Combined microscopy-PCR EURL-AP Proficiency Test 2015

Final version

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Summary

The European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP) organised the present proficiency test for assessing the ability of the NRL network with respect to the detection of processed animal proteins (PAPs) in feed using both light microscopy and PCR according the Commission Regulation EU/51/2013. It is the first time that the proficiency of the NRLs is assessed for the two methods within the same test. Even if the results are still independent, it is a first step to evaluate the process of the sample management within the labs.

Total number of participants was 31 (27 NRLs and 4 labs outside the NRL network). The study was based on a set of 10 blind samples (4 samples to be analysed by light microscopy only, 5 samples to be analysed by PCR only and the remaining 3 samples to be analysed both by microscopy and PCR) consisting of blank feed matrices or feeds fortified with terrestrial processed animal proteins and/or fishmeal or contaminated feed sent to the participants.

Twenty six of the 27 NRLs provided all their results in due time. The last NRL was unable to provide PCR results in time and considered as underperforming for the PCR method. All participants received after the closure of the results an individual table giving them a feedback of their results.

Regarding the detection of PAP by light microscopy the overall results indicate an excellent level of global performance with 93 % out of the NRL participants performing excellently for this method. The PCR results reflect also an excellent level of performance. 84.6 % of the 26 NRLs submitting results in time had no false result. The remaining four labs (15.4 %) obtained satisfactory level of performance by providing only one incorrect result.

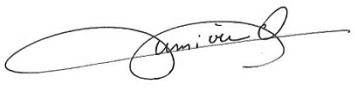
Keywords :

Processed animal proteins – Light microscopy – PCR - Proficiency test – Qualitative analysis

This report identified by an ISBN has been prepared from a draft version sent for revision and comments to the participants on the 19th April 2016. After reception of the comments on the 2nd May 2016, it was amended accordingly and approved by the signature of the organisers.

ISO 17043 coordinators signature for approval:

Pascal Veys *Olivier Fumière*



Wallonie

1. Foreword

European Union Reference Laboratories (EURL) were created in order to ensure a high level of quality and a uniformity of the results provided by European control laboratories. On 29th April 2004, the European Parliament and the Council adopted Regulation EC/882/2004 [1], 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 March 2011, Commission Regulation EC/208/2011 [2] renewed the nomination of the Walloon Agricultural Research Centre as European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP, http://eurl.craw.eu). It 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 (light microscopy, near infrared microscopy, PCR, 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.

In this framework, the EURL-AP is organising since 2006 yearly proficiency tests for the assessment of the implementation of the reference methods for the detection of animal proteins in feed as described by Commission Regulation EU/51/2013 [3] amending Annex VI of Commission Regulation EC/152/2009 [4]. The present study report is part of this activity scope.

2. Introduction

According to modified Annex VI of Commission Regulation EC/152/2009 [4] official controls for the detection of animal proteins in feed inside the EU are performed by light microscopy and/or PCR.

Until now the evaluations of the proficiency of the 27 NRLs of the network were operated at separate time schedule for each method; thus on a year basis, two proficiency tests were organised: one for the microscopic method and another one for the biomolecular method. For the first time, this study is intended to assess the performance of the participants to detect animal proteins in feed by both methods.

The objective of the present proficiency test is strictly to evaluate the performance of the network of 27 NRLs to detect the presence of processed animal proteins in feed by light microscopy and PCR.

On proposal of the Commission, invitations to participate to this test were also sent to a limited number of official control labs outside the EU. Non-EU participants were asked to apply also light microscopy and PCR although strict following of Annex VI of Commission Regulation EC/125/2009 was not imposed to them.

3. Material and methods

3.1. Study organisation

Participants were the 27 NRLs and 4 laboratories outside this EU network. A detailed list of the 31 participating labs is included in Annex 1.

Official announcement (Annex 2) of the study was made on the 30th October 2015 to all participants.

On the 11th December 2015, the sample sets were shipped to the participants. On the same day the Excel report forms containing the instructions (Annex 3) were communicated to all participants – downloadable from the EURL-AP intranet for the NRLs or sent by email to the non-EU participants who do not have access to this intranet.

The deadline for the delivery of the results was fixed in the announcement and in the instructions at the 15th January 2016. In order to allow enough time for the analyses the participants were informed before Christmas of an extension of the deadline at the 22nd January 2016 (Annex 2).

Within the instructions, some general recommendations were delivered to the participants:

- Laboratories participating to the proficiency test were themselves responsible to reach appropriate homogeneity of the sample sub-portions that had to be taken from the whole sample vial for analysis. Precautions to avoid laboratory cross-contamination were also highlighted.
- Results had to be encoded by way of an Excel report form (Annex 3). 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.
- Participants were asked to sign the summarized results sheet that is automatically generated when filling the form and to return it by email to the EURL-AP. Only when both the Excel file and a copy of the summarized results sheet were received by the EURL-AP were results taken into consideration.
- Deadline for providing results in the *ad hoc* forms to the EURL-AP was fixed at 22nd January 2016. Notification has been done that results arriving later would not be accepted.

27 NRL participants delivered their results on time. One NRL only delivered results for microscopic analyses, it was thus considered as underperforming for the PCR method. Concerning the 4 other non-EU participants three delivered their results on time. One non-EU participant delivered its results about one month beyond the deadline and was thus excluded. All of them performed microscopic analyses whereas only 2 labs returned PCR results. The proficiencies of NRLs and other participants were analysed separately in this report.

3.2. Material

3.2.1. Description of the samples

Ten different test materials were prepared for the proficiency test.

The composition of the sample set was established taking into account the following considerations:

- Target concentrations of mammalian PAPs around 0.1 % considered for time being as the adulteration level that light microscopy should be able to detect.
- Use of fishfeeds as matrices for assessing the detection capabilities of terrestrial PAPs because since the 1st June 2013 non-ruminant PAPS are authorized in aquafeed according to Commission Regulation EU/56/2013 [5].
- Use of feed matrices containing or adulterated with microscopically almost undetectable materials from terrestrial origin (blood meal) but able to deliver positive responses by PCR.

- Use of pelleted and ground test materials to investigate on the grinding effect on cross-contamination.

Each participating lab received about 50g of 12 blind samples to which a unique random number was assigned. Details of the samples are indicated in Table 1.

Table 1: Composition of the blind sample set used.

Colour code	Sample	Material	Nr of replicates	Expected results * Microscopy Terrestrial particles	Microscopy Fish particles	PCR Ruminant
	1	Feed I	1	-	-	
	2	Feed I + 0.05 % terrestrial PAP	1	+	-	
	3	Feed I + 0.1 % fishmeal	1	-	+	
	4	Feed I + 0.1 % fishmeal + 0.05 % terrestrial PAP	1	+	+	
	5	Fishfeed	1	-	+	-
	6	Fishfeed + 0.05 % ruminant PAP	1	+	+	+
	7	Fishfeed + 1 % ruminant blood	1	-	+	+
	8	Feed II	2			-
	9	Feed II + 0.1 % ruminant PAP	2			+
	10	Feed II + 0.1 % pig PAP	1			-
	Total		**12**	**3**	**5**	**3**

(* Explanations on expected results are described in section 3.4)

The expected results were internally determined based on the known composition of the samples (presence or absence of PAP) and the results obtained during the homogeneity study.

As mentioned in Table 1, the four first samples were intended to be analysed by light microscopy only (red code); the three following ones had to be analysed both by light microscopy and PCR (green code) whereas the three last ones (5 samples, orange code) were to be analysed by PCR only.

For avoiding ambiguities colour labels, stuck on the vials, were used to indicate which method or method combination had to be used.

3.2.2. Materials used in the preparation of the samples

Three matrices were used:

- Feed I was a **compound feed for horses** bought from a local producer. It was composed of barley flakes, alfalfa pellets, corn, soybean hulls, corn flakes, lin seeds, wheat bran, wheat, molasses, sunflower cake, calcium carbonate, barley, soybean cake, plant oil, feed complements (salts, vitamins, minerals). Its sediment content was about 2.0 %. This compound feed was pre-treated by grinding at 4 mm. This feed was used for preparing samples 1, 2, 3 and 4.
- The fishfeed was an industrial **compound feed for trout farming**. It consisted of fishmeal, soybean hull, toasted wheat, fish oil, corn gluten, spray dried haemoglobin powder, palm oil, wheat gluten, vitamins, minerals and antioxydants. The sediment content of the mixture was about 0.9 %. It was ground at 2 mm and used for preparing samples 5, 6 and 7.
- Feed II was a **compound feed for pigs** bought from an organic feed producer. It consisted of triticale, barley, oat, horse bean, pea, sunflower cake, potato proteins, soybean oil and feed complements (vitamins, salts, minerals). This compound feed was pre-treated by grinding at 2 mm. It was used for preparing samples 8, 9 and 10.

Adulterant material used:

- A **terrestrial PAP of unknown composition** was used for preparing sample 2 and 4. Its final bone content was of about 36.9 %. Its composition was investigated by microscopy and PCR. It was microscopically exclusively presenting terrestrial features. PCR revealed it as positive for pork and chicken and negative for bovine and fish.
- A **pure fishmeal** from Peru was used for preparing sample 3. Its sediment content was of about 15.2 %. Microscopic analyses showed it was only of fish origin.
- A **pure bovine PAP** was used for preparing sample 6 and 9. This PAP was produced by a pilot plant. Its bone content reached 52.4 %. Its purity was controlled by microscopy and PCR. By PCR the mean Ct values for ruminant PCR test was about 20 cycles corresponding to an estimated copy number of 850 000 copies. Some traces of porcine and poultry DNA were however detected.
- A mixed **ruminant blood meal** from a producer was used for sample 7. The blood meal was declared to contain 80 % ruminant blood and 20 % blood from other origin. It did not contain bones. Microscopic analyses did not allow detecting anything else but blood particles. PCR results confirmed the mixed origin of the blood meal.
- A **pure porcine PAP** was used for preparing sample 10. Its bone content was of about 14.0 % and its purity was checked by microscopy and PCR.

3.2.3. Description of the mixing procedures and pelleting

Prior to sample preparation, mixing of the materials and filling the vials, the rooms where those activities were performed were cleaned to avoid presence of interfering material.

Adulteration of the different samples was performed by successive dilutions.

For pelleting, corn starch and powdered sugar were added to the matrices as binding agent. This addition of binder was made before the adulteration process and the added amount was taken into account for obtaining correct levels of adulteration. A 6 mm pelleting machine was used.

3.3. Qualitative analysis

Analyses of qualitative proficiency testing were applied following ISO 13528 [6].

3.3.1. Light microscopy

Qualitative analysis concerned the detection of terrestrial animal and/or fish material.

Results are expressed by the participants in three formulations according to regulation EU/51/2013 [3] amending regulation EC/152/2009 [4]:

- Positive (= presence of animal material microscopically detectable)
- Negative (= absence of any animal material microscopically detectable)
- Below LOD (= low level presence of animal material microscopically detectable with a risk of false positive result)

Considering the risk of false positive results, all results expressed as below LOD have to be assimilated to negative ones as by definition they cannot be certified as positive *sensu stricto*. This allows an on-off, or binary result analysis.

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; it 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, it was calculated as follows:

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

Specificity is the ability of classifying negative results as negative, it 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 (detection of terrestrial animal material, detection of 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 in order to estimate the performance of the network.

3.3.2. PCR

Qualitative analysis concerned the detection of ruminant DNA.

The participants delivered Ct values (in cycles) to compare to a cut-off value (in cycles) set at 15 copies of the target and validated by a quality criterion (the cut-off Ct value must correspond to a number of copies of the target > 9.00 copies). For each sample, DNA is extracted from 2 test portions. The results obtained from the 2 test portions must be consistent. A Ct value < cut-off value corresponds to a positive result. Respectively, a Ct value ≥ cut-off value corresponds to a negative result. Results are expressed by the participants in two formulations:

- Present (= presence of ruminant DNA detected)
- Absent (= no ruminant DNA detected)

As for the light microscopy, these binary results were analysed by classical statistics (accuracy, sensitivity and specificity) with the same formulae as presented in 3.3.1.

3.4. Performance criteria

Evaluation of the performance and scoring were applied as recommended by ISO 13528 [6].

3.4.1. Light microscopy

Considering the sample set composition, the expected results are indicated on Table 1.

Sample 5 (Fishfeed) is considered to be declared negative for terrestrial particles detection although it is containing spray dried haemoglobin powder. Such ingredient is microscopically almost undetectable and is an authorized product.

Sample 7 (Fishfeed + 1 % ruminant blood) is considered to be declared negative for terrestrial particles as blood meal particles do not present identifiable features or bone fragments allowing to classify this material as from terrestrial origin.

Nevertheless participants that would be able to disclose in samples 5 and 7 this presence of blood products and would therefore declare the sample as positive for terrestrial material (as this type of product is not known to be obtained from fish) should logically not be penalised, therefore such results have to be assimilated to a correct negative assignment.

Based on these considerations, the following performance criteria were decided for the light microscopy:

- **Excellent** level of global performance = consolidated AC superior or equal to 0.90, i.e. having no more than 1 wrong result.
- **Satisfying** level of global performance = consolidated AC below 0.90 and having no more than 3 wrong results including a maximum of 1 ND for terrestrial material.
- **Underperforming** level of global performance = consolidated AC below 0.90 and having more than 3 wrong results –or 2 ND for terrestrial material.

3.4.2. PCR

Sample 6 (Fishfeed + 0.05 % ruminant PAP) is considered to be declared positive for the presence of ruminant DNA. The ruminant PAP content is below 0.1 %. The method is usually sensitive enough to detect the presence of ruminant DNA in that sample but it can be considered as a more challenging sample for the participants.

Concerning the PCR, the performance criteria were decided as:

- **Excellent** level of global performance = no wrong result for the detection of ruminant DNA.
- **Satisfying** level of global performance = no more than 3 wrong results and a maximum of 2 ND or 2 PD for the detection of ruminant DNA.
- **Underperforming** level of global performance = more than 3 wrong results or 3 ND or 3 PD for the detection of ruminant DNA.

3.5. Homogeneity study

Homogeneity study has been carried out for all materials used. Table 2 summarizes the results.

Table 2: Homogeneity study – Results.

Sample	Material	Light microscopy			NIRM		PCR				
		Nr of replicates	Terrestrial	Fish	Nr of replicates	Animal	Nr of replicates	Ruminant	Porcine	Fish	Universal plant
1	Feed I	10	-	-	5	-					
2	Feed I + 0.05 % terrestrial PAP	10	+	-	5	-					
3	Feed I + 0.1 % fishmeal	10	-	+	5	-					
4	Feed I + 0.1 % fishmeal + 0.05 % terrestrial PAP	10	+	+	5	+					
5	Fishfeed	10	-*	+	5	+	10	-	+	+	
6	Fishfeed + 0.05 % ruminant PAP	10	+	+	5	+	10	+			
7	Fishfeed + 1 % ruminant blood	10	-*	+	5	+	10	+			
8	Feed II						10	-	-		+
9	Feed II + 0.1 % ruminant PAP						10	+			
10	Feed II + 0.1 % pig PAP						10	-	+		

(Legend: blank cells = not tested, + = systematically detected, - = systematically not detected, NIRM = near infrared microscopy, * = blood particles detected)

The homogeneity was studied by light microscopy on 10 g of sample material for each replicate. Analyses of replicates were performed following strictly EC/152/2009. For PCR analysis of each replicate a double extraction was performed on 100 mg of sample material. Near infrared microscopy has also been performed on sediments of the samples and materials used for this study in complement to the official methods.

Sample 1 (Feed I) was systematically negative for any animal particle traces.

Sample 2 (Feed I + 0.05 % terrestrial PAP) revealed the sample always positive for terrestrial particles and always negative for fish.

Sample 3 (Feed I + 0.1 % fishmeal) systematically showed fish particles and never terrestrial particles.

Sample 4 (Feed I + 0.1 % fishmeal + 0.05 % terrestrial PAP) was always positive for both terrestrial and fish particles.

Sample 5 (Fishfeed) was always positive for fish presence and no terrestrial particles, such as bones or feathers, were observed. Blood particles were however systematically detected (always more than 5 particles per determination were recorded from the flotate). PCR analyses revealed the sample only positive for fish and porcine DNA. No ruminant DNA was detected.

Sample 6 (Fishfeed + 0.05 % ruminant PAP) was always found positive for both terrestrial and fish particles. PCR analyses always detected ruminant DNA.

Sample 7 (Fishfeed + 1 % ruminant blood) was always positive for fish particle presence and negative for terrestrial particles. Blood particles were systematically observed. By PCR, results were always positive for ruminant DNA.

Sample 8 (Feed II) PCR analyses led to negative results for ruminant and porcine DNA. The presence of amplifiable DNA was confirmed with the universal plant target.

Sample 9 (Feed II + 0.1 % ruminant PAP) PCR analyses revealed the sample positive for ruminant DNA.

Sample 10 (Feed II + 0.1 % pig PAP) led systematically to negative results for ruminant DNA and positive results for porcine DNA.

For samples 6, 7 and 9, it was checked that the mean copy number of the ruminant target from these samples were separated from the copy number of the cut-off by a value of at least three times the standard deviation of the measured copy number in 10 replicates of these sample. The mean copy number and standard deviation of the measured copy number are calculated twice on 20 data (DNA tested at the 1-fold dilution and the 10-fold dilution).

Near infrared microscopy analyses did not reveal inconsistencies in the materials used and the samples prepared this considering the adulteration levels and the fact that spectral records were only made on the sediments. As a standard for each sample 400 spectra were recorded on a fraction of 5 different sediments. Thus only 2000 spectra were obtained. This explains why for instance negative results for the presence of animal remains were obtained for samples 2 and 3 while for sample 4 a positive result was found but with only 5 animal records on the 2000. When the whole sediment was analysed and 10000 spectra were recorded, for sample 2 and 3, animal records were found: 4 for sample 2 and 3 for sample 3.

Results from the homogeneity study allowed declaring the samples as fit for their purpose.

4. Results

Gross results for microscopy and PCR from all participants are to be found in Annex 4 and 5 respectively.

4.1. Microscopy results

4.1.1. Qualitative analyses from the NRLs

4.1.1.1. On the respect of the instructions

NRLs respected the instructions related to the proficiency test itself.

Regarding the respect of EU regulation, as compared to a remark made last year on the respect of the number of determinations [7] still some few laboratories do not respect the diagrams. Most of them concerned correctly identified negative results for both terrestrial and fish presence (Sample 1) but based on an unauthorised number of determinations, i.e. two instead of a single. This represents only 4 % of superfluous repetitions over the total number of results (last year 8 %).

More worrying are the following cases. Lab 15 delivered an erroneous "<LOD" result for sample 6 while having only made a single determination. Similarly, lab 23 delivered an erroneous "<LOD" result but for sample 3 while also having performed only a single determination. EU regulation was not followed as it imposes a minimum of two determinations before using the "<LOD" result expression.

4.1.1.2. Results and performance of the network

Table 3 summarizes the results submitted by the 27 NRLs for the seven sample types submitted to qualitative analysis.

Table 3: Global results expressed as accuracy (AC) for the seven materials

Sample	Material	n	AC	
			Terrestrial	Fish
1	Feed I	27	1.000	1.000
2	Feed I + 0.05 % terrestrial PAP	27	0.963 (1)	1.000
3	Feed I + 0.1 % fishmeal	27	0.963 (1)	0.889 (3)
4	Feed I + 0.1 % fishmeal + 0.05 % terrestrial PAP	27	0.926 (2)	1.000
5	Fishfeed	27	1.000	1.000
6	Fishfeed + 0.05 % ruminant PAP	27	0.963 (1)	0.926 (2)
7	Fishfeed + 1 % ruminant blood	27	1.000	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 results).

The overall results, expressed in terms of global accuracy (AC), revealed the excellent global performance of the participants.

Sample 1: Feed I

No errors were noted. However one case of <LOD occurred:

- Lab 5: unknown few number of fish particles from 2 determinations

Sample 2: Feed I + 0.05 % terrestrial PAP

ND for terrestrial particles:

- Lab 22: less than 15 bones (but possibly confused with calcium phosphate) detected on 3 determinations

Sample 3: Feed I + 0.1 % fishmeal

PD for terrestrial particles:

- Lab 8: bones detected on one determination

ND for fish particles:

- Lab 3: “bones”, cartilage and gill fragments, but on total less than 15 as detected from 3 determinations
- Lab 22: less than 15 “bones” detected on 3 determinations
- Lab 23: 3 fish bones (but limited to a single determination)

Sample 4: Feed I + 0.1 % fishmeal + 0.05 % terrestrial PAP

ND for terrestrial particles:

- Lab 16
- Lab 22: less than 15 bones detected on 3 determinations

Sample 5: Fishfeed

No errors were noted.

This sample was declared to contain haemoglobin powder. According to the performance criteria it had to be considered as negative for terrestrial. Positive results for terrestrial under the condition that blood was described were also deliberated as correct. About 10 on 27 participants (37 %) were able to properly identify the presence of blood meal, whether or not they declared for this reason the sample as positive or negative for terrestrial.

Sample 6: Fishfeed + 0.05 % ruminant PAP

ND for terrestrial particles:

- Lab 15: less than 5 bones (but limited to a single determination)

In this sample, using the same matrix as sample 5, blood was also reported by 10 participants. Among these participants 9 out of them were the same that disclosed blood from sample 5.

ND for fish particles :

- Labs 9 and 23

Sample 7: Fishfeed + 1 % blood meal

No errors were noted. However some <LOD were reported :

- Lab 8: less than 10 terrestrial bones based on 2 determinations
- Labs 17 and 22: less than 15 terrestrial bones based on 3 determinations

In this sample again, a majority of participants this time were able to report the presence of blood : 15 out of 27 participants (55 %) detected this 1 % addition of blood meal.

4.1.1.3. Individual performances of NRLs in qualitative analysis

Individual performance parameters were assessed for each participant by calculating the accuracy, sensitivity and specificity over the blind sample set. This was calculated separately for both the detection of terrestrial material and of fish material. Results are to be found in Tables 4 and 5. A ranking of the labs was prepared based on the consolidated accuracy.

Tables 4 (left) and 5 (right): NRL proficiencies regarding the detection of terrestrial and fish material respectively. Ranking follows AC values for primary key and SE for second key. Underlined lab codes refer to NRLs that were able to identify blood presence in both sample 5 and 7.

Terrestrial

lab code	AC	SE	SP
1	1.000	1.000	1.000
2	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
6	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
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	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
8	0.857	1.000	0.750
15	0.857	0.667	1.000
16	0.857	0.667	1.000
22	0.714	0.333	1.000

Fish

lab code	AC	SE	SP
1	1.000	1.000	1.000
2	1.000	1.000	1.000
4	1.000	1.000	1.000
5	1.000	1.000	1.000
6	1.000	1.000	1.000
7	1.000	1.000	1.000
8	1.000	1.000	1.000
10	1.000	1.000	1.000
11	1.000	1.000	1.000
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	1.000	1.000	1.000
15	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
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
3	0.857	0.800	1.000
9	0.857	0.800	1.000
22	0.857	0.800	1.000
23	0.714	0.600	1.000

Details of the results were commented in section 4.2.3.

A general ranking of the NRLs was performed on a consolidated evaluation including their proficiency in detecting both terrestrial and fish materials through the set of blind samples (Table 6).

25 labs out of 27 NRLs or in other words for 93 % of the NRLs performed very well (2014 : 78 % [7] and 2013 : 63 % [8]). One NRL performed satisfyingly. Only one NRL was classified according the ranking criteria as underperforming for the present proficiency test. This lab requires improvement of proficiency. In agreement with the EURL-AP SOP for managing underperformances (available on the EURL-AP intranet since 18 January 2012), this underperforming participant is asked to report on the origin of his multiple errors as well as on the actions he will undertake in order to solve the problems.

Table 6: General NRL proficiency regarding the detection of terrestrial and fish material. Ranking follows AC values as primary key and SE as second key. Cells in blue refer to satisfying NRLs, cells in red refer to underperforming NRLs.

Consolidated			
lab code	AC	SE	SP
1	1.000	1.000	1.000
2	1.000	1.000	1.000
4	1.000	1.000	1.000
5	1.000	1.000	1.000
6	1.000	1.000	1.000
7	1.000	1.000	1.000
10	1.000	1.000	1.000
11	1.000	1.000	1.000
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	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
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
8	0.929	1.000	0.833
9	0.929	0.875	1.000
15	0.929	0.875	1.000
16	0.929	0.875	1.000
3	0.929	0.875	1.000
23	0.857	0.750	1.000
22	0.786	0.625	1.000

4.1.2. Qualitative analyses and individual performances the non-EU participants

Individual performances from the 4 participants outside the EU were assessed exactly as in previous section (4.1.1.3). A ranking of those labs was prepared as well based on the consolidated accuracy.

Results are to be found in Tables 7 and 8.

Tables 7 (left) and 8 (right): non-EU lab proficiencies regarding the detection of terrestrial and fish material respectively. Ranking follows AC values for primary key and SE for second key. (Legend: n.a. = not applicable)

Terrestrial			
lab code	AC	SE	SP
31	1.000	1.000	1.000
30	0.857	0.667	1.000
34	0.857	0.667	1.000
33	0.429	0.667	0.250

Fish			
lab code	AC	SE	SP
31	1.000	1.000	1.000
30	0.857	0.800	1.000
34	0.857	0.800	1.000
33	n.a.	n.a.	n.a.

Lab 33 did not report any results related to the possible presence or absence of fish particle. It has to be noted that none of the non-EU participating labs was able to mention the presence of blood.

The error details are described per sample:

Sample 2: Feed I + 0.05 % terrestrial PAP

ND for terrestrial particles

- Lab 30
- Labs 33 and 34: few bones (on 2 and 1 determination(s) respectively)

One <LOD case for fish particles by lab 34, but also based on a single determination.

Sample 3: Feed I + 0.1 % fishmeal

PD for terrestrial particles:

- Lab 33: mention of bones

Sample 4: Feed I + 0.1 % fishmeal + 0.05 % terrestrial PAP

ND for fish particles:

- Lab 30
- Lab 34: less than 5 fish bones but on a single determination

Sample 5: Fishfeed

PD for terrestrial particles:

- Lab 33: mention of bones

Sample 7: Fishfeed + 1 % blood meal

PD for terrestrial particles:

- Lab 33: mention of bones

As for the NRL participants, an indicative ranking of the non-EU participants was also realized on a consolidated evaluation including their proficiency in detecting both terrestrial and fish materials based on the same criteria as defined for the NRLs (Table 9).

Table 9: General non-EU 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**
31	1.000	1.000	1.000
30	0.857	0.750	1.000
34	0.857	0.750	1.000
33	0.429	0.667	0.250

One participant performed excellently, two performed satisfyingly (line in blue in Table 9).

Only one participant was classified as underperforming (line in red in Table 9) according to the applied criteria.

4.2. PCR results

4.2.1. Qualitative analyses from the NRLs

4.2.1.1. Overview of results and global performance of the test

Table 10 summarizes the results provided by the 26 NRLs for the six sample types submitted to qualitative analysis.

Table 10: Global results expressed as accuracy (AC) for the six sample types

Sample	Material	n	AC
5	Fishfeed	26	0.923 (2)
6	Fishfeed + 0.05 % ruminant PAP	26	1.000
7	Fishfeed + 1 % ruminant blood	26	1.000
8	Feed II	52	1.000
9	Feed II + 0.1 % ruminant PAP	52	1.000
10	Feed II + 0.1 % pig PAP	26	0.923 (2)

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

For all the samples, the overall results, expressed in terms of global accuracy (AC), are quite good. The occurrence of false positive results remains limited (3.8 %) whereas the presence of 0.1 % of ruminant PAP is always detected. For the first time, a sample at a level of 0.05 % of ruminant PAP was included in the sample set. In this case too, the presence of ruminant DNA was detected by all the participants showing not only the good sensitivity of the method but also the correct implementation of the method through the network.

4.2.1.2. Individual performances of NRLs in qualitative analysis

Individual performances were assessed for each participant by calculating the accuracy, sensitivity and specificity over the blind samples. A ranking of the labs was prepared based on the accuracy. Results are to be found in Table 11 that summarizes the results obtained by the participants for the eight analyses of the samples.

Table 11: NRL proficiencies regarding the detection of ruminant material starting from the eight samples. Ranking follows AC values. Cells in blue refer to satisfying NRLs.

Ranking	Lab code	AC	SE	SP
1	1	1.000	1.000	1.000
	2	1.000	1.000	1.000
	3	1.000	1.000	1.000
	5	1.000	1.000	1.000
	6	1.000	1.000	1.000
	7	1.000	1.000	1.000
	8	1.000	1.000	1.000
	9	1.000	1.000	1.000
	10	1.000	1.000	1.000
	12	1.000	1.000	1.000
	13	1.000	1.000	1.000
	14	1.000	1.000	1.000
	15	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
	22	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
23	4	0.875	1.000	0.750
	11	0.875	1.000	0.750
	21	0.875	1.000	0.750
	27	0.875	1.000	0.750

Table 11 illustrates the excellent level of global performance for 22 labs out of 26 NRLs (84.6 % of the NRLs) having no false result. Four labs (lines in blue in Table 11) out of the 26 (15.4 %) obtained satisfactory level of performance by providing only one incorrect result (1 false positive deviation).

4.2.1.3. Cut-off quality control

A quality control for the number of copies of the ruminant target reached with the Ct value of the cut-off, was developed to minimize the risk of false positive result. A minimum of 9.00 copies at the cut-off was required. Indeed, depending on the variability of the lab (PCR platform + operator), the cut-off value can correspond to a too low number of copies.

All the participants reached the minimum criterion of 9.00 copies. The percentage of the labs with a cut-off corresponding to a number of copies > 10 for this proficiency test is 65.4 % (70.4 % in 2014 [9] ; 55.6 % in 2013 [10]). The very few deviations observed are not due to a cut-off problem.

4.2.2. Qualitative analyses from the non-EU participants

4.2.2.1. Overview of results

There were only two non-EU labs providing results for the six sample types submitted to qualitative PCR analysis. The global accuracy is 1.000 for all the samples except for sample 9 (Feed II + 0.1 % ruminant PAP) for which one deviation was recorded (AC = 0.750).

4.2.2.2. Individual performances

Individual performances were assessed for each of these two participants by calculating the accuracy, sensitivity and specificity over the blind samples. Their results are to be found in Table 12.

Table 12 : Non NRL participant proficiencies regarding the detection of ruminant material starting from the eight samples. Ranking follows AC values.

Ranking	Lab code	AC	SE	SP
1	31	1.000	1.000	1.000
2	30	0.875	0.750	1.000

Lab 31 obtained excellent results (no deviation). Concerning Lab 30, one negative deviation is recorded with the sample 9 (Feed II + 0.1 % ruminant PAP). This participant probably uses another method than the one described in the EURL-AP SOP as no cut-off value nor Ct values were reported. Nevertheless, none of the two participants was underperforming.

4.2.2.3. Assessment of the cut-off values

Lab 30 gave no information about the cut-off value and the Ct values probably indicating the use of another PCR method than the official one in EU. Lab 31 has a cut-off that complies with the minimum criterion of 9 copies set by the EURL-AP.

5. Conclusions

Regarding the detection of PAP by light microscopy the overall results indicate an excellent level of global performance and a correct implementation of the official microscopic method. 93 % out of the NRL participants performed excellently for this method. This is the highest score ever obtained by this network.

Regardless of this, results demonstrated that the detection of fish particles still revealed some sensitivity problems. Detailed analysis showed that for one of the sample, the feed adulterated at 0.1 % fishmeal, it is more likely linked to a recovery issue of the sediment: some few fish particles are detected but not enough for allowing the results to be declared as positive. For the other sample, the fishfeed adulterated with 0.05 % ruminant PAP, the situation is unexplained.

For the detection of terrestrial particles problems of sensitivity and specificity were limited. Sensitivity issues were in some cases linked a possible recovery issue or the masking effect of fishmeal, as for the sample of feed adulterated with both fishmeal and terrestrial PAP. Erroneous bone detection accounted for the sole specificity issue observed for terrestrial PAP detection.

Once again the exercise of detecting blood particles in a feed showed interesting perspectives. Whereas last year microscopy proficiency test [7] revealed that about one third of the NRLs were able to identify blood meal at a level of 1 %, this year about 37 % out of them disclosed it from a real industrial fishfeed matrix and 55 % were able to detect it at a level comparable to that of last year. This increase in the NRL skills to identify blood within feed is demonstrating that this type of product, over the past recognised as almost undetectable, is actually no longer that difficult for a well-trained network of control laboratories. Nevertheless if this detection ability would have been mandatory in this test, hence modifying the performance criteria, it still would had impacted the proficiency level of the still 18 NRLs that were unable to detect blood in the two samples used for this purpose. Only paying extra attention on routine analysis will prevent from such situation in the future.

Concerning the non-EU participants, only one out of four performed excellently. None of the non-EU participants succeeded in detecting blood particles. However absence of details about the methods used by these participants does not allow in depth comparison with results of the NRL network.

The PCR results reflect also an excellent level of performance. 84.6 % of the 26 NRLs submitting results in time had no false result. The remaining four labs (15.4 %) obtained satisfactory level of performance by providing only one incorrect result (1 false positive deviation). It is the first proficiency test during which no under-performance is recorded among the participants submitting results.

The same conclusions can be made concerning the two non-EU participants: both of them are proficient and perform excellently or satisfactorily.

Although this proficiency test combines for the very first time both EU official methods for the detection of PAP in feed, light microscopy and PCR, any further combined performance assessment should not be authorised. The reasons for this are that each method has been implemented through this test on different sample types and sample numbers. Moreover for a given number of NRLs the analyses were performed by different teams on different locations. Nevertheless a large majority of participants performed both methods at one location and by a same team. The level of excellence achieved through this first combined proficiency test is demonstrating the maturity of the NRL network for both methods.

Acknowledgment

We are grateful to the EURL-AP technical staff that succeeded at addressing the challenge of a combined proficiency test. We also thank the participants for their fruitful collaboration.

References

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[2] EU. 2006. Commission Regulation (EU) No 208/2011 of 2 March 2011 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council and Commission Regulations (EC) No 180/2008 and (EC) No 737/2008 as regards lists and names of EU reference laboratories. Official Journal of the European Union L 58, 3/3/2011: 29–35.

[3] EU. 2013. Commission Regulation (EU) No 51/2013 of 16 January 2013 amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed. Official Journal of the European Union L 20, 23/01/2013: 33-43.

[4] EU. 2009. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union L 54, 26/2/2009: 1-130.

[5] EU. 2013. Commission Regulation (EU) No 56/2013 of 16 January 2013 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Official Journal of the European Union L 21, 24/1/2013: 3-16.

[6] ISO 13528, Statistical methods for use in proficiency testing by interlaboratory comparison.

[7] Veys P, Baeten V and Berben G. 2015. EURL-AP Proficiency Test Microscopy 2014: Final version. CRA-W, Gembloux, Belgium.

[8] Veys P, Baeten V and Berben G. 2014. EURL-AP Proficiency Test Microscopy 2013: Final version. CRA-W, Gembloux, Belgium.

[9] Fumière O., Marien A. and Berben G. 2013. EURL-AP PCR Proficiency Test 2013: Final version. CRA-W, Gembloux, Belgium.

[10] Fumière O., Marien A. and Berben G. 2014. EURL-AP PCR Proficiency Test 2014: Final version. CRA-W, Gembloux, Belgium.

Annex 1

List of participants (Laboratories that do not belong to the NRL network are *in italics*).

Country	Institute Name
Argentina	*Senasa Dilacot*
Australia	*Biosecurity Sciences Laboratory*
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
Croatia	Croatian Veterinary Institute
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 Centre for the Surveillance and Monitoring of Animal Feed
Japan	*Food and Agricultural Materials Inspection Center*
Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
Lithuania	National Food and Veterinary Risk Assessment Institute
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Norway	*LabNett AS and National Institute of Nutrition and Seafood Research*
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, Department of Animal Feed
United Kingdom	Animal and Plant Health Agency

Annex 2

Announcement letter and change of deadline

European Union Reference Laboratory for Animal Proteins in feedingstuffs

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Announcement of the EURL-AP proficiency test 2015/01 for the determination of Processed Animal Proteins (PAPs) in feed and Implementation test for porcine DNA detection

Introduction

The use of processed animal by-products as ingredient for animal feedingstuffs within the European Union is regulated by the TSE Regulation (Regulation EC N°999/2001), as amended. In particular, Article 7 imposes a prohibition to use processed animal proteins in the feeding of farmed animals (extended feed ban).
Commission Regulation (EU) No 51/2013 of 16 January 2013, amending Annex VI of Regulation (EC) No 152/2009, imposes the methods of analysis for the determination of constituents of animal origin for the official control of feed

Objective

The objective of the present proficiency test is to assess the performance of the NRLs to detect the presence of PAPs in feed by the reference methods using light microscopy and PCR as stated in Regulation EC 152/2009 as amended by Commission Regulation EU 51/2013 and related SOPs.
In addition to this proficiency test, an implementation study for the detection of porcine DNA in feed is asked to be performed by the NRLs. This implementation study is not intended for proficiency assessment.

The organizer team

The test will be coordinated by the European Union Reference Laboratory for Animal Proteins in feedingstuffs (EURL-AP).

Test material

Samples containing typical compound feed fortified with processed animal proteins (PAPs) will be prepared.
The EURL-AP will endorse the homogeneity of the samples. Nevertheless, each laboratory participating to the test is sole responsible to reach appropriate homogeneity for the sample sub-portions taken for analysis.
Each participant will receive :

1. *Proficiency test:* a maximum of 12 samples, each of about 50g. According to the instructions and colour codes, some samples will have to be analysed by microscopy only, by PCR only and by combining both microscopy and PCR.

2. *Implementation test for porcine DNA detection:* a maximum of 9 samples, each of about 10g

General outline of the exercise

- The light microscopic and PCR methods to use are described in Annex VI of Commission Regulation EC 152/2009 and related SOPs. These methods, alone or combined, shall be applied for the analyses.
- The EURL-AP will provide participants with an Excel file for reporting the results of the proficiency test analyses. A second separate Excel file will be provided for the implementation test for porcine DNA detection.
- Each participating laboratory will be assigned a unique code and only the organizer of the study knows the key to this code. After completing the test each laboratory will get a report including its results and lab code.
- The participation in this proficiency study and implementation test is mandatory and free of charge for national reference laboratories within Member States of the European Union.

European Union Reference Laboratory for Animal Proteins in feedingstuffs

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Time schedule

- Official announcement of the study to the NRLs by way of the intranet and e-mail : **30 October 2015**
- Sending of the sample boxes and communication of the instructions : **11 December 2015**

> ***By default, samples will be sent to the NRL microscopy contact person referred on the intranet. You are asked to check if this person is still your contact and to inform the organizer from any change.***

- Deadline for returning of results to the organizer :
  - Proficiency test : **15 January 2016**
  - Implementation test for porcine DNA detection : **5 February 2016**

Further information

- Refer to the address and coordinates mentioned in the heading,

or

- Dr Pascal VEYS
  EURL-AP NRL Network Manager
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or

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Change of results return date for the EURL-AP proficiency test 2015/01 for the determination of Processed Animal Proteins (PAPs) in feed

15th December 2015

Dear Participant,

The EURL-AP organising team decided to extend the deadline for returning of results to the organizer.
One more week is granted.
The **new deadline** is fixed at the **22nd January 2016**.

We wish you a very successful participation.

Further information

- Refer to the address and coordinates mentioned in the heading,

  or

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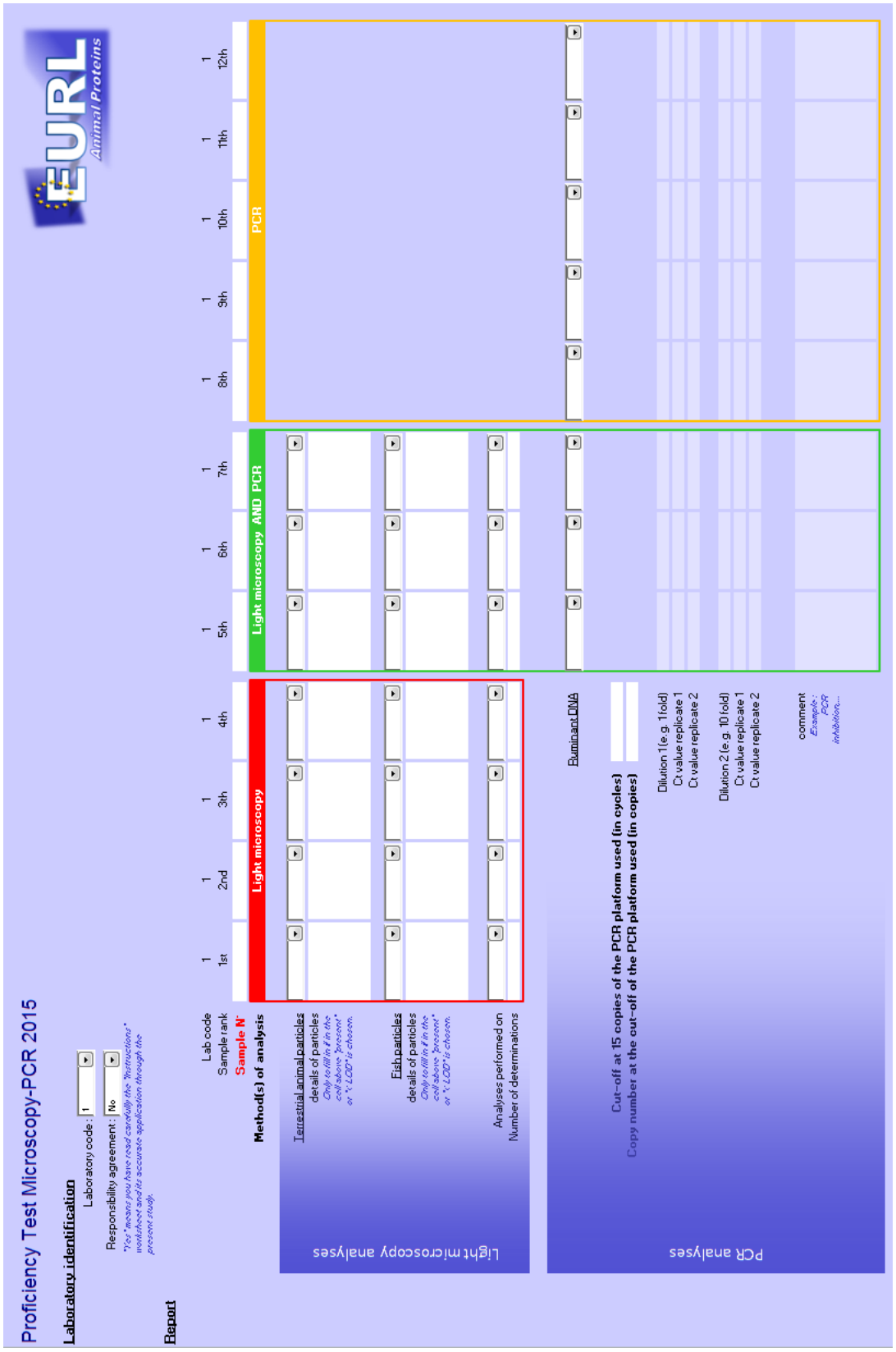
Annex 3

Excel result report form



Wallonie

Annex 4

Gross results of participants for microscopy (in numerical order of lab ID).

Laboratory identification code : **1**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	242	Absent		Absent		Sed. + Raw	1
2	484	Present	terr. bone particles 28	Absent		Sed. + Raw	1
4	568	Present	terr. bone particles c. 40	Present	fish bone, scale, cartilage, gill and otolith particles	Sed. + Raw	1
3	1586	Absent		Present	fish bone particles > 50	Sed. + Raw	1
5	1430	Absent		Present	fish bone, scale, cartilage, gill and otolith particles	Sed. + Raw	1
7	1754	Present	blood meal particles, picked & tested	Present	fish bone, scale, cartilage, gill and otolith particles	Sed. + Raw	1
6	1972	Present	terr. bone particles >10	Present	fish bone, scale, cartilage, gill and otolith particles	Sed. + Raw	1

Laboratory identification code : **2**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	348	Present	bones	Present	bones	Sed. + Flot.	2
2	704	Present	bones	Absent		Sed. + Raw	2
1	802	Absent		Absent		Sed. + Raw	2
3	1786	Absent		Present	bones, cartilages	Sed. + Flot.	2
5	270	Absent		Present	bones, cartilages, muscles	Sed. + Flot.	2
7	1434	Absent		Present	bones, cartilages	Sed. + Raw	2
6	1912	Present	bones	Present	bones	Sed. + Flot.	2

Laboratory identification code : **3**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	388	Present	bones	Present	bones, muscles, cartilage, scale	Sed. + Flot.	1
2	544	Present	bones	Absent		Sed. + Flot.	1
1	1722	Absent		Absent		Sed. + Flot.	1
3	1826	Absent		< LOD	bones, cartilage, gill	Sed. + Flot.	3
6	472	Present	bones, blood	Present	bones, muscles, cartilage, gill, scale	Sed. + Flot.	1
7	634	Present	blood	Present	bones, muscles, cartilage,gill, scale	Sed. + Flot.	1
5	710	Present	blood	Present	bones, muscles, cartilage, gill, scale	Sed. + Flot.	1

Laboratory identification code : **4**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	64	Present	bones	Absent		Sed. + Flot.	3
4	928	Present	bones	Present	fish bones, scales, gills	Sed. + Flot.	3
3	1646	Absent		Present	fish bones, scales, gills	Sed. + Flot.	3
1	1802	Absent		Absent		Sed. + Flot.	3
6	652	Present	bones	Present	fish bones, scales, gills	Sed. + Flot.	3
5	750	Absent		Present	fish bones, scales, gills	Sed. + Flot.	3
7	894	Absent		Present	fish bones, scales, gills	Sed. + Flot.	3

Laboratory identification code : **5**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	248	Present	bones, muscle	Present	bones, muscle	Sed. + Flot.	1
2	404	Present	bones	Absent		Sed. + Flot.	1
1	562	Absent		< LOD		Sed. + Flot.	2
3	1146	Absent		Present	bones	Sed. + Flot.	1
5	970	Absent		Present	bones, muscle	Sed. + Flot.	1
6	1332	Present	bones, muscle	Present	bones, muscle	Sed. + Flot.	1
7	1474	Present	blood meal	Present	bones, muscle	Sed. + Flot.	1

Laboratory identification code : **6**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	102	Absent		Absent		Sed. + Flot.	1
2	584	Present	Bones	Absent		Sed. + Flot.	1
3	1326	Absent		Present	Fishbones, muscles, gills	Sed. + Flot.	1
4	1648	Present	Bones, muscles	Present	Fishbones, muscles, gills	Sed. + Flot.	1
5	370	Present	Blood	Present	Fishbones, muscles, gills, scale, blood	Sed. + Flot.	1
7	1134	Present	Blood	Present	Fishbones, muscles, gills, scale, blood	Sed. + Flot.	1
6	2032	Present	Blood, bones	Present	Fishbones, muscles, gills, scale, blood	Sed. + Flot.	1

Laboratory identification code : **7**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	148	Present	bones	Present	fishbones, scales, cartilage	Sed. + Flot.	1
1	302	Absent		Absent		Sed. + Flot.	1
2	1224	Present	bones	Absent		Sed. + Flot.	1
3	2026	Absent		Present	fishbones, scales, muscles	Sed. + Flot.	1
5	90	Present	blood	Present	fishbones, scales, gills, cartilage, muscles	Sed. + Flot.	1
6	612	Present	bones, blood	Present	fishbones, scales, gills, cartilage, muscles	Sed. + Flot.	1
7	1334	Present	blood	Present	fishbones, scales, gills, cartilage, muscles	Sed. + Flot.	1

Laboratory identification code : **8**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	408	Present	Bones	Present	Fishbones; scales; gills; cartilages	Sed. + Flot.	1
1	902	Absent		Absent		Sed. + Flot.	1
2	1324	Present	Bones	Absent		Sed. + Flot.	1
3	1906	Present	Bones	Present	Fishbones; scales; gills; cartilages	Sed. + Flot.	1
7	954	< LOD	Bones	Present	Fishbones; scales; gills; cartilages	Sed. + Flot.	2
5	1870	Absent		Present	Fishbones; scales; gills; cartilages	Sed. + Flot.	1
6	2092	Present	Bones	Present	Fishbones; scales; gills; cartilages	Sed. + Flot.	1

Laboratory identification code : **9**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	168	Present	bone fragments	Present	fishbones, muscle fibres	Sed. + Flot.	1
1	682	Absent		Absent		Sed. + Flot.	1
2	1764	Present	bone fragments	Absent		Sed. + Flot.	1
3	1946	Absent		Present	fishbones, muscle fibres	Sed. + Flot.	1
5	130	Absent		Present	fishbones, muscle fibres	Sed. + Flot.	1
6	272	Present	bone fragments	Absent		Sed. + Flot.	1
7	1594	Present	blood particles	Present	fishbones, muscle fibres	Sed. + Flot.	1

Laboratory identification code : **10**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	468	Present	bones, muscle fibres no diff. between MBM- and FM muscle fibres possible	Present	fishbones, scales, muscle fibres no diff. between MBM- and FM fibres possible	Sed. + Flot.	1
1	722	Absent		Absent		Sed. + Flot.	1
3	1746	Absent		Present	fishbones, scales, muscle fibres it can't be excludet, that the muscle fibres only derive from FM	Sed. + Flot.	1
2	1964	Present	bones, muscle fibres it can't be excludet, that the muscle fibres only derive from MBM	Absent		Sed. + Flot.	1
5	330	Absent		Present	fishbones, scales, muscle fibres it can't be excludet, that the muscle fibres only derive from FM	Sed. + Flot.	1
6	1452	Present	bones, muscle fibres no diff. between MBM- and FM fibres possible	Present	fishbones, scales, muscle fibres no diff. between MBM- and FM fibres possible	Sed. + Flot.	1
7	1994	Absent		Present	fishbones, scales, muscle fibres, blood meal it can't be excludet, that the muscle fibres / blood found, only derive from FM	Sed. + Flot.	1

Laboratory identification code : **11**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	528	Present	terrestrial bones, (cartilage), (meat fiber)	Present	fish bones, scales, gills, (meat fiber), (cartilage)	Sed. + Raw	2
1	782	Absent		Absent		Sed. + Raw	2
2	944	Present	terrestrial bones, (cartilage), teeth	Absent		Sed. + Raw	3
3	1606	Absent		Present	fish bones, scales, gills, meat fiber	Sed. + Raw	2
5	470	Present	blood	Present	fish bones, scales, gills, meat fiber, cartilage	Sed. + Flot.	2
7	514	Present	blood	Present	fish bones, scales, gills, meat fiber, cartilage	Sed. + Flot.	2
6	1932	Present	Terrestrial bones, blood, (meat fiber)	Present	fish bones, scales, gills,(meat fiber), cartilage	Sed. + Flot.	2

Laboratory identification code : **12**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	864	Present	bones, muscles	Absent		Sed. + Flot.	1
3	1486	Absent		Present	fishbones, muscles, gills	Sed. + Flot.	1
1	1862	Absent		Absent		Sed. + Flot.	2
4	2008	Present	bones, muscles	Present	fishbones, muscles	Sed. + Flot.	1
6	212	Present	bones, blood	Present	fishbones, muscles,gills,scale	Sed. + Flot.	1
5	510	Present	blood, feather	Present	fishbones, muscles,gills,scale	Sed. + Flot.	2
7	694	Present	blood	Present	'fishbones, muscles,gills,scale	Sed. + Flot.	2

Laboratory identification code : **13**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	748	Present	bones	Present	bones, scales, gills	Sed. + Flot.	1
1	762	Absent		Absent		Sed. + Flot.	1
3	1306	Absent		Present	bones, cartilage, gills, scales	Sed. + Flot.	1
2	1684	Present	bones	Absent		Sed. + Flot.	1
5	50	Absent		Present	bones, cartilage, gills, scales	Sed. + Flot.	1
6	332	Present	bones	Present	bones, cartilage, gills, scales	Sed. + Flot.	1
7	1534	Absent		Present	bones, cartilage, gills, scales	Sed. + Flot.	2

Laboratory identification code : **14**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	108	Present	bones, muscle fibres	Present	bones, scales, gills, muscle fibres	Sed. + Flot.	1
1	122	Absent		Absent		Sed. + Flot.	1
3	1986	Absent		Present	bones, scales, gills, muscle fibres, cartilage	Sed. + Flot.	1
2	2084	Present	bones, muscle fibres, feathers	Absent		Sed. + Flot.	1
5	30	Present	blood	Present	bones, scales, gills, muscle fibres, cartilage	Sed. + Flot.	1
7	574	Present	blood	Present	bones, scales, gills, muscle fibres, cartilage	Sed. + Flot.	1
6	1272	Present	bones, muscle fibres, blood	Present	bones, scales, gills, muscle fibres	Sed. + Flot.	1

Laboratory identification code : **15**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	324	Present	Terrestrial bone	Absent		Sed. + Raw	1
1	602	Absent		Absent		Sed. + Raw	1
4	1288	Present	Terrestrial bone, muscle fibres	Present	muscle fibres, fish bone, scale, gill, skin	Sed. + Raw	1
3	1686	Absent		Present	muscle fibres, fish bone, scale, gill	Sed. + Raw	1
6	232	< LOD	Terrestrial bone	Present	muscle fibres, fish bone, cartilage, skin, scale, gill	Sed. + Raw	1
5	670	Absent		Present	muscle fibres, fish bone,scale, gill, tooth, skin	Sed. + Raw	1
7	834	Absent		Present	muscle fibres, fish bone, scale, gill, skin, mollusc	Sed. + Raw	1

Laboratory identification code : **16**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	48	Absent		Present	fishbones, cartilage, gills, muscles	Sed. + Raw	2
1	202	Absent		Absent		Sed. + Raw	2
2	644	Present	bones, muscles, hair	Absent		Sed. + Raw	2
3	1366	Absent		Present	fishbones, cartilage, muscles	Sed. + Raw	2
5	810	Absent		Present	fishbones, cartilage, gills, muscles	Sed. + Raw	2
7	1254	Absent		Present	fishbones, cartilage, tooth, muscles	Sed. + Raw	2
6	1672	Present	bones, cartilage, muscles	Present	fishbones, cartilage, tooth, muscles	Sed. + Raw	2

Laboratory identification code : **17**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	988	Present	bones	Present	fishbones, splinters, muscle fibres	Sed. + Flot.	2
2	1384	Present	bones	Absent		Sed. + Flot.	2
3	1546	Absent		Present	fishbones, splinters, scale	Sed. + Flot.	2
1	1782	Absent		Absent		Sed. + Flot.	2
5	610	Absent	blood	Present	fishbones, splinters, muscle fibres	Sed. + Flot.	2
6	792	Present	bones, blood	Present	fishbones, splinters, scale, muscle fibres	Sed. + Flot.	2
7	1714	< LOD	bones, blood	Present	fishbones, splinters, scale, muscle fibres	Sed. + Flot.	3

Laboratory identification code : **18**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	162	Absent		Absent		Sed. + Raw	1
2	1004	Present	bones	Absent		Sed. + Raw	1
3	1166	Absent		Present	fish bones, scales	Sed. + Raw	1
4	1468	Present	bones	Present	fish bones, scales	Sed. + Raw	1
5	170	Absent		Present	fish bones, cartilage, gills, scales	Sed. + Raw	1
6	672	Present	bones	Present	fish bones, cartilage, gills, scales	Sed. + Raw	1
7	1914	Absent		Present	fish bones, cartilage, scales	Sed. + Raw	1

Laboratory identification code : **19**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	184	Present	bones, muscles	Absent		Sed. + Flot.	1
4	588	Present	bones	Present	bones, cartilages, gills, otoliths, scales, muscles	Sed. + Flot.	1
1	862	Absent		Absent		Sed. + Flot.	1
3	1406	Absent		Present	bones,gills, scales, muscles	Sed. + Flot.	1
5	230	Present	blood	Present	bones, cartilages, gills, otoliths, scales, muscles	Sed. + Flot.	1
6	692	Present	bones, blood	Present	bones, cartilages, gills, otoliths, scales, muscles	Sed. + Flot.	1
7	1174	Present	blood	Present	bones, cartilages, gills, otoliths, scales, muscles	Sed. + Flot.	1

Laboratory identification code : **20**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	228	Present	bones, muscles fibres	Present	fishbones, scales, muscles fibres	Sed. + Raw	1
3	406	Absent		Present	fishbones, scales	Sed. + Raw	1
1	462	Absent		Absent		Sed. + Raw	1
2	1524	Present	bones	Absent		Sed. + Raw	1
5	950	Present	blood	Present	fishbones, scales, muscle fibres, blood	Sed. + Raw	1
6	1072	Present	bones, muscles fibres,blood	Present	fishbones, scales, muscles fibres	Sed. + Raw	1
7	1874	Present	blood	Present	fishbones, scales, muscles fibres	Sed. + Raw	1

Laboratory identification code : **21**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	402	Absent		Absent		Sed. + Flot.	1
3	546	Absent		Present	bone	Sed. + Flot.	1
2	904	Present	bone	Absent		Sed. + Flot.	1
4	1068	Present	bone, muscle	Present	bone, muscle	Sed. + Flot.	1
6	952	Present	bone, muscle	Present	gill, bone, cartilage, muscle	Sed. + Flot.	1
7	1294	Present	1 bone, blood	Present	gill, bone, cartilage	Sed. + Flot.	3
5	1650	Present	blood	Present	gill, bone, cartilage, scale	Sed. + Flot.	1

Laboratory identification code : **22**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	264	< LOD	Bones (calcium phosphate?)	Absent		Sed. + Flot.	3
4	708	< LOD	Bones	Present	"Bones, cartilage, gills, scales, muscles	Sed. + Flot.	3
3	886	Absent		< LOD	Bones	Sed. + Flot.	3
1	1702	Absent		Absent		Sed. + Flot.	1
7	134	< LOD	Bones	Present	'Bones, cartilage, gills, scales, muscles	Sed. + Flot.	3
5	430	Absent		Present	'Bones, cartilage, gills, scales, muscles	Sed. + Flot.	3
6	1732	Present	Bones, muscles	Present	Bones, cartilage, gills, scales, muscles	Sed. + Flot.	1

Laboratory identification code : **23**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	986	Absent		< LOD	We have found 3 fish bones	Sed. + Flot.	1
2	1624	Present	We have found 9 bones and 2 muscles	Absent		Sed. + Flot.	2
4	1788	Present	we have found 6 bones	Present	fish bones, scale, muscle	Sed. + Flot.	2
1	2042	Absent		Absent		Sed. + Flot.	2
6	552	Present	5 bones, muscle and blood we use the staining reagent tetrametilbenzidine	Absent		Sed. + Flot.	2
7	734	Absent		Present	fish bones, muscles and blood.We use the staining reagent tetrametil benzidine	Sed. + Flot.	2
5	1410	Absent		Present	fish bones, scale, muscle.There was red particles, we use tretrametil bendine and the result was negative	Sed. + Flot.	2

Laboratory identification code : **24**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	346	Absent		Present	gills,muscle,scales.	Sed. + Flot.	1
2	1584	Present	bones	Absent		Sed. + Flot.	1
4	1728	Present	bones	Present	gills,muscle,scales.	Sed. + Flot.	1
1	1762	Absent		Absent		Sed. + Flot.	1
7	934	Absent		Present	gills,muscle,scales.	Sed. + Flot.	2
6	1172	Present	bones	Present	gills,muscle,scales.	Sed. + Flot.	1
5	1470	Absent		Present	gills,muscle,scales.	Sed. + Flot.	1

Laboratory identification code : **25**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	266	Absent		Present	Bones	Sed. + Flot.	1
4	888	Present	Bones	Present	Bones	Sed. + Flot.	1
2	1824	Present	Bones	Absent		Sed. + Flot.	1
1	2002	Absent		Absent		Sed. + Flot.	1
7	354	Present	Blood	Present	Bones	Sed. + Flot.	1
5	1050	Absent		Present	Bones	Sed. + Flot.	1
6	1392	Present	Bones	Present	Bones	Sed. + Flot.	1

Laboratory identification code : **26**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	586	Absent		Present	Bone, Gills, Cartilage, Muscle, Scales	Sed. + Raw	1
2	744	Present	Bone	Absent		Sed. + Raw	1
1	822	Absent		Absent		Sed. + Raw	2
4	1968	Present	Bone	Present	Bone, Gills, Cartilage, Muscle, Scales	Sed. + Raw	1
7	14	Absent		Present	Bone, Gills, Cartilage, Muscle, Scales	Sed. + Raw	1
6	2072	Present	Bone	Present	Bone, Gills, Cartilage, Muscle, Scales	Sed. + Raw	1
5	2090	Absent		Present	Bone, Gills, Cartilage, Muscle, Scales	Sed. + Raw	1

Laboratory identification code : **27**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	206	Absent		Present	Fish bones, Fish scales, Gill, Cartilage, Muscle fibers	Sed. + Flot.	1
2	824	Present	Terrestrial bones	Absent		Sed. + Flot.	1
4	1128	Present	Terrestrial bones, Muscle fibers, Cartilage	Present	Fish bones, Fish scales, Gill, Fish skin, Cartilage, Muscle fibers	Sed. + Flot.	1
1	1842	Absent		Absent		Sed. + Flot.	1
6	1212	Present	Terrestrial bones, Muscle fibers, Cartilage	Present	Fish bones, Fish scales, Gill, Cartilage, Muscle fibers	Sed. + Flot.	1
7	1494	Present	Blood meal, Muscle fibers, Cartilage	Present	Fish bones, Fish scales, Gill, Cartilage, Muscle fiber, Otholith, Blood meal	Sed. + Flot.	1
5	1850	Absent		Present	Fish bones, Fish scales, Gill, Fish skin, Cartilage, Muscle fibers	Sed. + Flot.	1

Laboratory identification code : **30**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	486	Absent		Present	scales	Sed. + Raw	1
2	1184	Absent		Absent		Sed. + Raw	1
4	1248	Present	muscle	Absent		Sed. + Raw	1
1	2022	Absent		Absent		Sed. + Raw	1
6	352	Present	feathers	Present	scales fishbones	Sed. + Raw	1
7	394	Absent		Present	scales fishbones	Sed. + Raw	1
5	1810	Absent		Present	scales fishbones	Sed. + Raw	1

Laboratory identification code : **31**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	24	Present		Absent		Sed. + Flot.	2
3	126	Absent		Present		Sed. + Flot.	2
4	648	Present		Present		Sed. + Flot.	2
1	2062	Absent		Absent		Sed. + Flot.	2
6	1132	Present		Present		Sed. + Flot.	2
5	1350	Absent		Present		Sed. + Flot.	2
7	1834	Absent		Present		Sed. + Flot.	2

Laboratory identification code : **33**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
6	12	Present	bone			Sed. + Flot.	1
7	254	Present	bone			Sed. + Flot.	1
3	846	Present	bone			Sed. + Flot.	1
5	1250	Present	bone			Sed. + Flot.	1
2	1284	< LOD	bone			Sed. + Flot.	2
4	1668	Present	bone			Sed. + Flot.	1
1	1882	Absent				Sed. + Flot.	2

Laboratory identification code : **34**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	1308	Present	bones	< LOD	fishbones	Sed. + Flot.	1
2	1864	< LOD	bones	< LOD	fishbones	Sed. + Flot.	1
1	1662	Absent		Absent		Sed. + Flot.	1
3	966	Absent		Present	fishbones	Sed. + Flot.	1
6	1012	Present	bones	Present	fishbones	Sed. + Flot.	1
5	1710	Absent		Present	fishbones	Sed. + Flot.	1
7	2034	Absent		Present	fishbones	Sed. + Flot.	1

Annex 5

Gross results of participants for PCR (in numerical order of lab ID).

Laboratory identification code:	1
Cut-off at 15 copies :	36.00 cycles
Copy number at the cut-off	10.70 copies

Sample type	Sample N°	Ruminant DNA
5	1430	Absent
7	1754	Present
6	1972	Present
9	698	Present
9	898	Present
8	1616	Absent
10	1780	Absent
8	1936	Absent

Laboratory identification code:	2
Cut-off at 15 copies :	34.27 cycles
Copy number at the cut-off	11.30 copies

Sample type	Sample N°	Ruminant DNA
5	270	Absent
7	1434	Present
6	1912	Present
9	78	Present
9	718	Present
10	1100	Absent
8	1276	Absent
8	1796	Absent

Laboratory identification code:	3
Cut-off at 15 copies :	35.92 cycles
Copy number at the cut-off	10.76 copies

Sample type	Sample N°	Ruminant DNA
6	472	Present
7	634	Present
5	710	Absent
9	18	Present
9	958	Present
8	1396	Absent
10	1840	Absent
8	1956	Absent

Laboratory identification code:	4
Cut-off at 15 copies :	38.17 cycles
Copy number at the cut-off	12.17 copies

Sample type	Sample N°	Ruminant DNA
6	652	Present
5	750	Present
7	894	Present
9	338	Present
10	480	Absent
8	836	Absent
8	1356	Absent
9	1958	Present

Laboratory identification code:	5

Cut-off at 15 copies :	35.85 cycles
Copy number at the cut-off	9.65 copies

Sample type	Sample N°	Ruminant DNA
5	970	Absent
6	1332	Present
7	1474	Present
9	998	Present
10	1520	Absent
8	1636	Absent
8	1736	Absent
9	1898	Present

Laboratory identification code:	6

Cut-off at 15 copies :	35.62 cycles
Copy number at the cut-off	9.25 copies

Sample type	Sample N°	Ruminant DNA
5	370	Absent
7	1134	Present
6	2032	Present
10	300	Absent
9	358	Present
9	978	Present
8	1216	Absent
8	1596	Absent

Laboratory identification code:	7

Cut-off at 15 copies :	35.05 cycles
Copy number at the cut-off	10.70 copies

Sample type	Sample N°	Ruminant DNA
5	90	Absent
6	612	Present
7	1334	Present
9	238	Present
10	360	Absent
8	676	Absent
9	1018	Present
8	1296	Absent

Laboratory identification code:	8

Cut-off at 15 copies :	37.00 cycles
Copy number at the cut-off	9.74 copies

Sample type	Sample N°	Ruminant DNA
7	954	Present
5	1870	Absent
6	2092	Present
10	220	Absent
9	458	Present
9	1138	Present
8	1896	Absent
8	2096	Absent

Laboratory identification code:	9

Cut-off at 15 copies :	33.95 cycles
Copy number at the cut-off	11.99 copies

Sample type	Sample N°	Ruminant DNA
5	130	Absent
6	272	Present
7	1594	Present
9	158	Present
9	538	Present
8	1236	Absent
8	1416	Absent
10	1560	Absent

Laboratory identification code:	10

Cut-off at 15 copies :	35.72 cycles
Copy number at the cut-off	11.68 copies

Sample type	Sample N°	Ruminant DNA
5	330	Absent
6	1452	Present
7	1994	Present
9	58	Present
10	160	Absent
9	438	Present
8	896	Absent
8	1876	Absent

Laboratory identification code:	11

Cut-off at 15 copies :	35.91 cycles
Copy number at the cut-off	9.26 copies

Sample type	Sample N°	Ruminant DNA
5	470	Absent
7	514	Present
6	1932	Present
9	558	Present
8	916	Absent
10	1120	Present
8	1716	Absent
9	1938	Present

Laboratory identification code:	12

Cut-off at 15 copies :	35.82 cycles
Copy number at the cut-off	9.28 copies

Sample type	Sample N°	Ruminant DNA
6	212	Present
5	510	Absent
7	694	Present
9	298	Present
9	318	Present
10	1000	Absent
8	1316	Absent
8	1696	Absent

Laboratory identification code:	13

Cut-off at 15 copies :	36.27 cycles
Copy number at the cut-off	9.25 copies

Sample type	Sample N°	Ruminant DNA
5	50	Absent
6	332	Present
7	1534	Present
10	80	Absent
9	198	Present
9	918	Present
8	1916	Absent
8	2056	Absent

Laboratory identification code:	14

Cut-off at 15 copies :	35.57 cycles
Copy number at the cut-off	11.25 copies

Sample type	Sample N°	Ruminant DNA
5	30	Absent
6	574	Present
7	1272	Present
10	98	Present
9	478	Present
9	720	Absent
8	816	Absent
8	1656	Absent

Laboratory identification code:	15

Cut-off at 15 copies :	36.43 cycles
Copy number at the cut-off	11.02 copies

Sample type	Sample N°	Ruminant DNA
6	232	Present
5	670	Absent
7	834	Present
9	758	Present
9	938	Present
10	1020	Absent
8	1336	Absent
8	1856	Absent

Laboratory identification code:	16

Cut-off at 15 copies :	34.30 cycles
Copy number at the cut-off	11.17 copies

Sample type	Sample N°	Ruminant DNA
5	810	Absent
7	1254	Present
6	1672	Present
9	178	Present
10	880	Absent
9	1058	Present
8	1076	Absent
8	1256	Absent

Laboratory identification code:	17

Cut-off at 15 copies :	35.24 cycles
Copy number at the cut-off	10.10 copies

Sample type	Sample N°	Ruminant DNA
5	610	Absent
6	792	Present
7	1714	Present
10	680	Absent
8	1116	Absent
9	1678	Present
8	1756	Absent
9	1758	Present

Laboratory identification code:	18

Cut-off at 15 copies :	36.57 cycles
Copy number at the cut-off	11.03 copies

Sample type	Sample N°	Ruminant DNA
5	170	Absent
6	672	Present
7	1914	Present
10	420	Absent
9	618	Present
9	1858	Present
8	1976	Absent
8	1996	Absent

Laboratory identification code:	19

Cut-off at 15 copies :	35.34 cycles
Copy number at the cut-off	9.58 copies

Sample type	Sample N°	Ruminant DNA
5	230	Absent
6	692	Present
7	1174	Present
10	500	Absent
9	878	Present
8	956	Absent
8	1576	Absent
9	1778	Present

Laboratory identification code:	20

Cut-off at 15 copies :	36.98 cycles
Copy number at the cut-off	9.27 copies

Sample type	Sample N°	Ruminant DNA
5	950	Absent
6	1072	Present
7	1874	Present
9	678	Present
8	796	Absent
10	1300	Absent
8	1496	Absent
9	1818	Present

Laboratory identification code:	21

Cut-off at 15 copies :	38.50 cycles
Copy number at the cut-off	12.60 copies

Sample type	Sample N°	Ruminant DNA
5	952	Present
6	1294	Present
7	1650	Present
9	696	Absent
8	1178	Present
10	1516	Absent
8	1698	Present
9	1860	Absent

Laboratory identification code:	22

Cut-off at 15 copies :	37.28 cycles
Copy number at the cut-off	9.05 copies

Sample type	Sample N°	Ruminant DNA
7	134	Present
5	430	Absent
6	1732	Present
8	96	Absent
9	398	Present
9	578	Present
8	1456	Absent
10	1580	Absent

Laboratory identification code:	23

Cut-off at 15 copies :	36.33 cycles
Copy number at the cut-off	10.13 copies

Sample type	Sample N°	Ruminant DNA
6	552	Present
7	734	Present
5	1410	Absent
8	16	Absent
9	278	Present
8	336	Absent
10	2040	Absent
8	2058	Present

Laboratory identification code:	24

Cut-off at 15 copies :	36.33 cycles
Copy number at the cut-off	10.13 copies

Sample type	Sample N°	Ruminant DNA
7	934	Present
6	1172	Present
5	1470	Absent
8	156	Absent
9	378	Present
10	1360	Absent
8	1676	Absent
9	2038	Present

Laboratory identification code:	25

Cut-off at 15 copies :	34.24 cycles
Copy number at the cut-off	11.08 copies

Sample type	Sample N°	Ruminant DNA
7	354	Present
5	1050	Absent
6	1392	Present
9	418	Present
9	738	Present
8	936	Absent
10	1760	Absent
8	2076	Absent

Laboratory identification code:	27

Cut-off at 15 copies :	35.83 cycles
Copy number at the cut-off	10.62 copies

Sample type	Sample N°	Ruminant DNA
6	1212	Present
7	1494	Present
5	1850	Absent
9	258	Present
8	396	Absent
10	640	Present
8	1476	Absent
9	1878	Present

Laboratory identification code:	30

Cut-off at 15 copies :	cycles
Copy number at the cut-off	copies

Sample type	Sample N°	Ruminant DNA
6	352	Present
7	394	Present
5	1810	Absent
8	416	Absent
9	778	Present
10	840	Absent
8	876	Absent
9	1298	Absent

Laboratory identification code:	31

Cut-off at 15 copies :	35.62 cycles
Copy number at the cut-off	12.17 copies

Sample type	Sample N°	Ruminant DNA
6	1132	Present
5	1350	Absent
7	1834	Present
8	276	Absent
10	1140	Absent
8	1156	Absent
9	1338	Present
9	1418	Present