Combined microscopy-PCR EURL-AP Proficiency Test 2020

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 to Commission Regulation EU/51/2013.

The total number of participating laboratories was 35 (26 NRLs and 9 labs outside the NRL network). On the exception of one non-EU participant, all laboratories delivered results. The study was based on a set of six samples (to be analysed both by light microscopy and PCR) consisting of blank feed matrices or feed fortified with processed animal proteins from terrestrial vertebrates and/or from fish.

Regarding the detection of PAPs by light microscopy the overall results indicated an excellent and satisfactory level of global performance for 100 % of the NRLs; no underperformance was notified. The composition of the sample set allowed pointing a sensitivity issue for terrestrial vertebrate remains in an aquafeed adulterated at 0.1 % with a pig PAP. For this sample, the positive detection of terrestrial vertebrates accounted for only 54 % of the NRL participants. This problem was not found within the non-EU participants for whom specificity issues were the major problem.

Concerning the PCR results, 77 % of the NRLs (20 out of 26) performed excellently. Two NRLs (8 %) returned satisfying results and 4 laboratories (15 %) were considered as underperforming. For two of these 4 laboratories, the underperformances were due to a misunderstanding of the instructions; respectively 3 and 5 samples were not analysed by PCR. The two other underperforming labs had problems of contaminations leading to multiple false positive results.

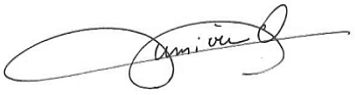
Keywords :

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 10th March 2021. After reception of the comments on the 26th March 2021, it was amended accordingly and approved by the signature of the organisers.

ISO 17043 coordinators signature for approval:

Olivier Fumière *Pascal Veys*



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 15th March 2017, the European Parliament and the Council adopted Regulation EU/625/2017 [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, www.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 has been 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]. Since 2016, the proficiency tests conducted by the EURL-AP are organised under the ISO17043 standard.

The present study report this part of the 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 have to be performed by light microscopy and/or PCR since June 2013. Standard Operating Procedures (SOP) are supporting the implementation of the two methods.

The objective of the present proficiency test was strictly to evaluate within the network of 26 NRLs the analytical performance to detect processed animal proteins (PAPs) in feed by light microscopy and PCR. Participation of the NRLs is mandatory.

In addition and 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/152/2009 and related SOPs was not imposed to them.

3. Material and methods

3.1. Study organisation

Twenty six NRLs and nine laboratories outside this EU network participated to the study. A detailed list of the 35 participating labs is included in Annex 1.

Official announcement (Annex 2) of the study was made on the 11th September 2020 to all invited participants.

On the 9th October 2020, the sample sets were shipped to the participants. On the same day the Excel report forms containing the instructions (Annex 4) were communicated to all participants – downloadable from the EURL-AP intranet for the NRLs or sent by email to the non-EU participants who have no access to this intranet.

The deadline for the delivery of the results was fixed in the announcement and in the instructions at the 6th November 2020. However on the 28th October 2020 participants were also informed on an extra delay of the timing initially planned for the study (Annex 3). This delay as requested by some participants was mainly linked to the Covid-19 pandemic. The new deadline for results delivery was fixed at the 16th November 2020.

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 4). 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. Results were taken into consideration only when both the Excel file and a copy of the summarized results sheet were received by the EURL-AP.
- Participants were notified that results arriving later would not be accepted.

On the exception of one participant outside the EU, which did not deliver its results, all results were delivered on time to the organiser.

Twenty nine participants returned results for both microscopic and PCR analyses. The proficiencies of NRLs and other participants were evaluated separately in this report.

3.2. Material

3.2.1. Description of the samples

Six different test materials were prepared for the study. The composition of the sample set was established taking into account the following considerations:

- Use of feed matrices intended to different farmed animals ;
- Use of aquafeeds as matrices for assessing the detection capabilities of terrestrial PAPs because since the 1st June 2013 non-ruminant PAPs are authorized in aquafeeds according to Commission Regulation EU/56/2013 [5] ;
- Use of ruminant and non-ruminant PAPs as well as authorised animal ingredients (e.g. milk powder).

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

Table 1: Composition of the sample set

Sample	Material	Nr of replicates	Expected results * Microscopy: Terrestrial particles	Expected results * Microscopy: Fish particles	Expected results * PCR: Ruminant DNA
1	Poultry feed	1	-	-	-
2	Poultry feed + 0.5 % poultry PAP	2	+	-	-
3	Poultry feed + 0.5 % poultry PAP + 0.5 % milk powder	2	+	-	+
4	Aquafeed	1	-	+	-
5	Aquafeed + 0.1 % ruminant PAP	1	+	+	+
6	Aquafeed + 0.1 % pig PAP	1	+	+	-
Total		**8**	**6**	**3**	**3**

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

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.

3.2.2. Materials used in the preparation of the samples

Two commercial matrices were used:

- **Poultry feed** was a complete feed for chicken made of maize, wheat, soybean defatted cake, bran flour, calcium carbonate, soya oil, premix, salts, lysine, methionine and essential oils. This feed was analytically free of any terrestrial PAP (Table 2). Its sediment was of 2.55 %. It was used for preparing sample 1, 2 and 3.
- **Aquafeed** was a complete feed for trout. It was composed of decorticated bean, fish meal, rapeseed oil, maize gluten, expeller soybean meal, soya protein concentrate, fish oil, wheat gluten, lysine, methionine, premix, vitamins, minerals and dicalcium phosphate. Its sediment content was of 1.18%. This feed used for preparing sample 4, 5 and 6 was free of terrestrial PAP (Table 2).

Adulterant material used:

- A **pure poultry PAP** was used for preparing sample 2 and 3. This PAP was used in implementation test for the detection of poultry. This PAP was processed according to method 7. In this case, the PAP was heated at a temperature of minimum 90°C during 30 minutes on the cooking side. With the drying treatment, the material was heated at approximately 95°C during 60 minutes. Its sediment was of 43 %. PCR analyses revealed it from poultry origin and free from ruminant and porcine DNA.
- A **pure ruminant PAP** was used for preparing sample 5. This PAP presenting a high bone content of 60.7 % was used in previous proficiency test 2017, 2018 and 2019 [6, 7, 8]. Its purity was controlled by PCR.
- A **skimmed milk powder** was used for sample 3. PCR analyses proved detection of ruminant DNA.
- A **pure porcine PAP** was used for preparing sample 6. This PAP was used in previous proficiency test of 2015 [9], 2016 [10], 2017 [6], 2018 [7], 2019 [8]. Its bone content was of about 14 % and its purity was checked by microscopy and PCR.

3.2.3. Description of the mixing procedures and pelleting

To avoid presence of interfering material, a cleaning of the rooms where the samples were handled was performed prior to sample preparation, mixing of the materials and filling of the vials.

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.

Blank matrix was conditioned first in order to avoid contamination.

Adulteration of samples 2 and 3 was performed by successive dilutions.

Samples 5 and 6 were directly spiked with the adulterant.

3.3. Qualitative analysis

Analyses of qualitative proficiency testing were applied following ISO 13528 [11].

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 microscopically detectable animal material)
- Negative (= absence of any microscopically detectable animal material)
- Below LOD (= low level presence of microscopically detectable animal material 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, in the sense that both Ct values should be close enough to each other and on the same side compared to the cut-off value. 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 [11].

This year, since all samples had to be analysed by both light microscopy and PCR, no assessment of the correct implementation of the legislation (i.e. choice of method to apply in accordance with the SOP on operational protocol) was realised. The only evaluation done was on the reported analytical results.

3.4.1. Light microscopy

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

Sample 1 is to be declared negative for both terrestrial and fish material presence.

Samples 2 and 3 are to be declared positive for terrestrial material presence and negative for fish material presence.

Sample 4 is to be declared negative for terrestrial material presence and positive for fish material presence.

Samples 5 and 6 are to be declared positive for both terrestrial and fish material presence.

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 with no ND for terrestrial material.
- **Satisfying** level of global performance = consolidated AC superior or equal to 0.90 with one ND for terrestrial material OR a consolidated AC superior to 0.85 with no ND for terrestrial material.
- **Underperforming** level of global performance = consolidated AC equal or inferior to 0.85.

3.4.2. PCR

As for light microscopy, the expected results are indicated in Table 1.

Samples 1, 2, 4 and 6 are considered to be declared negative for the presence of ruminant DNA.

Samples 3 and 5 are considered to be declared positive for the presence of ruminant DNA. Sample 3 contains 0.5 % of milk powder. Sample 5 was adulterated with 0.1 % of ruminant PAP.

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 1 wrong result for the detection of ruminant DNA.
- **Underperforming** level of global performance = 2 wrong results or more 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				PCR			
		Nr of replicates	Terrestrial	Fish	Nr of replicates	Ruminant	Porcine	Poultry	Fish
1	Poultry feed	10	-	-	10	-	ND	ND	ND
2	Poultry feed + 0.5 % poultry PAP	10	+	-	10	-	ND	+	ND
3	Poultry feed + 0.5 % poultry PAP + 0.5 % milk powder	10	+	-	10	+	ND	+	ND
4	Aquafeed	10	-	+	10	-	ND	ND	+
5	Aquafeed + 0.1 % ruminant PAP	10	+	+	10	+	ND	ND	+
6	Aquafeed + 0.1 % pig PAP	10	+	+	10	-	+*	ND	+

(Legend: ND = not tested, + = systematically detected, - = systematically not detected)
*** not systematically detected – explanations in the text on sample 6**

The homogeneity was studied by light microscopy on 10 g of sample material for 10 replicates. Analyses of replicates were performed following strictly EC/152/2009 regulation. For PCR analysis, a DNA extraction was performed on 2 test portions of 100 mg of sample material for each of the 10 replicates.

Sample 1 (poultry feed) was microscopically free from any trace of animal origin. The PCR analyses confirmed the absence of ruminant DNA.

Sample 2 (poultry feed + 0.5 % poultry PAP) showed systematically the presence of terrestrial bones (on each slide more than 5 bones). On the exception of one test portion from one replicate giving a late signal before the cut-off value, all test portions tested gave negative results for the presence of ruminant DNA. A second extraction of two test portions from the same replicate was performed and the PCR analyses confirmed the absence of ruminant DNA. PCR analyses showed the sample positive for poultry DNA.

Sample 3 (poultry feed + 0.5 % poultry PAP + 0.5 % milk powder) showed systematically the presence of terrestrial bones (on the exception of 3 slides, the number of bones per slides was superior to 5). This sample was positive for the presence of ruminant and poultry DNA.

Sample 4 (aquafeed) showed systematically the presence of fish particles such as fishbones, placoid scales, otoliths, gill fragments and scales. No particle that could be interpreted as from terrestrial animal origin was observed. PCR analyses revealed the sample as negative for ruminant DNA and positive for fish DNA.

Sample 5 (aquafeed + 0.1 % ruminant PAP) was systematically positive for the presence of both fish and terrestrial animal particles. The bone numbers of each type, fish and terrestrial, were all superior to 5 for each slide. Ruminant and fish DNA were systematically detected using PCR.

Sample 6 (aquafeed + 0.1 % pig PAP) was positive for both terrestrial particles and fish fragments. 80% of the slides presented both terrestrial bones and fishbones. In all cases, the number of terrestrial bones observed for each replicate allowed to declare it as positive for this parameter. PCR analyses revealed the sample as negative for ruminant DNA and positive for fish DNA. Porcine DNA was not detected systematically. Among the 10 replicates analysed twice, 4 replicates were positive (both test portions are positive), 4 were ambiguous (1 positive test portion on two) and 2 were negative. For the test portions giving a negative result (8), the Ct values were systematically close but after the cut-off except for one test

portion with no signal at all. However the detection of porcine DNA was not evaluated in the framework of this proficiency test, therefore the samples were not discarded.

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

3.6. Stability of the samples

Internal stability studies performed on similar samples from past studies have demonstrated that such samples were stable over time (years) for both light microscopic and PCR analyses. There are no reasonable elements which would indicate that present samples should be unstable.

4. Results

Gross results for microscopy and PCR from all participants are to be found in Annexes 5 and 6 respectively.

4.1. Microscopy results

4.1.1. Qualitative analyses from the NRLs

4.1.1.1. Results and performance of the network

Table 3 summarizes the results reported by the 26 NRLs for the sample types submitted to microscopic analysis.

The overall results, expressed in terms of global accuracy (AC) reveal the high quality of the NRL network for the detection of PAPs. The percentage of total error only accounted for 8% of the total responses.

Table 3: Global results expressed as accuracy (AC) – light microscopy

Sample	Material	n	AC	
			Terrestrial	Fish
1	Poultry feed	26	1.000	1.000
2	Poultry feed + 0.5 % poultry PAP	52	1.000	0.981 (1)
3	Poultry feed + 0.5 % poultry PAP + 0.5 % milk powder	52	1.000	1.000
4	Aquafeed	26	0.885 (3)	1.000
5	Aquafeed + 0.1 % ruminant PAP	26	1.000	1.000
6	Aquafeed + 0.1 % pig PAP	26	0.538 (12)	1.000

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

Some specificity issues are observed: one case of false positive result for fish in sample 2 and three cases of false positive results for terrestrial animal in sample 4, an aquafeed.

The major source of error for the present study is found in sample 6, the aquafeed adulterated at 0.1% with a pig PAP. This sample is peculiarly subject to sensitivity issues for terrestrial animal findings ; its rate of false negatives results reaches 46%.

The origin of this sensitivity issue is probably due to the low bone content of the pig PAP used, only a few 14%. This PAP, commercially referred as a porcine protein, was nevertheless already used over several past studies: in 2015 [9], 2016 [10], 2017 [6], 2018 [7] and 2019 [8]. Over this last 5 years period, it had to be detected by light microscopy at three occasions with sensitivity score ranging from 100% (in 2016), 92% (in 2017) and 81% (in 2018). The unusual low level of correct answers will be discussed in the conclusion.

4.1.1.2. Detailed review of results per sample

Sample 1 : poultry feed

No error occurred.

Sample 2: poultry feed + 0.5 % poultry PAP

PD for fish particles :

- Lab 4 reported the presence of fish bones in one of the replicates but not in the other one.

Among the correct results for terrestrial animal particles, some avian structures were identified and reported :

- Lab 23 reported the finding of feathers in one of the replicates but not in the other one,
- Lab 6 reported the presence of feathers in both replicates.

Sample 3: poultry feed + 0.5 % poultry PAP + 0.5 % milk powder

No error occurred.

From the reported results some avian structures were identified :

- Lab 6 reported the presence of feathers in only one of the two replicates.

Sample 4: aquafeed

PD for terrestrial particles :

- Labs 1 and 3 reported terrestrial bones and cartilage,
- Lab 9 reported terrestrial bones only.

Lab 4 reported the sample as < LOD after the finding of one terrestrial bone (on two determinations).

Among the comments received on this sample, lab 19 reported some few atypical fishbones described as possibly originating from salmon.

Sample 5: aquafeed + 0.1 % ruminant PAP

No error occurred.

Sample 6: aquafeed + 0.1 % pig PAP

ND for terrestrial particles :

- Labs 3, 6, 12, 13, 16, 20, 21, 23, 25 and 26 failed at detecting any terrestrial particles

Two <LOD cases were reported for terrestrial material presence by labs 4 and 14 :

- Lab 4 reported 4 bones but declared to have found blood (on two determinations),
- Lab 14 found 1 terrestrial bone for the first determination and 3 terrestrial bones for the second determination.

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 of next page. 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

Terrestrial			
lab code	AC	SE	SP
2, 5, 7, 8, 10, 11, 15, 17, 18, 19, 22 and 24	1.000	1.000	1.000
1 and 9	0.875	1.000	0.500
4, 6, 12, 13, 14, 16, 20, 21, 23, 25 and 26	0.875	0.833	1.000
3	0.750	0.833	0.500

Fish			
lab code	AC	SE	SP
1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26	1.000	1.000	1.000
4	0.875	1.000	0.800

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

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. Lines in black refer to excellent results and lines in blue to satisfying results

Consolidated			
lab code	AC	SE	SP
2, 5, 7, 8, 10, 11, 15, 17, 18, 19, 22 and 24	1.000	1.000	1.000
1 and 9	0.938	1.000	0.857
6, 12, 13, 14, 16, 20, 21, 23, 25 and 26	0.938	0.889	1.000
3 and 4	0.875	0.889	0.857

Fourteen labs out of 26 NRLs (54 %) performed very well.

Twelve NRLs performed satisfyingly (46 %) and there was no situation of underperformance.

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

Individual performances from the 8 participants outside the EU were assessed exactly as in the 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 (next page).

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: na, not applicable)

Terrestrial			
lab code	**AC**	**SE**	**SP**
31, 35 and 37	1.000	1.000	1.000
29, 33, 36 and 38	0.875	1.000	0.500
32	0.625	0.500	1.000

Fish			
lab code	**AC**	**SE**	**SP**
29, 31, 35, 36 and 37	1.000	1.000	1.000
38	0.750	1.000	0.600
32	0.500	1.000	0.200
33	na	na	na

The error details are described per sample:

Sample 1 : poultry feed

No error occurred.

One participant, lab 38, reported the sample as < LOD for fish based on the finding of less than 5 particles of fishbones.

Sample 2: poultry feed + 0.5 % poultry PAP

ND for terrestrial particles :

- Lab 32 failed at detecting terrestrial particles in one of the replicates but not in the other one.

PD for fish particles :

- Lab 32 reported structures misidentified as fishbones, scales, gills and thorns in both replicates,
- Lab 38 reported less than 10 fishbones in one of the replicates, while in the other replicate less than 5 particles from fish origin were reported and therefore reported as < LOD.

Another case of < LOD was reported by lab 37 for one of the replicates.

Sample 3: poultry feed + 0.5 % poultry PAP + 0.5 % milk powder

PD for fish particles :

- Lab 32 reported structures misidentified as fishbones, scales, gills in both replicates,
- Lab 38 reported less than 10 fishbones in one of the replicates, while in the other replicate less than 5 particles from fish origin were reported and therefore reported as < LOD.

Sample 4: aquafeed

PD for terrestrial particles :

- Labs 29, 36 and 38 reported terrestrial bones,
- Lab 33 declared the sample as positive for terrestrial particles presence without details.

Sample 5: aquafeed + 0.1 % ruminant PAP

ND for terrestrial particles :

- Lab 32 failed at identifying terrestrial structures.

Sample 6: aquafeed + 0.1 % pig PAP

ND for terrestrial particles :

- Lab 32 failed at identifying terrestrial structures.

A general ranking as for the NRL network was established (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. Lines in black refer to excellent results, lines in blue refer to satisfying results and lines in red refer to underperforming results

Consolidated			
lab code	**AC**	**SE**	**SP**
31, 35 and 37	1.000	1.000	1.000
29 and 36	0.938	1.000	0.857
33	0.875	1.000	0.500
38	0.813	1.000	0.571
32	0.563	0.667	0.429

Five participants performed excellently, one performed satisfyingly (line in blue in Table 9). Two other participants were classified as underperforming (lines 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. On the respect of the instructions

The NRLs seem to stick generally to the SOPs. No deviation is to be noticed.

4.2.1.2. Overview of results and global performance of the network

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

Table 10: Global results expressed as accuracy (AC) – PCR

Sample	Material	n	AC
1	Poultry feed	26	0.88 (3)
2	Poultry feed + 0.5 % poultry PAP	52	0.87 (7)
3	Poultry feed + 0.5 % poultry PAP + 0.5 % skimmed milk powder	52	0.96 (2)
4	Aquafeed	26	0.92 (2)
5	Aquafeed + 0.1 % ruminant PAP	26	1.00
6	Aquafeed + 0.1 % pig PAP	26	0.88 (3)

Accuracy means sensitivity in case of ND and specificity in case of PD.
The absence of a PCR result is considered as a deviation (ND or PD).
In brackets the absolute number of false results. (Legend: n = number of results)

On the overall, 17 deviations (11% of the 156 results) were recorded. Among the 15 positive deviations, 8 (53 %) are due to the absence of some results (Labs 21 and 23) and 7 (47%) came from 2 underperforming labs (Labs 2 and 8) having problems of cross-contaminations. There were 2 negative deviations due to missing results observed with sample 3 (Lab 21) but for the rest the presence of ruminant DNA was systematically detected. Only 2 positive deviations observed with sample 2 are difficult to explain as they come from two labs showing no other deviation (Labs 3 and 10).

Sample 1 was a poultry feed. The PCR result expected for the presence of ruminant DNA was negative. Two of the three positive deviations were due to missing results (Labs 21 and 23) and the last one can be attributed to a contamination (Lab 8).

Sample 2 was the same poultry feed adulterated with 0.5 % of poultry PAP. This sample concentrates almost half (41 %) of the deviations but 5 out of the 7 deviations are due to missing results (Lab 21) or probable contaminations (Labs 2 and 8).

Sample 3 was very comparable to sample 2 but contained milk powder. The 2 negative deviations recorded were due to missing results (Lab 21).

Sample 4 was an aquafeed without any trace of ruminant DNA. One of the 2 positive deviations was due to a missing result (Lab 23). The other deviation was obtained by a lab having cross-contamination problems (Lab 2).

Sample 5 was the same aquafeed as sample 4 but containing 0.1 % in mass fraction of ruminant PAP. No negative deviation was recorded for this sample.

Sample 6 was the same aquafeed as sample 4 in which 0.1 % in mass fraction of pig PAP was added. Three positive deviations were recorded: one is a missing result (Lab 23) and the 2 others were reported by the 2 labs having cross-contaminations (Labs 2 and 8).

4.2.1.3. Individual performances of NRLs in qualitative analysis

Individual performances were assessed for each participant by calculating the accuracy, sensitivity and specificity over the 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.

Table 11: NRL proficiencies regarding the detection of ruminant material. Ranking follows AC values. Cells in black refers to excellent NRLs. Cells in blue refers to satisfying NRLs. Cells in red refer to underperforming NRLs

Lab code	AC	SE	SP
1	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
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
22	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
3	0.875	1.000	0.800
10	0.875	1.000	0.800
23*	0.625	1.000	0.400
8	0.571	1.000	0.250
2	0.500	1.000	0.200
21*	0.375	0.333	0.400

* Absence of a PCR result is assimilated to a deviation

Excellent performances were recorded for 20 labs out of 26 NRLs (77 % of the NRLs) having no false result. Two labs were satisfying: Labs 3 and 10 reported a PD for sample 2. Four labs were underperforming: Labs 2 and 8 reported 4 and 3 PD respectively. Labs 21 and 23 did not reported 5 and 3 PCR results respectively.

4.2.1.4. 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.

One participant (Lab 9) did not reach the minimum criterion of 9.00 copies. Its cut-off is at 8.77 copies but this had no impact on the results. The percentage of the labs with a cut-off corresponding to a number of copies > 10 for this proficiency test was 57.7 %. It is similar as for the PT 2019 (56.0 %) [8] but lower than the 2 previous years (65.4 % in 2018 [10]; 64.0 % in 2017 [9]).

4.2.2. Qualitative analyses from the non-EU participants

4.2.2.1. Individual performances

Individual performances were assessed for three non-EU participants who reported PCR results by calculating the accuracy, sensitivity and specificity over the samples. Their results are to be found in Table 12.

Table 12 : Non-EU participant proficiencies regarding the detection of ruminant material. Ranking follows AC values. Cells in black refers to excellent labs. Cells in blue refers to satisfying labs.

Lab code	AC	SE	SP
29	1.000	1.000	1.000
31	1.000	1.000	1.000
37	0.875	0.727	0.384

Labs 29 and 31 obtained excellent results (no deviation).

Concerning Lab 37, only one negative deviation was recorded with sample 5, the aquafeed containing 0.1 % of a ruminant PAP.

4.2.2.2. Assessment of the cut-off values

Labs 29 and 31 have cut-off values that comply with the minimum criterion of 9 copies set by the EURL-AP.

No statement can be made for Lab 37 as they did not communicate this kind of result.

5. Discussion and conclusions

This combined proficiency test involving both the detection of animal traces by light microscopy and PCR delivered good expected scores.

Concerning the microscopic results, the number of excellent and satisfactory scores obtained within the network of NRLs reached respectively 54 % and 46 %. There were no underperforming NRL for the present study.

On the exception of some few specificity issues resulting in false positive findings of terrestrial animal particles in a fishfeed matrix which is a usual background level, the major problem encountered is related to a lack of sensitivity for the detection of terrestrial material in the fishfeed matrix adulterated at 0.1 % of porcine PAP. The performance of the NRL network for this sample barely scored 54 %. Although the bone content of this PAP is rather low compared to other terrestrial vertebrates PAP, this PAP was used in previous proficiency tests [6, 7, 10] as an adulterant of fishfeeds without, so far, any noticeable problem (Table 13).

Table 13 : Overview of sensitivity scores of the same porcine PAP used in fishfeeds from past EURL-AP proficiency tests

Year	Sample	Sensitivity score
2016	Fishfeed + 1 % porcine PAP	1.000
2017	Fishfeed + 0.1 % porcine PAP	0.920
2018	Fishfeed + 0.05 % porcine PAP	0.808

The level of adulteration used this year with this porcine PAP was the same as in the study of 2017 but the sensitivity score by the NRL network was the worst ever. A reason possibly explaining this situation might be linked to a matrix effect of the fishfeed composition. Nevertheless after investigations no elements could be identified to support this assumption, furthermore the score obtained by non-EU participants on this sample (0.875 or one ND on eight results) indicates that this sample was not an issue for them. Even more, all non-EU participants disclosed the presence of terrestrial vertebrates remains based on one determination only. The weaker performance of the NRL network on this sample lacks sound explanation. More precisely the reason may be a combination of several associated parameters : the low bone content of the PAP used associated to a masking effect of the matrix which had to be ground prior to observation, and finally maybe observations performed after staining by Alizarin red or on unstained sediment. However the information delivered by the participants does not allow more detailed investigation on it. Therefore NRLs having failed at disclosing the terrestrial vertebrates presence in this sample are invited to investigate on what went wrong with this sample and to come back to the organiser with their results of a new determination.

However a positive point is that such situation should be less frequent in the future. Effectively it has been discerned that all lack of sensitivity for this sample resulted from a single determination ; with the modifications brought into Annex VI of EC/152/2009 by Commission Implementation Regulation EU/1560/2020 [12] the new observation flowcharts from now on impose, in absence of declaration (the present case), to perform two determinations. This will undoubtedly improve the detection capabilities of the laboratories and prevent false negative results.

Concerning non-EU participants, they encountered problems which were different from the ones of the NRL network. They are mainly linked to specificity issues for fish in the poultry feed matrix as well for terrestrial vertebrates in unadulterated aquafeed. For these participants the percentage of perfect and satisfying results reached 75 % while 25% were, according to the applied performance criteria, categorised as underperforming.

For the PCR results, 15 NRLs out of 25 (60%) performed excellently and 6 NRLs (24%) were satisfying. Four NRLs (16%) were considered as underperforming. These results are comparable to last year. Whereas a lack of sensitivity of the light microscopy leading to stop the investigations was a major reason of the deviations observed in 2019, the two main reasons are this year problems of cross-contaminations for 2 labs and a misunderstanding of the instructions for 2 other labs. The analysis of all the samples by

both methods was clearly mentioned in the announcement letter and in the “Instructions” sheet of the reporting Excel file. Even if the general guidelines are almost the same from year to year, a careful reading of the instructions remains important and mandatory.

Among the 9 participants outside the NRL network, only 3 reported PCR results. They are all excellent or satisfactory.

Acknowledgment

We are grateful to the EURL-AP technical staff for their preparation work and the efforts made to meet the ISO 17043 requirements: A. Cordonnier, L. Plasman and J. Maljean. We also thank the participants for their fruitful collaboration.

References

[1] EU. 2017. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation). Official Journal of the European Union L 95, 7/4/2017: 1-142.

[2] EU. 2011. 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] Veys P, Fumière O, Marien A, Baeten V and Berben G. 2018. Combined microscopy-PCR EURL-AP Proficiency Test 2017: Final version. CRA-W, Gembloux, Belgium.

[7] Fumière O, Veys P, Marien A, Baeten V and Berben G. 2019. Combined microscopy-PCR EURL-AP Proficiency Test 2018: Final version. CRA-W, Gembloux, Belgium

[8] Veys P, Fumière O and Marien A. 2020. Combined microscopy-PCR EURL-AP Proficiency Test 2019: Final version – version 02. CRA-W, Gembloux, Belgium

[9] Veys P, Fumière O, Marien A, Baeten V and Berben G. 2016. Combined microscopy-PCR EURL-AP Proficiency Test 2015: Final version. CRA-W, Gembloux, Belgium.

[10] Fumière O, Veys P, Marien A, Baeten V and Berben G. 2017. Combined microscopy-PCR EURL-AP Proficiency Test 2016: Final version. CRA-W, Gembloux, Belgium.

[11] ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparison.

[12] EU. 2020. Commission Implementing Regulation (EU) 2020/1560 of 26 October 2020 amending Annex VI to Regulation (EC) No 152/2009 laying down 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 357, 27/10/2020: 17–23.

Annex 1

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

Country	Institute Name
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
Botswana	*Botswana National Veterinary Laboratory*
China	*China Agricultural University*
Croatia	Croatian Veterinary Institute
Cyprus	Cyprus Veterinary Services
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	*FAMIC*
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	Wageningen Food Safety Research
Norway	*LabNett AS and Institute of Marine Research*
Peru	*Inspectorate Services PerúRÚ SAC*
Poland	National Veterinary Research Institute
Portugal	Instituto Nacional de Investigacao Agraria e Veterinaria
Romania	Hygiene Institute of Veterinary Health
Serbia	*Institute of Veterinary Medicine of Serbia*
Slovakia	State Veterinary and Food Institute
Slovenia	Veterinary faculty - National Veterinary Institute - Institute of Food Safety, Feed and Environment - Department of Environment, Animal Nutrition, Welfare and Hygiene
South Africa	*Stellenbosch University, Department of Animal Sciences*
Spain	Laboratorio Arbitral Agroalimentario
Sweden	National Veterinary Institute, Department of Animal Feed
Thailand	*Bureau of Quality Control of Livestock Products*
United Kingdom	Animal and Plant Health Agency

Annex 2

Announcement letter

European Union Reference Laboratory for Animal Proteins in feedingstuffs

Walloon Agricultural Research Centre, Knowledge and Valorisation of Agricultural Products Department

Henseval building
Chaussée de Namur 24, B – 5030 GEMBLOUX
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Announcement of the EURL-AP proficiency test 2020/01 for the determination of Processed Animal Proteins (PAPs) in feed

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.

Objectives

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 (excluding the SOP on operational protocols for the combination of methods due to ongoing revision work).

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 a maximum of 8 samples, each of about 50g. Each sample shall be analysed by microscopy and PCR.

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 (excluding the SOP on operational protocols for the combination of methods). These methods 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.
- 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 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

Walloon Agricultural Research Centre, Knowledge and Valorisation of Agricultural Products Department

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Time schedule

- Official announcement of the study to the NRLs by way of the intranet and e-mail: 11 September 2020
- Sending of the sample boxes and communication of the instructions: 9 October 2020

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

- Deadline for returning of results to the organizer: 6 November 2020

Further information

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

or

- Dr Pascal VEYS
  EURL-AP Deputy Director
  ☎32 (0) 81 87 52 28
  32 (0) 81 87 40 19
  E-mail: p.veys@cra.wallonie.be

or

- Dr Olivier FUMIERE
  EURL-AP Director
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Annex 3

Change of agenda

European Union Reference Laboratory for Animal Proteins in feedingstuffs

Walloon Agricultural Research Centre, Knowledge and Valorisation of Agricultural Products Department

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Change of timing for the EURL-AP proficiency test 2020/01 for the determination of Processed Animal Proteins (PAPs) in feed

NEW DEADLINE for results delivery

Gembloux, 28th October 2020

Dear Participant,

Due to the exceptional situation of the second wave of COVID-19 spreading through the European Union and the linked uncertainty of delivering the results on due time expressed by some of you, we have decided to postpone the deadline for results delivery.

The new deadline for returning of results to the organizer is the 16th November 2020.

We hope that this new dates suits you.

Meanwhile stay safe.

Yours Truly.

Further information

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

  or

- Dr Pascal VEYS
  EURL-AP Deputy Director

  ☎32 (0) 81 87 52 28

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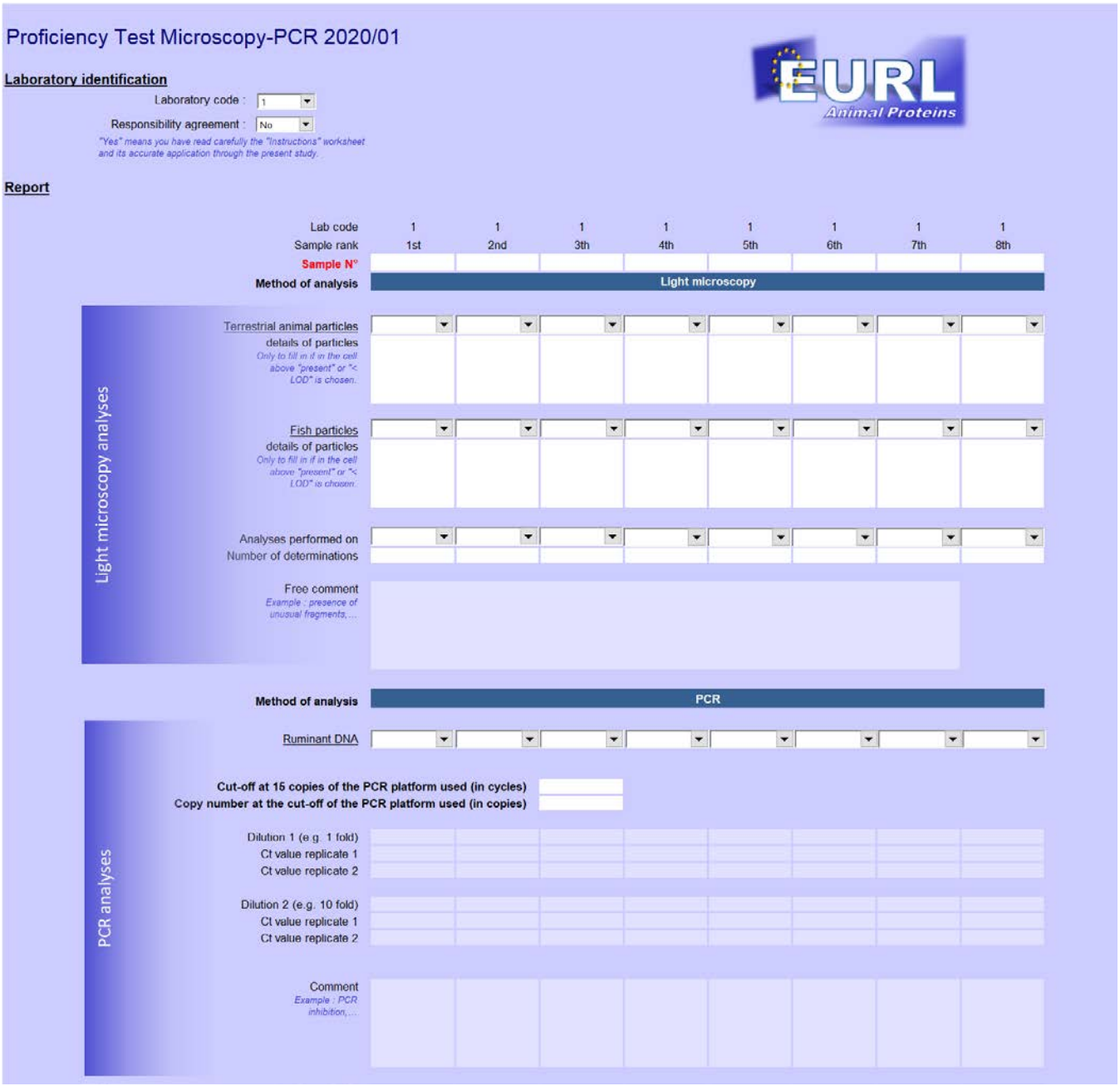
Annex 4

Excel result report form



Proficiency Test Microscopy-PCR 2020/01

EURL Animal Proteins

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	1	1	1	1
Sample rank	1st	2nd	3th	4th	5th	6th	7th	8th
Sample N°								
Method of analysis	Light microscopy							
Light microscopy analyses: Terrestrial animal particles								
details of particles (*Only to fill in if in the cell above "present" or "< LOD" is chosen.*)								
Fish particles								
details of particles (*Only to fill in if in the cell above "present" or "< LOD" is chosen.*)								
Analyses performed on								
Number of determinations								
Free comment (*Example : presence of unusual fragments,...*)								
Method of analysis	PCR							
PCR analyses: Ruminant DNA								
Cut-off at 15 copies of the PCR platform used (in cycles)								
Copy number at the cut-off of the PCR platform used (in copies)								
Dilution 1 (e.g. 1 fold)								
Ct value replicate 1								
Ct value replicate 2								
Dilution 2 (e.g. 10 fold)								
Ct value replicate 1								
Ct value replicate 2								
Comment (*Example : PCR inhibition,...*)								

Annex 5

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
5	234	Present	Bone, cartilage, muscle fibres.	Present	Bone, scale, gill, skin, cartilage, muscle fibres	Sed. + Flot.	1
2	246	Present	Bone	Absent		Sed. + Flot.	1
3	682	Present	Bone, cartilage, mucle fibres.	Absent		Sed. + Flot.	1
1	698	Absent		Absent		Sed. + Flot.	1
4	758	Present	Bone, muscle fibres, cartilage.	Present	Bone, skin, scale, gill, cartilage, muscle fibres.	Sed. + Flot.	1
3	994	Present	bone, muscle fibres.	Absent		Sed. + Flot.	1
6	1030	Present	Bone, cartilage, muscle fibres.	Present	Bone, scale, gill, cartilage, muscle fibres.	Sed. + Flot.	1
2	2118	Present	Bone, cartilage.	Absent		Sed. + Flot.	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
2	54	Present	bone fragments	Absent		Sed. + Flot.	1
5	666	Present	bone fragments	Present	bone, otolithe, scale and muscle fibers fragments	Sed. + Flot.	1
6	790	Present	bone fragments	Present	bone, otolithe, scale and muscle fibers fragments	Sed. + Flot.	1
4	806	Absent		Present	bone, otolithe, scale and muscle fibers fragments	Sed. + Flot.	1
1	866	Absent		Absent		Sed. + Flot.	1
3	922	Present	bone fragments	Absent		Sed. + Flot.	1
3	2194	Present	bone fragments	Absent		Sed. + Flot.	1
2	2262	Present	bone fragments, very few muscle fibers fragments	Absent		Sed. + Flot.	1

Free comment

Unusual is: the presence of numerous bone fragments and no muscle fibers in samples 54, 922 and 2194. In sample 2262 also, we found a lot of bone fragments and only 2 small fragments of muscle fibers.
We also noted presence of crystals in the flotate of samples 666, 790 and 806. We did not check further, but they could be lactose (or other sugar) crystals.

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	278	Present	Bone, cartilage	Present	Bone, cartliage, gills, tooth, otolith, muscle	Sed. + Flot.	1
2	366	Present	Bone, cartilage, muscle	Absent		Sed. + Flot.	1
5	402	Present	Bone, cartilage, insct particles	Present	Bone, cartliage, gills, tooth, otolith, muscle	Sed. + Flot.	1
6	430	Absent		Present	'Bone, cartliage, gills, tooth, otolith, muscle	Sed. + Flot.	1
1	1154	Absent		Absent		Sed. + Flot.	1
2	1878	Present	Bone, cartilage	Absent		Sed. + Flot.	1
3	2098	Present	Bone, cartilage	Absent		Sed. + Flot.	1
3	2602	Present	Bone, cartilage	Absent		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	414	Present	bones , muscles	Absent		Sed. + Flot.	2
5	474	Present	Bones , muscles	Present	Fish bones, scales,	Sed. + Flot.	2
3	634	Present	Bones, muscles, 5 particles of blood	Absent		Sed. + Flot.	2
6	766	< LOD	4 bones and we found	Present	fish bones, muscles,scales	Sed. + Flot.	2
1	1010	Absent		Absent		Sed. + Flot.	2
4	1310	< LOD	1 bone	Present	fish bones , scales and muscles	Sed. + Flot.	2
2	2142	Present	bones and muscle	Present	fish bones , muscles	Sed. + Flot.	
3	2626	Present	bones, muscles	Absent		Sed. + Flot.	

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
2	150	Present	bones, cartilage	Absent		Sed. + Flot.	1
2	198	Present	bones, cartilage	Absent		Sed. + Flot.	1
4	374	Absent		Present	bones, cartilage, muscles, scales, gill	Sed. + Flot.	1
6	694	Present	bones	Present	bones, cartilage, muscles, scales	Sed. + Flot.	1
5	786	Present	bones	Present	bones, cartilage, muscles, scales	Sed. + Flot.	1
1	842	Absent		Absent		Sed. + Flot.	1
3	1330	Present	bones, muscle	Absent		Sed. + Flot.	1
3	2002	Present	bones	Absent		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
4	86	Absent		Present	muscle fibers, fish bones, scales	Sed. + Flot.	1
2	222	Present	muscle	Absent		Sed. + Flot.	1
5	306	Present	bones	Present	muscle fibers, fish bones, scales	Sed. + Flot.	1
2	486	Present	muscle	Absent		Sed. + Flot.	1
1	674	Absent		Absent		Sed. + Flot.	1
6	910	Absent		Present	muscle fibers, fish bones, scales	Sed. + Flot.	1
3	2218	Present	muscle fibers,bones,	Absent		Sed. + Flot.	1
3	2506	Present	muscle fibers,bones,	Absent		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
2	78	Present	terestrial bones	Absent		Sed. + Flot.	1
6	502	Present	terestrial bones	Present	fish bones	Sed. + Flot.	1
3	850	Present	terestrial bones	Absent		Sed. + Flot.	1
5	858	Present	terestrial bones	Present	fish bones	Sed. + Flot.	1
3	1186	Present	terestrial bones	Absent		Sed. + Flot.	1
1	1250	Absent		Absent		Sed. + Flot.	1
4	1262	Absent		Present	fish bones	Sed. + Flot.	1
2	1302	Present	terestrial bones	Absent		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
2	294	Present	Bones	Absent		Sed. + Flot.	1
5	378	Present	Bones, cartiladges, muscle fibers	Present	Bones, scales, cartiladges, muscle fibers	Sed. + Flot.	1
6	574	Present	Bones, muscle fibers	Present	Bones, scales, muscle fibers	Sed. + Flot.	1
3	610	Present	Bones	Absent		Sed. + Flot.	1
1	1106	Absent		Absent		Sed. + Flot.	1
4	1286	Absent		Present	Bones, scales, cartiladges, gills, muscle fibers	Sed. + Flot.	1
3	1306	Present	Bones, muscle fibers	Absent		Sed. + Flot.	1
2	1686	Present	Bones	Absent		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
6	286	Present	Bones	Present	Fish bones, scales, gills	Sed. + Flot.	3
1	650	Absent		Absent		Sed. + Flot.	3
3	778	Present	Bones	Absent		Sed. + Flot.	3
5	834	Present	Bones	Present	Fish bones, scales	Sed. + Flot.	3
4	998	Present	Bones	Present	Fish bones, scales, gills	Sed. + Flot.	3
2	1134	Present	Bones	Absent		Sed. + Flot.	3
2	1638	Present	Bones	Absent		Sed. + Flot.	3
3	2530	Present	Bones	Absent		Sed. + Flot.	3

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
5	186	Present	bone fragments, muscle fibres	Present	fishbone fragments, scales, gills, muscle fibres	Sed. + Raw	1
4	662	Absent		Present	fishbone fragments, scales, gills, muscle fibres	Sed. + Raw	1
3	802	Present	bone fragments, muscle fibres	Absent		Sed. + Raw	1
6	958	Present	bone fragments, muscle fibres	Present	fishbone fragments, scales, gills, muscle fibres	Sed. + Raw	1
2	1278	Present	bone fragments, muscle fibres	Absent		Sed. + Raw	1
1	1298	Absent		Absent		Sed. + Raw	1
3	1354	Present	bone fragments, muscle fibres	Absent		Sed. + Raw	1
2	1614	Present	bone fragments	Absent		Sed. + Raw	1

Free comment

The attribution of muscle fibres to either terrestrial animals or to fish in samples 186 and 958 is unsecure.

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
5	354	Present	Bones	Present	Bones, gills	Sed. + Flot.	1
2	582	Present	Bones	Absent		Sed. + Flot.	1
6	598	Present	Bones	Present	Bones, gills, scales	Sed. + Flot.	1
4	974	Absent		Present	Bones, cartilage, gills	Sed. + Flot.	1
1	1130	Absent		Absent		Sed. + Flot.	1
3	1162	Present	Bones	Absent		Sed. + Flot.	1
2	1374	Present	Bones	Absent		Sed. + Flot.	1
3	2026	Present	Bones	Absent		Sed. + Flot.	1

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	6	Present	bones	Absent		Sed. + Flot.	1
1	578	Absent		Absent		Sed. + Flot.	1
4	614	Absent		Present	muscles, fishbones, cartilage, scales, gills, otoliths	Sed. + Flot.	1
3	658	Present	bones, muscles	Absent		Sed. + Flot.	1
6	934	Absent		Present	muscles, fishbones, scales, gills, teeth	Sed. + Flot.	1
5	1050	Present	bones, cartilage, muscles	Present	muscles, fishbones, cartilage, scales, gills, otoliths	Sed. + Flot.	1
3	1378	Present	bones	Absent		Sed. + Flot.	1
2	1422	Present	bones	Absent		Sed. + Flot.	1

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
3	442	Present	bone fragments, cartilage	Absent		Sed. + Flot.	1
4	566	Absent		Present	bone fragments, cartilage, muscle fibres	Sed. + Flot.	1
1	602	Absent		Absent		Sed. + Flot.	1
2	606	Present	bone fragments	Absent		Sed. + Flot.	1
6	886	Absent		Present	bone fragments, cartilage, muscle fibers	Sed. + Flot.	2
5	1002	Present	bone fragments, cartilage, muscle fibers	Present	bone fragments, cartilage, muscle fibres	Sed. + Flot.	1
3	1282	Present	bone fragments	Absent		Sed. + Flot.	1
2	2430	Present	bone fragments	Absent		Sed. + Flot.	1

Free comment

Muscle fibres detected in samples 1002 could be from terrestrial and/or fish origin.

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	38	Absent		Present	Muscle/Bone/Scale/Gill	Sed. + Raw	1
2	102	Present	Ter Bone	Absent		Sed. + Raw	1
1	794	Absent		Absent		Sed. + Raw	1
6	1102	< LOD	Ter Bone First Determination (1 bone).	Present	Bone/Scale/Gill/Otolith	Sed. + Raw	2
5	1146	Present	Ter Bone	Present	Bone/Scale/Gill/Otolith	Sed. + Raw	1
2	1326	Present	Ter Bone	Absent		Sed. + Raw	1
3	1882	Present	Ter Bone	Absent		Sed. + Raw	1
3	2362	Present	Ter Bone	Absent		Sed. + Raw	1

Free comment

It is assumed that the decision rule for PCR does not apply as there is no "N/A" in the drop down box. Due to this the lab tested all samples by PCR to satisfy "Absent/Present" drop down for PCR section. We believe this to be the intention from reading section 2.2 of the instructions.

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
1	50	Absent		Absent		Sed. + Flot.	3
3	490	Present	bones, muscles	Absent		Sed. + Flot.	3
2	510	Present	bones	Absent		Sed. + Flot.	3
5	522	Present	bones	Present	muscles, fishbones, fishscale	Sed. + Flot.	3
4	590	Absent		Present	muscles, fishbones, fishscale	Sed. + Flot.	3
6	1054	Present	bones	Present	muscles, fishbones, fishscale	Sed. + Flot.	3
3	1258	Present	bones, muscles	Absent		Sed. + Flot.	3
2	1446	Present	bones	Absent		Sed. + Flot.	3

Free comment

In sample No 50, in the sieved fraction I found 4 bones, 4 muscles and feathers.

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
5	90	Present	bones, cartilage	Present	bones, scales	Sed. + Flot.	1
4	110	Absent		Present	bones, scales	Sed. + Flot.	1
2	438	Present	bones, cartilage, musles	Absent		Sed. + Flot.	1
1	770	Absent		Absent		Sed. + Flot.	1
6	1270	Absent		Present	scales, bones	Sed. + Flot.	1
2	1470	Present	bones	Absent		Sed. + Flot.	1
3	1834	Present	bones	Absent		Sed. + Flot.	1
3	2578	Present	bones	Absent		Sed. + Flot.	1

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
2	174	Present	bones, cartilage	Absent		Sed. + Flot.	1
6	214	Present	bones, cartilage, muscle fibres, feather meal	Present	fish bones, cartilage, fish scales, fish skin, muscle fibres, gill	Sed. + Flot.	3
5	450	Present	bones, cartilage, muscle fibres	Present	fish bones, cartilage, fish scales, fish skin, muscle fibres, gill	Sed. + Flot.	1
4	638	Absent		Present	fish bones, cartilage, fish scales, fish skin, muscle fibres, gill	Sed. + Flot.	1
1	1058	Absent		Absent		Sed. + Flot.	1
3	1138	Present	bones, cartilage	Absent		Sed. + Flot.	1
2	1398	Present	bones, cartilage	Absent		Sed. + Flot.	1
3	1858	Present	bones, cartilage, muscle fibre	Absent		Sed. + Flot.	1

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
3	34	Present	bones, cartilage.	Absent		Sed. + Raw	1
6	238	Present	bones, cartilages, muscle fibers.	Present	scales, fishbones, cartilages, muscle fibers,	Sed. + Raw	1
1	386	Absent		Absent		Sed. + Raw	1
5	642	Present	bones, muscle fibers, cartilages.	Present	scales, fishbones, cartilages, muscle fibers,	Sed. + Raw	1
2	942	Present	bones, cartilages.	Absent		Sed. + Raw	1
3	946	Present	bones, cartilages.	Absent		Sed. + Raw	1
4	1190	Absent		Present	scales, fishbones, muscle fibers, seashells, otholithes,	Sed. + Raw	1
2	2022	Present	bones, cartilages.	Absent		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
5	42	Present	bones, muscle fibers	Present	fishbones, muscle fibers	Sed. + Flot.	1
6	46	Present	bones, muscle fibers	Present	fishbones, muscle fibers, scales, gills	Sed. + Flot.	1
2	126	Present	bones	Absent		Sed. + Flot.	1
3	298	Present	bones	Absent		Sed. + Flot.	1
4	1070	Absent		Present	fishbones, muscle fibers	Sed. + Flot.	1
1	1082	Absent		Absent		Sed. + Flot.	1
3	1210	Present	bones	Absent		Sed. + Flot.	1
2	2046	Present	bones	Absent		Sed. + Flot.	1

Free comment

Sample 1070: few (less than 5) atypical fishbones, salmon meal?

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	62	Absent		Present	cartilages, fish bones, gills, muscle fiibres	Sed. + Raw	1
5	258	Present	bones, cartilages	Present	cartilages, fish bones, gills, muscle fiibres	Sed. + Raw	1
3	322	Present	bones, cartilages	Absent		Sed. + Raw	1
2	462	Present	bones, cartilages	Absent		Sed. + Raw	1
1	938	Absent		Absent		Sed. + Raw	1
6	1318	Absent		Present	cartilages, fish bones, gills, muscle fiibres	Sed. + Raw	1
2	1518	Present	bones, cartilages	Absent		Sed. + Raw	1
3	2458	Present	bones	Absent		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
4	230	Absent		Present	meat, bones, gills, fish scales, cartilage	Sed. + Flot.	1
2	270	Present	bones	Absent		Sed. + Raw	1
6	454	Absent		Present	meat, bones, gills, fish scales, cartilage, tooth	Sed. + Flot.	1
1	890	Absent		Absent		Sed. + Raw	1
5	1314	Present	bones	Present	meat,bones, gills,fish scales,cartilage, otholith	Sed. + Flot.	1
3	1570	Present	bones, traces of cartilage	Absent		Sed. + Raw	1
2	1830	Present	Bones, traces of cartilage	Absent		Sed. + Raw	1
3	2314	Present	bones, cartilage, traces of blood	Absent		Sed. + Raw	1

Free comment

1314 possible traces of roller blood but negative in blood-test.

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
6	142	Present	Bones	Present	Fishbones, gills, scales	Sed. + Flot.	1
4	302	Absent		Present	Fishbones, muscles, cartilage, gills, scales	Sed. + Flot.	1
5	954	Present	Bones	Present	Fishbones, gills, scales	Sed. + Flot.	1
1	962	Absent		Absent		Sed. + Flot.	1
2	1158	Present	Bones	Absent		Sed. + Flot.	1
3	1402	Present	Bones	Absent		Sed. + Flot.	1
2	1494	Present	Bones	Absent		Sed. + Flot.	1
3	2290	Present	Bones	Absent		Sed. + Flot.	1

Free comment

In the sediment and flotate of the samples 142 and 954 were detected cartilages (sediment) and muscles (flotate) but are not mentioned in the table as we can't determined if they originate from fish or terrestrial.

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
4	326	Absent		Present	Bones, gills, scale	Sed. + Flot.	1
2	342	Present	bones, fethers	Absent		Sed. + Flot.	1
5	882	Present	bones	Present	Bones, gills, scale	Sed. + Flot.	1
1	914	Absent		Absent		Sed. + Flot.	1
3	1090	Present	bones	Absent		Sed. + Flot.	1
6	1246	Absent		Present	Bones, gills, scale	Sed. + Flot.	1
3	1474	Present	bones	Absent		Sed. + Flot.	1
2	1662	Present	bones	Absent		Sed. + Flot.	1

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
1	74	Absent		Absent		Sed. + Raw	1
6	310	Present	bones; cartilages and muscles of unknown origin	Present	bones; cartilages and muscles of unknown origin	Sed. + Flot.	2
4	446	Absent		Present	bones; cartilages and muscles of unknown origin	Sed. + Flot.	2
5	546	Present	bones; cartilages and muscles of unknown origin	Present	bones; cartilages and muscles of unknown origin	Sed. + Flot.	1
3	754	Present	bones; cartilages and muscles of unknown origin	Absent		Sed. + Flot.	2
2	846	Present	bones; cartilages and muscles of unknown origin	Absent		Sed. + Flot.	2
3	1906	Present	bones; cartilages and muscles of unknown origin	Absent		Sed. + Flot.	2
2	2166	Present	bones; cartilages and muscles of unknown origin	Absent		Sed. + Flot.	2

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	58	Present	bones	Absent		Sed. + Raw	1
4	518	Absent		Present	fishbones, cartilage,	Sed. + Raw	1
5	618	Present	bones	Present	fishbones, cartilage, gills, muscles	Sed. + Raw	1
6	670	Absent		Present	fishbones,gills, muscles	Sed. + Raw	1
2	798	Present	bones	Absent		Sed. + Raw	1
3	1114	Present	bones	Absent		Sed. + Raw	1
1	1226	Absent		Absent		Sed. + Raw	1
2	2214	Present	bones	Absent		Sed. + Raw	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
6	94	Absent		Present	fishbones, scales, muscle fibres it can't be excludet, that the muscle fibres found only derive from fish	Sed. + Flot.	1
2	390	Present	bones	Absent		Sed. + Flot.	1
5	498	Present	bones, muscle fibres no diff. between terestrial animal and fish muscle fibres possible	Present	fishbones, scales, muscle fibres no diff. between terestrial animal and fish muscle fibres possible	Sed. + Flot.	1
3	514	Present	bones, muscle fibres it can't be excludet, that the muscle fibres found only derive from terestrial animals	Absent		Sed. + Flot.	1
4	542	Absent		Present	fishbones, scales, muscle fibres it can't be excludet, that the muscle fibres found only derive from fish	Sed. + Flot.	1
1	986	Absent		Absent		Sed. + Flot.	1
3	1618	Present	bones	Absent		Sed. + Flot.	1
2	2454	Present	bones	Absent		Sed. + Flot.	1

Laboratory identification code : **29**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	122	Absent		Absent		Sed. + Flot.	1
6	526	Present	bone, muscle and cartilage (?)	Present	bone, scale, gill, tooth, muscle and cartilage(?)	Sed. + Flot.	1
5	810	Present	bone, muscle and cartilage(?)	Present	bone, scale, gill, tooth, otolite, muscle and	Sed. + Flot.	1
3	874	Present	bone	Absent		Sed. + Flot.	1
4	1118	Present	bone and muscle	Present	bone, scale, gill, tooth and muscle	Sed. + Flot.	1
3	1762	Present	bone and cartilage	Absent		Sed. + Flot.	1
2	1854	Present	bone and catilage	Absent		Sed. + Flot.	1
2	2574	Present	bone and muscle	Absent		Sed. + Flot.	1

Free comment

810: vegetal #526: insect #874: vegetal #1762: vegetal+insect #1854: vegetal and insect #2574: vegetal. We can not determine if catilage in 526 and 810 is from terrestial or fish origin.

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
5	66	Present	bones	Present	bones, gills, scale	Sed. + Flot.	1
1	170	Absent		Absent		Sed. + Flot.	1
6	334	Present	bones	Present	bones, gills, scale	Sed. + Flot.	1
3	394	Present	bones	Absent		Sed. + Flot.	1
4	734	Absent		Present	bones, gills, scale, muscles	Sed. + Flot.	1
2	1110	Present	bones	Absent		Sed. + Flot.	1
3	1546	Present	bones	Absent		Sed. + Flot.	1
2	2310	Present	bones	Absent		Sed. + Flot.	1

Laboratory identification code : **32**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	82	Present	Bones Hairs	Present	Bones, Scales, Gills	Sed. + Flot.	1
1	98	Absent		Absent		Sed. + Flot.	1
3	466	Present	Bones Hairs	Present	Bones, Scales, Gills	Sed. + Flot.	1
6	478	Absent		Present	Bones, Scales, Gills	Sed. + Flot.	1
5	762	Absent		Present	Bones, Scales, Gills, Thorns	Sed. + Flot.	1
2	822	Absent		Present	Bones, Scales, Gills, Otoliths	Sed. + Flot.	1
4	926	Absent		Present	Bones, Scales, Gills	Sed. + Flot.	1
2	1734	Present	Bones Hairs	Present	Bones, Scales, Gills, Thorns	Sed. + Flot.	1

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
5	162	Present					
4	254	Present					
6	1198	Present					
1	1202	Absent					
2	1758	Present					
3	1810	Present					
2	2070	Present					
3	2482	Present					

Laboratory identification code : **35**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
6	22	Present	Bones	Present	Bones, Scales	Sed. + Raw	1
1	242	Absent		Absent		Sed. + Raw	1
4	470	Absent		Present	Bones, Scales	Sed. + Raw	1
3	730	Present	Bones	Absent		Sed. + Raw	1
5	906	Present	Bones	Present	Bones, Scales	Sed. + Raw	2
2	1230	Present	Bones	Absent		Sed. + Raw	1
2	1806	Present	Bones	Absent		Sed. + Raw	1
3	2050	Present	Bones	Absent		Sed. + Raw	1

Laboratory identification code : **36**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	410	Absent		Absent		Sed. + Flot.	1
4	1238	Present	bones, muscle	Present	fishbones, scale	Sed. + Flot.	1
6	406	Present	bones, muscle	Present	fishbones, scale	Sed. + Flot.	1
5	594	Present	bones, muscle	Present	fishbones, scale	Sed. + Flot.	1
2	990	Present	bones	Absent		Sed. + Flot.	1
3	2074	Present	bones	Absent		Sed. + Flot.	1
3	1594	Present	bones	Absent		Sed. + Flot.	2
2	1782	Present	bones, muscle	Absent		Sed. + Flot.	2

Laboratory identification code : **37**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
6	190	Present	bones	Present	fishbones, scales	Sed. + Raw	1
5	210	Present	bones	Present	fishbones, scales	Sed. + Raw	1
1	746	Absent		Absent		Sed. + Raw	1
2	1038	Present	bones	Absent		Sed. + Raw	1
4	1046	Absent		Present	fishbones, scales	Sed. + Raw	1
2	1566	Present	bones	< LOD	squid cartilage	Sed. + Raw	1
3	1666	Present	bones	Absent		Sed. + Raw	1
3	2122	Present	bones	Absent		Sed. + Raw	1

Laboratory identification code : **38**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
5	114	Present	Bone, muscle	Present	Bone, muscle	Sed. + Flot.	1
1	434	Absent		< LOD	Bone < 5 particles	Sed. + Flot.	3
4	878	Present	Bone, muscle, few blood particles	Present	Bone, muscle	Sed. + Flot.	1
6	1126	Present	Bone, hair, blood	Present	Bone, muscle	Sed. + Flot.	1
2	1350	Present	Bone	< LOD	Bone < 5 particles	Sed. + Flot.	3
3	1522	Present	Bone, few blood particles	Present	Bone, very little (< 10 particles)	Sed. + Flot.	2
3	2410	Present	Bone	< LOD	Bone < 5 particles	Sed. + Flot.	3
2	2526	Present	Bone, muscle	Present	Bone, very little (< 10 particles)	Sed. + Flot.	2

Annex 6

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

Laboratory identification code : **1**

Cut-off at 15 copies :	37,66 cycles
Copy number at the cut-off :	10,31 copies

Sample type	Sample N°	Ruminant DNA	Comment
5	234	Present	No inhibition obsevered in the neat or 1 in 10 sample.
2	246	Absent	No inhibition obsevered in the neat or 1 in 10 sample.
3	682	Present	No inhibition obsevered in the neat or 1 in 10 sample.
1	698	Absent	No inhibition obsevered in the neat or 1 in 10 sample.
4	758	Absent	No inhibition obsevered in the neat or 1 in 10 sample.
3	994	Present	No inhibition obsevered in the neat or 1 in 10 sample.
6	1030	Absent	No inhibition obsevered in the neat or 1 in 10 sample.
2	2118	Absent	No inhibition obsevered in the neat or 1 in 10 sample.

Laboratory identification code : **2**

Cut-off at 15 copies :	33,53 cycles
Copy number at the cut-off :	9,14 copies

Sample type	Sample N°	Ruminant DNA	Comment
2	54	Present	
5	666	Present	
6	790	Present	
4	806	Present	
1	866	Absent	really really close to the cut-off. No PCR inhibition (inhibition test performed with the addition of
3	922	Present	
3	2194	Present	
2	2262	Present	

Laboratory identification code : **3**

Cut-off at 15 copies :	36,96 cycles
Copy number at the cut-off :	9,02 copies

Sample type	Sample N°	Ruminant DNA	Comment
4	278	Absent	
2	366	Absent	
5	402	Present	
6	430	Absent	
1	1154	Absent	
2	1878	Present	Weak
3	2098	Present	
3	2602	Present	

Laboratory identification code : **4**

Cut-off at 15 copies :	36,13 cycles
Copy number at the cut-off :	11,24 copies

Sample type	Sample N°	Ruminant DNA	Comment
2	414	Absent	low inhibition
5	474	Present	
3	634	Present	
6	766	Absent	
1	1010	Absent	
4	1310	Absent	
2	2142	Absent	low inhibition
3	2626	Present	

Laboratory identification code : **5**

Cut-off at 15 copies :	35,92 cycles
Copy number at the cut-off :	10,76 copies

Sample type	Sample N°	Ruminant DNA	Comment
2	150	Absent	
2	198	Absent	
4	374	Absent	
6	694	Absent	
5	786	Present	
1	842	Absent	
3	1330	Present	
3	2002	Present	

Laboratory identification code : **6**

Cut-off at 15 copies :	35,35 cycles
Copy number at the cut-off :	9,27 copies

Sample type	Sample N°	Ruminant DNA	Comment
4	86	Absent	
2	222	Absent	
5	306	Present	
2	486	Absent	
1	674	Absent	
6	910	Absent	
3	2218	Present	
3	2506	Present	

Laboratory identification code : **7**

Cut-off at 15 copies :	35,87 cycles
Copy number at the cut-off :	11,62 copies

Sample type	Sample N°	Ruminant DNA	Comment
2	78	Absent	
6	502	Absent	
3	850	Present	
5	858	Present	
3	1186	Present	
1	1250	Absent	
4	1262	Absent	
2	1302	Absent	

Laboratory identification code : **8**

Cut-off at 15 copies :	37,74 cycles
Copy number at the cut-off :	9,48 copies

Sample type	Sample N°	Ruminant DNA	Comment
2	294	Absent	
5	378	Present	
6	574	Present	
3	610	Present	
1	1106	Present	
4	1286	Absent	
3	1306	Present	
2	1686	Present	

Laboratory identification code : **9**

Cut-off at 15 copies :	37,31 cycles
Copy number at the cut-off :	8,77 copies

Sample type	Sample N°	Ruminant DNA	Comment
6	286	Absent	
1	650	Absent	
3	778	Present	
5	834	Present	
4	998	Absent	
2	1134	Absent	
2	1638	Absent	
3	2530	Present	

Laboratory identification code : **10**

Cut-off at 15 copies :	35,35 cycles
Copy number at the cut-off :	10,80 copies

Sample type	Sample N°	Ruminant DNA	Comment
5	186	Present	
4	662	Absent	analysis repeated, and again one positive result. Final concl.: absent.
3	802	Present	
6	958	Absent	
2	1278	Absent	
1	1298	Absent	
3	1354	Present	
2	1614	Present	

Laboratory identification code : **11**

Cut-off at 15 copies :	34,23 cycles
Copy number at the cut-off :	11,08 copies

Sample type	Sample N°	Ruminant DNA	Comment
5	354	Present	
2	582	Absent	
6	598	Absent	
4	974	Absent	
1	1130	Absent	
3	1162	Present	
2	1374	Absent	
3	2026	Present	

Laboratory identification code : **12**

Cut-off at 15 copies :	34,52 cycles
Copy number at the cut-off :	9,93 copies

Sample type	Sample N°	Ruminant DNA	Comment
2	6	Absent	no
1	578	Absent	replicate 1 have inhibition
4	614	Absent	replicate 2 have inhibition
3	658	Present	no
6	934	Absent	no
5	1050	Present	no
3	1378	Present	'replicate 2 have inhibition
2	1422	Absent	no

Laboratory identification code : **13**

Cut-off at 15 copies :	35,57 cycles
Copy number at the cut-off :	11,25 copies

Sample type	Sample N°	Ruminant DNA	Comment
3	442	Present	
4	566	Absent	
1	602	Absent	
2	606	Absent	
6	886	Absent	
5	1002	Present	
3	1282	Present	
2	2430	Absent	

Laboratory identification code : **14**

Cut-off at 15 copies :	36,22 cycles
Copy number at the cut-off :	10,75 copies

Sample type	Sample N°	Ruminant DNA	Comment
4	38	Absent	No Targets Detected
2	102	Absent	Poultry DNA detected
1	794	Absent	No Targets Detected
6	1102	Absent	Porcine DNA detected
5	1146	Present	Ruminant DNA detected. Poultry DNA detected.
2	1326	Absent	Poultry DNA detected
3	1882	Present	Ruminant DNA detected. Poultry DNA detected.
3	2362	Present	Ruminant DNA detected. Poultry DNA detected.

Laboratory identification code : **15**

Cut-off at 15 copies :	32,33 cycles
Copy number at the cut-off :	9,14 copies

Sample type	Sample N°	Ruminant DNA	Comment
1	50	Absent	inhibition
3	490	Present	
2	510	Absent	inhibition
5	522	Present	
4	590	Absent	
6	1054	Absent	
3	1258	Present	
2	1446	Absent	

Laboratory identification code : **16**

Cut-off at 15 copies :	31,37 cycles
Copy number at the cut-off :	13,27 copies

Sample type	Sample N°	Ruminant DNA	Comment
5	**90**	Present	
4	**110**	Absent	
2	**438**	Absent	
1	**770**	Absent	
6	**1270**	Absent	
2	**1470**	Absent	
3	**1834**	Present	
3	**2578**	Present	

Laboratory identification code : **17**

Cut-off at 15 copies :	36,35 cycles
Copy number at the cut-off :	9,46 copies

Sample type	Sample N°	Ruminant DNA	Comment
2	**174**	Absent	Significant inhibition excluded by spiking exp.
6	**214**	Absent	Significant inhibition excluded by spiking exp.
5	**450**	Present	
4	**638**	Absent	Significant inhibition excluded by spiking exp.
1	**1058**	Absent	Significant inhibition excluded by spiking exp.
3	**1138**	Present	
2	**1398**	Absent	Significant inhibition excluded by spiking exp.
3	**1858**	Present	

Laboratory identification code : **18**

Cut-off at 15 copies :	36,60 cycles
Copy number at the cut-off :	10,40 copies

Sample type	Sample N°	Ruminant DNA	Comment
3	**34**	Present	2020-1297
6	**238**	Absent	2020-1298
1	**386**	Absent	2020-1299
5	**642**	Present	2020-1300
2	**942**	Absent	2020-1301
3	**946**	Present	2020-1302
4	**1190**	Absent	2020-1303
2	**2022**	Absent	2020-1304

Laboratory identification code : **19**

Cut-off at 15 copies :	37,35 cycles
Copy number at the cut-off :	9,93 copies

Sample type	Sample N°	Ruminant DNA	Comment
5	42	Present	The used PCR platform was CFX Maestro version 4.0.2325.0418
6	46	Absent	
2	126	Absent	
3	298	Present	
4	1070	Absent	
1	1082	Absent	
3	1210	Present	
2	2046	Absent	

Laboratory identification code : **20**

Cut-off at 15 copies :	33,88 cycles
Copy number at the cut-off :	10,48 copies

Sample type	Sample N°	Ruminant DNA	Comment
4	62	Absent	
5	258	Present	
3	322	Present	
2	462	Absent	
1	938	Absent	
6	1318	Absent	
2	1518	Absent	
3	2458	Present	

Laboratory identification code : **21**

Cut-off at 15 copies :	34,30 cycles
Copy number at the cut-off :	9,26 copies

Sample type	Sample N°	Ruminant DNA	Comment
4	230	Absent	
2	270		Not done based on the type of feed in the sample
6	454	Absent	
1	890		Not done based on the type of feed in the sample
5	1314	Present	
3	1570		Not done based on the type of feed in the sample
2	1830		Not done based on the type of feed in the sample
3	2314		Not done based on the type of feed in the sample

Laboratory identification code : **22**

Cut-off at 15 copies :	35,34 cycles
Copy number at the cut-off :	11,08 copies

Sample type	Sample N°	Ruminant DNA	Comment
6	142	Absent	
4	302	Absent	
5	954	Present	
1	962	Absent	
2	1158	Absent	
3	1402	Present	
2	1494	Absent	
3	2290	Present	

Laboratory identification code : **23**

Cut-off at 15 copies :	31,91 cycles
Copy number at the cut-off :	10,32 copies

Sample type	Sample N°	Ruminant DNA	Comment
4	326		
2	342	Absent	
5	882	Present	
1	914		
3	1090	Present	
6	1246		
3	1474	Present	
2	1662	Absent	

Laboratory identification code : **24**

Cut-off at 15 copies :	35,76 cycles
Copy number at the cut-off :	9,09 copies

Sample type	Sample N°	Ruminant DNA	Comment
1	74	Absent	
6	310	Absent	
4	446	Absent	
5	546	Present	
3	754	Present	
2	846	Absent	
3	1906	Present	
2	2166	Absent	

Laboratory identification code : **25**

Cut-off at 15 copies :	36,45 cycles
Copy number at the cut-off :	11,34 copies

Sample type	Sample N°	Ruminant DNA	Comment
3	58	Present	
4	518	Absent	
5	618	Present	
6	670	Absent	
2	798	Absent	
3	1114	Present	
1	1226	Absent	
2	2214	Absent	

Laboratory identification code : **26**

Cut-off at 15 copies :	36,71 cycles
Copy number at the cut-off :	10,96 copies

Sample type	Sample N°	Ruminant DNA	Comment
6	94	Absent	
2	390	Absent	
5	498	Present	
3	514	Present	
4	542	Absent	
1	986	Absent	
3	1618	Present	
2	2454	Absent	

Laboratory identification code : **29**

Cut-off at 15 copies :	37,03 cycles
Copy number at the cut-off :	11,30 copies

Sample type	Sample N°	Ruminant DNA	Comment
1	122	Absent	
6	526	Absent	
5	810	Present	
3	874	Present	
4	1118	Absent	
3	1762	Present	
2	1854	Absent	
2	2574	Absent	

Laboratory identification code : **31**

Cut-off at 15 copies :	37,59 cycles
Copy number at the cut-off :	9,66 copies

Sample type	Sample N°	Ruminant DNA	Comment
5	66	Present	
1	170	Absent	
6	334	Absent	
3	394	Present	
4	734	Absent	
2	1110	Absent	
3	1546	Present	
2	2310	Absent	

Laboratory identification code : **37**

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

Sample type	Sample N°	Ruminant DNA	Comment
6	190	Absent	
5	210	Absent	
1	746	Absent	
2	1038	Absent	
4	1046	Absent	
2	1566	Absent	
3	1666	Present	
3	2122	Present	