedstuffs Control Laboratory
Hungary	Central Agricultural Office-Directorate Food and Feed Safety-Central Feed Investigation Lab.
Ireland	Department of Agriculture and Food Microscopy Laboratory - Seed Testing Station
Italy	National Reference Center for the Surveillance and Monitoring of Animal Feed
Latvia	National Diagnostic Centre of Food and veterinary Service
Lithuania	National Veterinary Laboratory
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of food safety, Wageningen UR
Poland	National Veterinary Research Institute
Portugal	Laboratorio Nacional de Investigaçao Veterinaria
Romania	Hygiene Institute of Veterinary Health
Slovakia	State veterinary and food institute
Slovenia	Veterinary Faculty-National Veterinary Institute-Unit for pathology of animal nutrition and environmental hygiene
Spain	Laboratorio arbitral agroalimentario
Sweden	National Veterinary Institute, Dept of Animal Feed
United Kingdom	Veterinary Laboratories Agency

Annex 2

Excel result report form.



Proficiency Test 2007

Laboratory identification

Laboratory code : 1

Responsibility agreement : No

"Yes" means you have read carefully the "Instructions" worksheet and its accurate application through the present study.

Report

Lab code	1	1	1	1
Sample rank	1st	2nd	3rd	4th
Sample II°				

Qualitative analysis

Terrestrial animal particles				
Fish particles				

Additional data

Number of slides observed				
Whole slide observed?	No	No	No	No
Sample weight (*W*)				
Sediment weight (*S*)				
Number terrestrial particles detected	0	0	0	0
if ≤ 5 *(cf. cell above)* please specify *(example : horn, hair, muscle, bone, cartilage, feather, egg scale, blood...)*				
Number fish particles detected	0	0	0	0
if ≤ 5 *(cf. cell above)* please specify *(example : fishbone, scale, gill, teeth, otolith...*				

Instructions | **Report form** | Report summary

Annex 3

Gross results of all participants (in numerical order of lab ID)

Laboratory identification code :			1							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
C	Present	Present	4	Yes	10	0,39	4 to 5	bone	> 50	
B	Not present	Not present	3	Yes	10	0,11	0		0	
D	Not present	Present	4	Yes	10	0,34	0		> 50	
A	Not present	Not present	4	Yes	10	0,31	0		0	

Laboratory identification code :			3							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
D	Not present	Present	6	Yes	10	0,36	0		> 50	
C	Present	Present	7	Yes	10	0,35	6 to 10	bones, see pictures attached	> 50	
A	Not present	Not present	6	Yes	10	0,29	0		2	1 fish bone, 1 scale, maybe contamination
B	Not present	Not present	6	Yes	10	0,12	0		0	

Laboratory identification code :			4							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
D	Not present	Present	3	No	10,1	0,247	0		> 50	
C	Present	Present	3	No	9,99	0,244	11 to 50		> 50	
B	Not present	Not present	3	No	10,07	0,062	0		0	
A	Not present	Not present	3	No	10,27	0,197	0		0	

Laboratory identification code :			5							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
B	Not present	Not present	6	Yes	5,01	0,07	0		0	
D	Not present	Present	6	Yes	5,007	0,194	0		11 to 50	
A	Not present	Not present	6	Yes	5,009	0,169	0		0	
C	Not present	Present	6	Yes	5,004	0,19	1	Is detected one bone in a sediment, but final result is free from bone, because too small concentratio n of ter. particles.	11 to 50	

Laboratory identification code :			7							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
D	Not present	Present	3	Yes	10	0,1987	0		> 50	fishbones and scales - muscle fibers (in sieved fraction)
B	Not present	Not present	3	Yes	10	0,0513	0		0	
C	Not present	Present	3	Yes	10	0,208	0		> 50	fishbones and scales - muscle fibers (in sieved fraction)
A	Not present	Not present	3	Yes	10	0,1778	0		0	

Laboratory identification code :			9							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
C	Present	Present	3	Yes	5,04	0,09	3	bone	> 50	
A	Not present	Not present	3	Yes	5,02	0,08	0		0	
D	Not present	Present	3	Yes	5,04	0,09	0		> 50	
B	Not present	Not present	1	Yes	5,03	0,04	0		0	

Laboratory identification code :			10							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
D	Present	Present	3	No	10	0,281 g	11 to 50		11 to 50	
B	Present	Not present	3	No	10,00 g	0,059 g	11 to 50		0	
C	Present	Present	3	No	10,00 g	0,279 g	6 to 10		11 to 50	
A	Not present	Not present	3	No	10,00 g	0,266 g	0		0	

Laboratory identification code :			11							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
C	Present	Present	2	Yes	10	0,3312	1	bone	> 50	
B	Not present	Not present	2	Yes	10	0,1077	0		0	
A	Not present	Not present	2	Yes	10	0,2453	0		0	
D	Not present	Present	2	Yes	10	0,2481	0		> 50	

Laboratory identification code :			15							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
B	Not present	Not present	5	Yes	10	0,151	0		0	
C	Present	Present	5	Yes	10	0,4	11 to 50		> 50	
D	Not present	Present	5	Yes	10	0,351	0		11 to 50	
A	Not present	Present	5	Yes	10	0,34	0		4 to 5	snails

Laboratory identification code :			16							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
B	Not present	Present	15	Yes	10	0,273	0		6 to 10	
D	Not present	Present	20	Yes	10	0,311	0		> 50	
C	Present	Present	20	Yes	10	0,292	11 to 50		> 50	
A	Not present	Not present	15	Yes	10	0,252	0		0	

Laboratory identification code :			17							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
D	Present	Present	20	Yes	5	0,18	3	bone	> 50	
C	Present	Present	20	Yes	5	0,18	11 to 50		> 50	
A	Not present	Not present	20	Yes	5	0,165	0		0	
B	Not present	Not present	20	Yes	5	0,044	0		0	

Laboratory identification code :			18							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
D	Not present	Present		Yes	10,08	0,37	0		0	
C	Present	Present		Yes	10,03	0,36	0		0	
B	Not present	Not present		Yes	10,07	0,1	0		0	
A	Not present	Not present		Yes	10,02	0,29	0		0	

Laboratory identification code :			19							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
B	Not present	Not present		Yes	5	0,103	0		0	
A	Not present	Not present		Yes	5	0,216	0		0	
C	Present	Present		Yes	5	0,203	6 to 10	bone	> 50	
D	Not present	Present		Yes	5	0,234	0		> 50	

Laboratory identification code :			20							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
D	Present	Present	7	No	5	0,23	6 to 10		> 50	
C	Not present	Present	5	No	5	0,19	0		> 50	
B	Not present	Not present	5	No	5	0,18	0		0	
A	Not present	Not present	5	No	5	0,18	0		0	

Laboratory identification code :			21							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
C	Not present	Present	2	Yes	10,00g	0,308g	0		> 50	
D	Not present	Present	2	Yes	10.00g	0,330g	0		> 50	
B	Present	Not present	2	Yes	10,00g	0,069g	11 to 50		0	
A	Not present	Not present	2	Yes	10,00g	0,306g	0		0	

Laboratory identification code :			23							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
C	Present	Present	6	Yes	5	0,202	4 to 5	bone	> 50	
A	Not present	Not present	5	Yes	5	0,21	0		0	
B	Not present	Not present	5	Yes	5	0,12	0		0	
D	Not present	Present	5	Yes	5	0,23	0		> 50	

Laboratory identification code :			24							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
B	Not present	Not present	3	Yes	10,147 g	0.092 g	0		0	
C	Present	Present	7	Yes	10,146 g	0,361 g	11 to 50		> 50	
A	Not present	Not present	4	Yes	10,077	0,262	0		0	
D	Not present	Present	7	Yes	10,06	0,343	0		> 50	

Laboratory identification code :			25							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
A	Not present	Not present		Yes	5,33	0,24	0		0	
D	Not present	Present		Yes	5,46	0,246	0		> 50	Bone fragments, muscle
C	Present	Present		Yes	5,65	0,266	6 to 10	Bone	> 50	Bone fragments, muscle
B	Not present	Not present		Yes	5,08	0,089	0		0	

Laboratory identification code :			26							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
D	Not present	Present	3	Yes	5,000 g	0,1718 g	0		> 50	
C	Present	Present	3	Yes	5,000 g	0,1764 g	6 to 10	bones	> 50	
B	Not present	Not present	2	Yes	5,000 g	0,0437 g	0		0	
A	Not present	Not present	3	Yes	5,000 g	0,1627 g	0		0	

Laboratory identification code :			27							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
B	Not present	Not present	6	Yes	10,0013	0,2262	0		0	
D	Not present	Present	3	Yes	10,0055	0,3646	0		> 50	
C	Present	Present	3	Yes	10,0006	0,3959	3	bones	> 50	
A	Not present	Not present	6	Yes	10,0022	0,3148	0		0	

Laboratory identification code :			29							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
D	Not present	Present	9	Yes	5,0005	0,1416	0		> 50	fishbones
C	Present	Present	6	Yes	5,0005	0,1432	11 to 50	bones	> 50	fishbones
B	Not present	Not present	6	Yes	5,0008	0,0311	0		0	
A	Not present	Not present	15	Yes	5,0004	0,1219	0		0	

Laboratory identification code :			30							
Sample	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
D	Not present	Present	6	Yes	5,12	0,231	0		> 50	
C	Present	Present	6	Yes	5,13	0,268	6 to 10		> 50	
B	Not present	Not present	2	Yes	5,06	0,071	0		0	
A	Not present	Not present	2	Yes	5,19	0,215	0		0	

Laboratory identification code :			31							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
B	Not present	Not present		Yes	5,101335	0,06515	0		0	
D	Not present	Present		Yes	5,163805	0,20785	0		0	
A	Not present	Not present		Yes	5,140245	0,16945	0		0	
C	Present	Present		Yes	5,078805	0,1875	0		0	

Laboratory identification code :			32							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
B	Not present	Not present	3	Yes	10,004	0,212	0		0	
C	Present	Present	3	Yes	10,1125	0,18	3	only bones	> 50	
D	Present	Present	4	Yes	10,0016	0,209	4 to 5	only bones	> 50	
A	Present	Not present	3	Yes	10,0077	0,165	3	bone, cartilage	0	

Laboratory identification code :			34							
Sample	**Terrestrial animal part.**	**Fish part.**	**Number of slides**	**Whole slide ?**	**W (g)**	**S (g)**	**Number of terrestrial part. detected**	**Comment if number of terr. part. ≤5**	**Number of fish part. detected**	**Comment if number of fish part. ≤5**
B	Not present	Not present	3	Yes	10,08	0,049	0		0	
A	Not present	Not present	3	Yes	10,05	0,19	0		0	
D	Not present	Present	3	Yes	10,06	0,223	0		> 50	
C	Present	Present	3	Yes	10,04	0,208	4 to 5	Bones	> 50	