EURL-AP Proficiency Test Microscopy 2011

Final version

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Summary

The European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP) organised the present proficiency study for assessing the ability of the NRL network for the detection of animal proteins in feed according to Annex VI of Commission Regulation EC/152/2009. This study was also open to some non-EU participants. Total number of participants was 31 of which 26 NRLs and 5 non-EU participating laboratories. The study was based on a set of 7 blind samples. The sample set consisted of blanks, feed matrices or fats, fortified with terrestrial meat and bone meal and hydrolysed feather meal.

Results from the NRLs indicated a very good global performance of the network. The disclosure of animal particles in fat according to an optional protocol from Annex VI occurred satisfactorily. Further analysis of the results revealed a serious lack of sensitivity for the detection of hydrolysed feather meal within fish feed. Results from non-EU participants were evaluated as for NRLs. The percentage of good performing labs was higher within the NRL network than among non-EU participants. However non-EU participants showed better sensitivity scores for hydrolysed feather meal detection than the NRLs.

The study showed that some participants, NRLs or non-EU, had underperforming results. For NRLs in this situation, an action plan to remediate to those underperformances was asked.

Keywords :

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

1. Foreword

European Union Reference Laboratories (EURL) – formerly referred to as Community Reference Laboratories (CRL) – were created in order to ensure a high level of quality and a uniformity of the results provided by European control laboratories. On 29 April 2004, the European Parliament and the Council adopted the 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, the Commission Regulation EC/208/2011 [2], renewed the Walloon Agricultural Research Centre as European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP, http://crl.cra.wallonie.be). 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 yearly proficiency tests for the assessment of the implementation of the reference microscopic method for the detection of animal proteins in feed as described in Annex VI of Commission Regulation EC/152/2009 [3]. The present study report is part of this activity scope.

This final report was prepared based on a working document version diffused through the NRL network and discussed during the 6th EURL-AP Annual Workshop held in Berlin from 17th to 19th April 2012.

2. Introduction

According to Annex VI of Commission Regulation EC/152/2009 official controls for the detection of animal proteins in feed inside the EU are performed by light microscopy. The objective of the present proficiency test is to evaluate performances of the network of 26 NRLs to detect the presence of processed animal proteins in feed for the year 2011.

On proposal of the Commission, invitations to participate to this test were also sent to official control labs outside the EU. Non-EU participants were asked to apply also light microscopic methods.

3. Material and methods

3.1. Study organisation

Official announcement of the study was made on the 1st September 2011 to all participants.

Participants were the 26 NRLs and 5 laboratories outside this EU network. These five foreign participants were the Croatian Veterinary Institute, the Servicio Nacional de Sanidad y Calidad Agroalimentaria from Argentina, the China Agricultural University, the Food and Agricultural Materials Inspection Center from Japan and LabNett AS from Norway. A detailed list of the 31 participating labs is included in Annex 1.

On the 31st October, the Excel report forms containing the instructions (Annex 2) were communicated to all participants – downloadable from the EURL-AP intranet for the NRLs or sent to the non-EU participants who do not have access to this intranet.

On the 7th November 2011, sets of 7 blind samples were sent by express shipment to the participants.

Some general recommendations were delivered to the participants:

- Mention was done that each participating laboratory was itself responsible to reach appropriate homogeneity of the sample sub-portions that had to be taken from the whole sample vial for analysis.
- Results had to be encoded by way of an Excel report form (Annex 2). Participants were asked to carefully read the instructions on how to fill in the result form and to testify they did it prior to encoding their results. No other support for communicating the results was accepted.
- A summarized results sheet was automatically generated. Participants were asked to sign the summarized results sheet and to return it by fax and email to the EURL-AP. Only when both the Excel file and the fax were received by EURL-AP were results taken into consideration.
- The results had to be sent in both forms concomitantly to the EURL-AP by the 25th November 2011. Notification has been done that this date was a deadline and that results arriving later would not be accepted.

All participants delivered their results on time and no participant had to be excluded. Results from NRLs and non-EU participants were analysed separately in this report.

3.2. Material

3.2.1. Description of the samples

Seven different materials containing typical feed ingredients and/or processed animal proteins (PAPs) from various animal origins at different concentration levels have been prepared as shown in table 1 (next page).

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

- Target concentrations of mammalian PAP largely inferior to the classical 0.1% considered for the time being as the adulteration level that the method should be able to detect.
- Presence of feather meal presenting a certain degree of hydroysis which leads to an alteration of the morphological features of feathers.

- Feed matrix conditioning (pelletized) that requires grinding before analysis as requested by Commission Regulation EC/152/2009 [3].
- The ability to handle fat as a matrix to which processed animal protein detection has to be performed (according to the optional protocol from Annex VI of EC/152/2009 or not).

Each participating lab received about 55g of 7 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 in the EURL-AP Proficiency Test 2011.

Sample	Material	Nr of replicates
1	blank I	1
2	beet pulp pellets	1
3	blank I + 0.05% PAP	1
4	fat (+ 0.1% of dicalcium phosphate)	1
5	fat + 0.1% PAP	1
6	fish feed (pellets)	1
7	fish feed (pellets) + 0.5% hydrolysed feather	1
Total		**7**

3.2.2. Materials used in the preparation of the samples

Different feed matrices were used for the preparation of the sample set:

- A **first feed matrix** used was a classical compound feed. The matrix was already used and described in a former EURL-AP proficiency test in 2007 [4]. Sediment content of this compound feed was about 2.2%. This feed matrix was used for preparing samples 1 and 3.
- A **second feed matrix** was a pelletized beet pulp for bovines from a local producer. Its sediment content was about 0.6%. This feed matrix was used for sample 2.
- A **third feed matrix** was a pelletized fish feed composed of Chili fish meal, soya, wheat, barley malt by product, corn gluten and pork blood meal. This feed matrix was only used for samples 6 and 7. Its sediment content was about 4.3%.
- The **fat** used for samples 4 and 5 was pure lard bought from a supermarket. It had no sediment. The 50°C heated fat was coloured with Sudan black B (sample 4) and an artificial lipophilic food colouring agent (sample 5). After staining the molten stained lard was filtered on a P16 (10-16µm) sintered glass Buchner funnel to remove the excess dye particles.

Prior to use, all matrix materials were tested by light microscopy and PCR (except the fat) in order to confirm the absence of any interfering substances from animal origin.

Adulterant material used:

- Commercial dicalcium phosphate presenting semi-transparent crystals.
- The **PAP** utilised for preparing samples 3 and 5 was a pure porcine meat and bone meal. Its final bone content was of about 36%. Its purity was controlled by PCR.
- The **feather meal** used is an industrial "hydrolysed" feather meal from poultry origin. Its bone content was about 0.9%. Its purity was controlled by PCR.

3.2.3. Description of the mixing procedures

The stepwise dilution procedure developed by CRA-W and JRC-IRMM was used to produce sample 3. This procedure has been successfully used in numerous former European interlaboratory studies aiming to evaluate different light microscopy protocols.

For samples 4, 5 and 7 direct spiking in the vials was performed. For samples 4 and 5 spiking was realised when the fat was melted at a temperature of about 50℃.

Quality control of the sample production was achieved by a homogeneity study (cf. section 4.1).

3.3. Qualitative analysis

Qualitative analysis concerned the presence or absence of terrestrial animal (PAP) and/or fish material. 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 method.

4. Results

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

4.1. Homogeneity study

Homogeneity study has been carried out for all materials used. The following table summarizes the results.

Table 2: Homogeneity study – Results.

Sample	Material	Light microscopy			% sed	PCR					
		Nr of replicates	Terrestrial particle	Fish particle		Nr of replicates	Bovine	Porcine	Poultry	Fish	Sheep
1	blank I	15	-	1 scale, 1 fish bone	2,23	5	+ (2/5)	-	-	-	-
2	beet pulp pellets	15	8 bones	-	0.64	5	-	- (traces 1/5)	-	-	-
3	blank I + 0.05% PAP	10	+	-	2.66						
4	fat (+ 0.1% dicalcium phosphate)	5	-	-	0.08						
5	fat + 0.1% PAP	5	+	-	0.03						
6	fish feed (pellets)	10	-	+	4.31	4	-	+ (3/4)	+ (3/4)	+	-
7	fish feed (pellets) + 0.5% hydrolysed feather	5	+	+	4.62						

(Legend: sed = sediment, + = present, - = not present, traces = signals below cut off values)

The homogeneity was studied on 10g of sample material for each replicate. For the homogeneity study both flotate and sediment fractions were analysed. Each time 6 slides were prepared.

Blank I (**sample 1**) was negative for any presence of terrestrial material by light microscopy. A single observation of one scale and one fishbone fragment was reported and hence considered as negative for fish. By PCR it revealed to be negative for all markers except for bovine DNA (2 positives on 5 tests). This could be explained by the fact that the plant produced another batch of feed containing whey powder before this batch.

Beet pulp pellets (**sample 2**) was free from any presence of fish material. The presence of terrestrial bones was reported for 6 replicates (five replicates with only one bone, one replicate with three bones) but

considered as negative because below the LOD [5]. Therefore this material was treated as a blank in this study. PCR traces of porcine DNA were reported for one replicate.

The blank I adulterated with 0.05% of PAP (**sample 3**) showed systematically terrestrial bones, with an average of 22 (± 7) particles per 6 slides.

The fat to which dicalcium phosphate was added (**sample 4**) did not reveal any traces of animal remains.

The fat adulterated with 0.1% of PAP (**sample 5**) showed systematically terrestrial bones.

The fish feed (**sample 6**) did not present any traces of terrestrial animal by light microscopy. According to its composition porcine DNA was detected. Poultry signal was detected as well without any explanation.

In the fish feed fortified with 0.5% of hydrolysed feather (**sample 7**) feather fragments were systematically observed on the microscope, in addition some few terrestrial bones were reported for two of the 5 replicates.

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

4.2. Qualitative analyses from the NRLs

4.2.1. On the respect of the instructions

Overall instructions have been respected through the study.

4.2.2. Overview of results and performance of the method

Table 3 summarizes the results submitted by the 26 NRLs for the seven sample types submitted to qualitative analysis.

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

Sample	Material	Nr	AC	
			Terrestrial	Fish
1	blank I	26	1.000	0.885 (3)
2	beet pulp pellets	26	0.923 (2)	0.923 (2)
3	blank I + 0.05% PAP	26	1.000	0.962 (1)
4	Fat	26	1.000	1.000
5	fat + 0.1% PAP	26	0.962 (1)	0.962 (1)
6	fish feed	26	0.962 (1)	1.000
7	fish feed II + 0.5% h. feather	26	0.308 (18)	1.000

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

The overall results, expressed in terms of global accuracy (AC), reveal a very good global performance. Nevertheless the number of false positive results for fish in blank I (sample 1) is reaching 12% but these results are based on the observations of numbers of particles below the LOD [5] (for details cf. next section). The study reveals an abnormally high number of false negative results for the detection of hydrolysed feathers; the lack of sensitivity is reaching 70%.

On the exception of one lab, all participants made the choice of using the optional protocol for the fat containing samples (samples 4 and 5), as described in section 9 of Annex VI of regulation EC/152/2009. Results obtained by those laboratories are quite satisfying. The only apparent difficulty was that of

defining the weight of the sample fraction being used: 32% of the labs did not mention the weight, 24% mentioned 5-10g, and the remaining 44% used subsamples ranging from 0.47g to 55g.

4.2.3. Detailed review of results for each sample material

Mainly incorrect results are detailed in this section.

Blank I

Fish particles were detected:

- Lab 3 : 3 fish bones
- Lab 7 : 2 fish bones.
- Lab 12 : 3 fish bones and 1 foram. Foraminifera cannot be assimilated as fish material as they belong to the Protist kingdom.

It has to be noted that all these results are based on numbers of particles that are inferior to the LOD [5] and that are not even repeatable. It cannot be excluded that those particles originate from laboratory cross contamination; the same conclusion could be made for the homogeneity study as PCR analysis did not reveal traces of fish.

Beet pulp pellets

Terrestrial animal particles were detected:

- Lab 17 : 5 sheep hair fragments. Carryover from clothes was first envisaged but the detected hairs were embedded in an unknown matrix. No explanation was found for this by the participant.
- Lab 23 : 2 bones (<LOD)

Presence of fish was also reported as follows:

- Lab 24 : 8 fish particles.
- Lab 27 : 7 fish particles consisting of bones and muscle fibres.

Blank I + 0.05% PAP

Fish particles were reported:

- Lab 27 : 1 fishbone (application of strict “zero tolerance” policy).

Some laboratories (Labs 23 and 25) reported correctly the presence of terrestrial material but the number of particles they had observed was abnormally low. This situation can generate a potential risk for false negative results.

Fat blank (+0.5% dicalcium phosphate)

No wrong results were noted. Almost all participants (25 on 26) used the optional protocol for fat analysis.

The presence of dicalcium phosphate which generated sediment did not impact on the results.

Fat +0.1% PAP

Lab 4 failed to detect the presence of terrestrial particles.

Lab 23 detected the presence of more than 10 particles identified as from fish origin.

Almost all participants (25 on 26) used the optional protocol for fat analysis.

Fish feed

Terrestrial animal particles were reported:

- Lab3 : detected 6 terrestrial bones

Lab 15 and 19 declared the sample as negative for terrestrial material as they observed respectively only one feather fragment and three bone particles, i.e. each time below the LOD.

Fish feed + 0.5% hydrolysed feather meal:

18 participants failed at detecting the presence of terrestrial particles.

This reflects the difficulty of distinguishing hydrolysed feather meal (with a very low percentage of bones) within a matrix presenting an abundance of muscle fibres. The ability to discriminate hydrolysed feather fragments, sometimes described as “cooked spaghetti” [6] from muscle fibres bundles still remains very weak. It is known to be easily misidentified with muscle tissues or even confused with fragments of fishbones [6]. For that reason, it is recommended to use the cystine reagent helping in the differentiation [6].

4.2.4. 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. This was performed separately for both the detection of terrestrial material and fish material. A ranking of the labs was prepared based on the consolidated accuracy.

Results are to be found in tables 4 and 5 (next page).

Concerning the ability to detect terrestrial animal constituents (table 4), 20 labs provided incorrect results according to the following details:

- PD for PAP in beet pulp pellets : labs 17 and 23
- ND for PAP in fat + 0.1% PAP : lab 4
- PD for PAP in fish feed : lab 3
- ND for PAP in fish feed + 0.5% hydrolysed feather : labs 1, 5, 6, 9, 11, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 26 and 27

Concerning the ability to detect fish material (table 5), 6 labs delivered incorrect results according to the following details:

- PD for fish in blank I : labs 3, 7 and 12
- PD for fish in beet pulp pellets : labs 24 and 27
- PD for fish in blank I + 0.05% PAP : lab 27
- PD for fish in fat + 0.1% PAP : lab 23

Tables 4 (left) and 5 (right): NRL proficiencies regarding the detection of terrestrial and fish material. Ranking follows AC values.

Terrestrial			
lab code	AC	SE	SP
2	1.000	1.000	1.000
7	1.000	1.000	1.000
10	1.000	1.000	1.000
12	1.000	1.000	1.000
20	1.000	1.000	1.000
25	1.000	1.000	1.000
3	0.857	1.000	0.750
1	0.857	0.667	1.000
4	0.857	0.667	1.000
5	0.857	0.667	1.000
6	0.857	0.667	1.000
9	0.857	0.667	1.000
11	0.857	0.667	1.000
13	0.857	0.667	1.000
14	0.857	0.667	1.000
15	0.857	0.667	1.000
16	0.857	0.667	1.000
18	0.857	0.667	1.000
19	0.857	0.667	1.000
21	0.857	0.667	1.000
22	0.857	0.667	1.000
24	0.857	0.667	1.000
26	0.857	0.667	1.000
27	0.857	0.667	1.000
17	0.714	0.667	0.750
23	0.714	0.667	0.750

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
9	1.000	1.000	1.000
10	1.000	1.000	1.000
11	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
22	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
3	0.857	1.000	0.800
7	0.857	1.000	0.800
12	0.857	1.000	0.800
23	0.857	1.000	0.800
24	0.857	1.000	0.800
27	0.714	1.000	0.600

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 next page)

Table 6 illustrates the very good level of global performance (= consolidated AC superior or equal to 0.90, i.e. having no more than one false result) for 21 labs out of 26 NRLs or in other words for 81% of the NRLs. A second category of NRLs (cells in blue in table 6) having a satisfying global performance is defined (= consolidated AC below 0.90 and having no more than two false results including a maximum of one ND for terrestrial material). Three NRLs fall into this category. NRLs included in this category are nevertheless asked to report to the EURL-AP on the possible source of these deviations. A third category (cells in red in table 6) includes NRLs that have underperforming results for the present test (= consolidated AC below 0.90 and having either at least three false results or two ND for terrestrial). For one of this lab (lab 27) the explanation is partly to be found in the fact that it applied a strict “zero tolerance” policy in the present study, i.e. declaring a sample as positive as soon as a single particle was found. In agreement with the EURL-AP SOP for managing underperformances (available on the EURL-AP intranet since 18 January 2012), these two participants are nevertheless asked to report on the origin of those multiple errors as well as on the actions they will undertake in order to solve this key issue.

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.

Consolidated			
lab code	AC	SE	SP
2	1.000	1.000	1.000
10	1.000	1.000	1.000
20	1.000	1.000	1.000
25	1.000	1.000	1.000
7	0.929	1.000	0.889
12	0.929	1.000	0.889
1	0.929	0.800	1.000
4	0.929	0.800	1.000
5	0.929	0.800	1.000
6	0.929	0.800	1.000
9	0.929	0.800	1.000
11	0.929	0.800	1.000
13	0.929	0.800	1.000
14	0.929	0.800	1.000
15	0.929	0.800	1.000
16	0.929	0.800	1.000
18	0.929	0.800	1.000
19	0.929	0.800	1.000
21	0.929	0.800	1.000
22	0.929	0.800	1.000
26	0.929	0.800	1.000
3	0.857	1.000	0.778
17	0.857	0.800	0.889
24	0.857	0.800	0.889
23	0.786	0.800	0.778
27	0.786	0.800	0.778

4.3. Qualitative analyses from the non-EU participants

4.3.1. Individual performances of non-EU participants in qualitative analysis

For reminder foreign participants were requested to realise the test by microscopic method.

Individual performances from the 5 participants outside the EU were assessed exactly as in previous section (4.2.4.). A ranking of those labs was prepared 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. Ranking follows AC values.

Terrestrial			
lab code	AC	SE	SP
30	1.000	1.000	1.000
35	0.857	0.667	1.000
34	0.714	0.667	0.750
32	0.714	0.333	1.000
33	0.571	1.000	0.250

Fish			
lab code	AC	SE	SP
32	1.000	1.000	1.000
33	1.000	1.000	1.000
35	1.000	1.000	1.000
34	0.857	1.000	0.800
30	0.714	1.000	0.600

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

- PD for PAP in blank I : lab 33
- PD for PAP in fat : lab 33
- ND for PAP in fat + 0.1% PAP : labs 32 and 34
- PD for PAP in fish feed : labs 33 and 34. Lab 34 based its results on the observation of 2 bones (<LOD).
- ND for PAP in fish feed + 0.5% hydrolysed feather : labs 32 and 35

Concerning the ability to detect fish material:

- PD for fish in blank I : lab 30
- PD for fish in beet pulp pellets : lab 30
- PD for fish in fat + 0.1% PAP : lab 34

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
35	0.929	0.800	1.000
30	0.857	1.000	0.778
32	0.857	0.600	1.000
33	0.786	1.000	0.667
34	0.786	0.800	0.778

One participant (lab 35) obtained a very good level of global performance.

Lab 30 obtained a satisfying result (cells in blue in table 9).

The other participants were underperforming (cells in red in table 9) according to the applied criteria.

5. Conclusions

This study confirmed the high proficiency level of the NRL network. With 81% of the NRLs obtaining a very good global performance the proficiency level is close to the record level of 85% obtained in 2010. According the performance criteria used in the present test, only two NRLs had underperforming results. They are asked to take actions to make progress in their proficiency.

The sample set used allowed focusing on the difficulty of detection and identification of hydrolysed feather meal in a classical fish feed. The lack of sensitivity for hydrolysed feather detection is reaching 70% of the participants, and accounts for 62% of the total errors of this study. Aside training and experience, the situation can easily be improved. As the pelletized fish feed sample was simply spiked directly with the hydrolysed feather meal, the separated analysis of a sieve fraction before grinding would have helped to better disclose the adulteration. As proposed recently [7], it is recommended to use through the microscopic observations at least one slide with the cystine reagent renowned for helping in the identification of feather and hairs.

The use of fat samples was partly intended to evaluate the optional protocol as described under section 9 of the Annex VI of regulation EC/152/2009. It revealed that almost all NRLs were choosing this protocol for fats and that the results were plenty satisfying. Nevertheless the study showed that this part of Annex VI was unclear in terms of quantity of material that should be used. Clearer information on the quantity of material to use would improve this optional protocol.

Some erroneous results, false positive results, once again are to be pointed out due to decisions taken on the observations of a too small number of suspicious particles or in order terms a number of particles being below the limit of detection. The proposed revision of Annex VI of EC/152/2009 should overcome this issue by allowing declaring a sample as negative if less than 5 particles of a given nature is found per determination. As regard to the pelletized beet pulp, the revision would also allow implementation of regulation EC/163/2009 [8] which states that feeds of plant origin containing insignificants amounts of bone spicules may be used to feed farmed animals. Up to now, as to our knowledge, only few Member States established a risk assessment for such type of feed from plant origin as it is however legally prescribed.

Concerning the non-EU participants, two out of five performed satisfyingly or excellently. Compared to the NRL network these non-EU participants had a better relative score for the detection of hydrolysed feather meal.

Acknowledgment

We are grateful to the EURL-AP staff and the participants for their fruitful collaboration.

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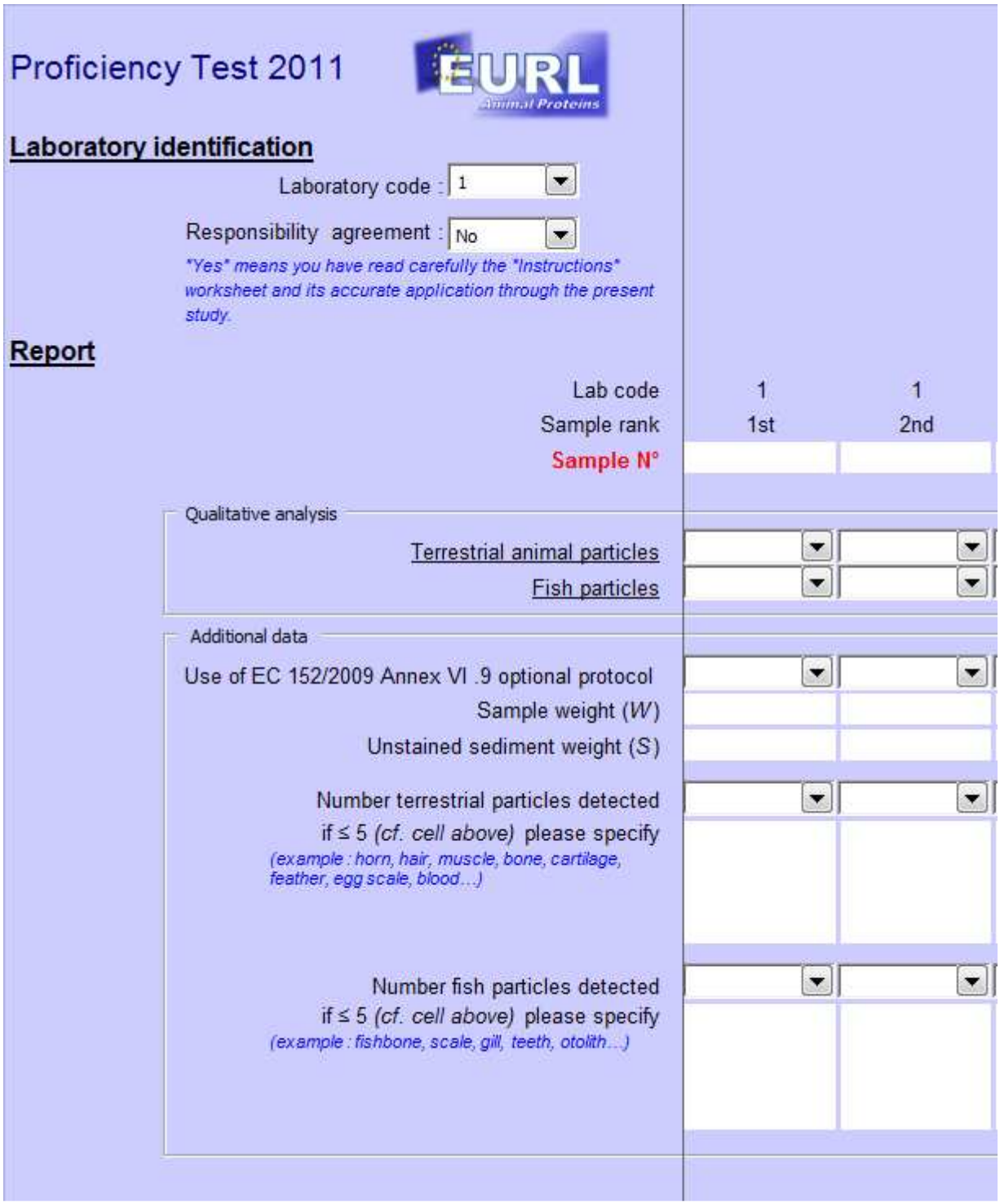
Annex 1

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

Country	Institute Name
Austria	Austrian Agency for Health and Food Safety
Argentina	*Servicio Nacional de Sanidad y Calidad Agroalimentaria*
Belgium	Federal Agency for the Safety of the Food Chain
Bulgaria	National Diagnostic Research Veterinary Medical Institute
China	*China Agricultural University Beijing*
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 Veterinary Laboratory
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Norway	*LabNett AS*
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 Health and Veterinary Laboratories Agency

Annex 2

Excel result report form



Proficiency Test 2011

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
Sample rank	1st	2nd
Sample N°		

Qualitative analysis

Terrestrial animal particles		
Fish particles		

Additional data

Use of EC 152/2009 Annex VI .9 optional protocol		
Sample weight (*W*)		
Unstained sediment weight (*S*)		
Number terrestrial particles detected		
if ≤ 5 *(cf. cell above)* please specify *(example : horn, hair, muscle, bone, cartilage, feather, egg scale, blood…)*		
Number fish particles detected		
if ≤ 5 *(cf. cell above)* please specify *(example : fishbone, scale, gill, teeth, otolith…)*		

Annex 3

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

Laboratory identification code : **1**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	No	10.00	0.183				
3	Present	Absent	No	10.00	0.319	> 10			
7	Absent	Present	No	10.00	0.484			> 10	
5	Present	Absent	Yes	10.00	0.006	> 10			
4	Absent	Absent	Yes	10.00	0.012				
6	Absent	Present	No	10.00	0.471			> 10	
1	Absent	Absent	No	10.00	0.303				

Laboratory identification code : **2**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	No	5.00	0.040				
4	Absent	Absent	Yes	5.00	0.000				
3	Present	Absent	No	5.00	0.132	> 10	bone muscle feather mousehair?		
7	Present	Present	No	5.00	0.102	> 10	feather	> 10	bone muscle scale cartilage gill teeth
6	Absent	Present	No	5.00	0.209			> 10	bone muscle scale gill cartilage teeth
1	Absent	Absent	No	5.00	0.142				
5	Present	Absent	Yes	5.00	0.001	> 10	muscle bone		

Laboratory identification code : **3**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Present	Present		10.38	0.431	7	Bone, Muscle, Hair	> 10	Bone, Scales, Gills, Cartilage, Muscle
2	Absent	Absent		10.17	0.045				
3	Present	Absent		10.39	0.305	10	Bone, Muscle, Cartilage		
1	Absent	Present		10.41	0.364			3	Bone
4	Absent	Absent	No	0.00	0.000				
5	Present	Absent	No	0.00	0.000	> 10	Bone		
6	Present	Present		10.12	0.629	6	Bone, Muscle	> 10	Bone, Muscle, Gills, Scales, Cartilage

Laboratory identification code : **4**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	No	10.03	0.052				
7	Present	Present	No	10.03	0.228	> 10	fearther	> 10	
3	Present	Absent	No	10.01	0.184	10	bone		
6	Absent	Present	No	10.03	0.231			> 10	
1	Absent	Absent	No	10.03	0.246				
4	Absent	Absent	Yes	0.00	0.000				
5	Absent	Absent	Yes	0.00	0.000				

Laboratory identification code : **5**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	No	5.55	0.057				
4	Absent	Absent	Yes	6.05	0.048				
5	Present	Absent	Yes	5.77	0.034	> 10	Bones, cartilage, muscles		
7	Absent	Present	No	5.52	0.070			> 10	Fishbones, cartilage, muscles, gill
6	Absent	Present	No	5.88	0.080			> 10	Fishbones, cartilage, muscles, gill
3	Present	Absent	No	5.59	0.170	8	bones, cartilage		
1	Absent	Absent	No	5.71	0.170				

Laboratory identification code : **6**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent		5.34	0.081				
7	Absent	Present		5.56	0.109			> 10	
3	Present	Absent		5.49	0.180	7			
1	Absent	Absent		5.81	0.188				
4	Absent	Absent	Yes	0.00	0.000				
6	Absent	Present		5.37	0.099			> 10	
5	Present	Absent	Yes	0.00	0.000	> 10			

Laboratory identification code : **7**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	32.28	0.000				
3	Present	Absent	No	10.00	0.291	> 10			
7	Present	Present	No	5.00	0.241	> 10		> 10	
5	Present	Absent	Yes	31.09	0.000	> 10			
6	Absent	Present	No	5.00	0.188			> 10	
1	Absent	Present	No	10.00	0.294			2	fishbone
2	Absent	Absent	No	10.00	0.046				

Laboratory identification code : **9**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Absent	Present		10.00	0.378			> 10	
4	Absent	Absent	Yes	0.00	0.000				
3	Present	Absent		10.00	0.283	7			
6	Absent	Present		10.00	0.385			> 10	
2	Absent	Absent		10.00	0.092				
5	Present	Absent	Yes	0.00	0.000	> 10			
1	Absent	Absent		10.00	0.279				

Laboratory identification code : **10**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	20.00	0.000				
1	Absent	Absent	Yes	5.00	0.152				
2	Absent	Absent	Yes	5.00	0.457				
7	Present	Present	Yes	5.00	0.193	2	feather	> 10	fishbone, scale, gill, cartilage, meatfiber
3	Present	Absent	Yes	5.00	0.159	> 10	bone, meatfiber		
6	Absent	Present	Yes	5.00	0.203			> 10	fishbone,meatfiber,cartilage, scale, gill
5	Present	Absent	Yes	20.00	0.000	> 10	bone		

Laboratory identification code : **11**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	2.55	0.247				
7	Absent	Present	Yes	5.08	0.355			> 10	
5	Present	Absent	Yes	7.70	0.875	> 10			
6	Absent	Present	Yes	5.08	0.325			> 10	
3	Present	Absent	Yes	10.11	0.372	> 10			
2	Absent	Absent	Yes	10.40	0.098				
1	Absent	Absent	Yes	10.53	0.442				

Laboratory identification code : **12**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
3	Present	Absent	Yes	9.94	0.430	> 10			
6	Absent	Present	Yes	10.88	0.620			> 10	
7	Present	Present	Yes	9.96	0.500	> 10	2 bones and the rest feathers	> 10	
5	Present	Absent	Yes	0.00	0.000	> 10			
4	Absent	Absent	Yes	0.00	0.000				
2	Absent	Absent	Yes	10.00	0.060				
1	Absent	Present	Yes	9.92	0.410			4	3 fishbones and 1 foraminifer

Laboratory identification code : **13**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	No	5.00	0.032				
3	Present	Absent	No	5.00	0.138	8			
4	Absent	Absent	Yes	0.00	0.000				
1	Absent	Absent	No	5.00	0.148				
7	Absent	Present	No	5.00	0.106			> 10	
6	Absent	Present	No	5.00	0.144			> 10	
5	Present	Absent	Yes	0.00	0.000	> 10			

Laboratory identification code : **14**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	0.00	0.000				
3	Present	Absent	Yes	10.02	0.282	> 10			
7	Absent	Present	Yes	10.10	0.273			> 10	
1	Absent	Absent	Yes	10.05	0.300				
2	Absent	Absent	Yes	10.12	0.039				
6	Absent	Present	Yes	10.06	0.211			> 10	
5	Present	Absent	Yes	0.00	0.000	> 10			

Laboratory identification code : **15**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	Yes	10.00	0.145				
7	Absent	Present	Yes	10.00	0.569	1	feather	> 10	fishbones, scales, muscle fibres, etc. it can't be excludet, that the muscle fibres found, only derive from FM
5	Present	Absent	Yes	whole sample	0.000	> 10	bones, muscle fibres it can't be excludet, that the muscle fibres found, only derive from MBM		
3	Present	Absent	Yes	10.00	0.124	> 10	bones, muscle fibres it can't be excludet, that the muscle fibres found, only derive from MBM		
4	Absent	Absent	Yes	whole sample	0.000				
6	Absent	Present	Yes	10.00	0.477	1	feather	> 10	fishbones, scales, muscle fibres, etc. it can't be excludet, that the muscle fibres found, only derive from FM
1	Absent	Absent	Yes	10.00	0.209	1	muscle fibre no diff. between MBM and FM possible		

Laboratory identification code : **16**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
3	Present	Absent	Yes	10.06	0.260	> 10			
4	Absent	Absent	Yes	0.00	0.000				
1	Absent	Absent	Yes	10.00	0.270				
7	Absent	Present	Yes	11.38	0.620			> 10	
2	Absent	Absent	Yes	12.81	0.120				
5	Present	Absent	Yes	0.00	0.000	> 10			
6	Absent	Present	Yes	10.33	0.620			> 10	

Laboratory identification code : **17**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	50.25	0.013				
3	Present	Absent	No	10.05	0.280	> 10			
2	Present	Absent	No	10.09	0.114	5	hair (sheep) particles		
5	Present	Absent	Yes	52.50	0.022	> 10			
7	Absent	Present	No	10.08	0.445			> 10	
1	Absent	Absent	No	10.06	0.324				
6	Absent	Present	No	10.15	0.390			> 10	

Laboratory identification code : **18**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Absent	Present	No	5.00	0.176			> 10	
3	Present	Absent	No	10.00	0.282	> 10			
5	Present	Absent	Yes	40.00	0.082	> 10			
1	Absent	Absent	No	10.00	0.313				
6	Absent	Present	No	5.00	0.162			> 10	
4	Absent	Absent	Yes	40.00	0.046				
2	Absent	Absent	No	10.00	0.068				

Laboratory identification code : **19**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	Yes	10.00	0.116				
7	Absent	Present	Yes	3.00	0.157			> 10	fish bones, scales, gills, cartilages
1	Absent	Absent	Yes	10.00	0.319				
6	Absent	Present	Yes	3.00	0.123	3	bones	> 10	fish bones, scales, gills, cartilages
4	Absent	Absent	Yes	40.00	0.000				
5	Present	Absent	Yes	40.00	0.000	> 10	bones, muscles		
3	Present	Absent	Yes	10.00	0.242	8	bones		

Laboratory identification code : **20**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
3	Present	Absent	No	10.00	0.260	> 10	bones 1 muscle fiber		
2	Absent	Absent	No	10.00	0.090				
4	Absent	Absent	Yes	0.00	0.000				
7	Present	Present	No	10.00	0.370	> 10	only few hydrolised feathers(?)	> 10	fishbones, scales, gills, (muscle fibers)
6	Absent	Present	No	10.00	0.390			> 10	fishbones, scales, gills (muscle fibers)
5	Present	Absent	Yes	0.00	0.000	> 10	bones, (muscle fibers)		
1	Absent	Absent	No	10.00	0.310				

Laboratory identification code : **21**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	0.47	0.001				
6	Absent	Present	No	5.05	0.244			> 10	
7	Absent	Present	No	5.02	0.247			> 10	
2	Absent	Absent	No	10.04	0.072				
1	Absent	Absent	No	9.97	0.074				
5	Present	Absent	Yes	0.94	0.004	> 10			
3	Present	Absent	No	9.97	0.220	> 10			

Laboratory identification code : **22**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent		10.00	0.183				
4	Absent	Absent	Yes	10.00	0.021				
1	Absent	Absent		10.00	0.295				
3	Present	Absent		10.00	0.275	9	after seeing 4 slides of the sediment		
7	Absent	Present		10.00	0.377			> 10	
5	Present	Absent	Yes	10.00	0.011	> 10			
6	Absent	Present		10.00	0.339			> 10	

Laboratory identification code : **23**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	10.00	0.002				
1	Absent	Absent	No	10.00	0.165				
3	Present	Absent	No	10.00	0.152	4	bone		
7	Absent	Present	No	10.00	0.454			> 10	
2	Present	Absent	No	10.00	0.078	2	bone		
6	Absent	Present	No	10.00	0.342			> 10	
5	Present	Present	Yes	10.00	0.008	> 10		> 10	

Laboratory identification code : **24**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Absent	Present	No	5.00	0.172			> 10	
3	Present	Absent	No	5.00	0.107	7			
1	Absent	Absent	No	5.00	0.125				
5	Present	Absent	Yes	5.00	0.956	> 10			
6	Absent	Present	No	5.00	0.303			> 10	
2	Absent	Present	No	5.00	0.050			8	
4	Absent	Absent	Yes	5.00	0.809				

Laboratory identification code : **25**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Present	Present	No	10.00	0.187	7	feather, 1 bone	> 10	
3	Present	Absent	No	10.00	0.161	3	2 muscle fibers, 1 bone		
6	Absent	Present	No	10.00	0.161			> 10	
4	Absent	Absent	Yes	10.00	0.361				
2	Absent	Absent	No	10.00	0.068				
1	Absent	Absent	No	10.00	0.176				
5	Present	Absent	Yes	10.02	0.367	> 10	muscle fibers, bones		

Laboratory identification code : **26**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	30.00	0.090				
3	Present	Absent	No	10.00	0.251	9			
7	Absent	Present	No	10.00	0.263			> 10	
1	Absent	Absent	No	10.00	0.269				
2	Absent	Absent	No	10.00	0.124				
6	Absent	Present	No	10.00	0.306			> 10	
5	Present	Absent	Yes	30.00	0.000	10			

Laboratory identification code : **27**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Absent	Present	No	10.00	0.454			> 10	bones and muscle fibres
2	Absent	Present	No	10.00	0.041			7	bones and few muscle fibres
6	Absent	Present	No	10.00	0.477			> 10	bones and muscle fibres
3	Present	Present	No	10.00	0.245	> 10	bones and muscle fibres	1	bones
4	Absent	Absent	Yes	20.00	0.001				
5	Present	Absent	Yes	20.00	0.001	> 10	bones and muscle fibres		
1	Absent	Absent	No	10.00	0.252				

Laboratory identification code : **30**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Present	No	10.00	0.045				muscle, gill
7	Present	Present	No	10.00	0.304	4	bone	> 10	muscle, fishbone, scale, gill
4	Absent	Absent	Yes	10.00	0.001		none		none
6	Absent	Present	No	10.00	0.314			> 10	muscle, fishbone, scale, gill
5	Present	Absent	Yes	10.00	0.002	8	bones, muscle		
3	Present	Absent	No	10.00	0.155	4	muscle, bone		
1	Absent	Present	No	10.00	0.200		muscle		muscle

Laboratory identification code : **32**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Absent	Present	No	10.01	0.544			> 10	
3	Present	Absent	No	10.00	0.260	> 10			
4	Absent	Absent	Yes	0.00	0.000				
6	Absent	Present	No	10.01	0.541			> 10	
2	Absent	Absent	No	10.00	0.124				
5	Absent	Absent	Yes	0.00	0.000				
1	Absent	Absent	No	10.00	0.270				

Laboratory identification code : **33**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	No	2.02	0.419				
6	Present	Present	No	2.01	0.160	> 10	feather	> 10	
7	Present	Present	No	2.01	0.161	> 10	feather	> 10	
4	Present	Absent	No	2.05	0.020	> 10			
1	Present	Absent	No	2.02	0.075	9	bone		
3	Present	Absent	No	2.01	0.083	7	bone		
5	Present	Absent	No	2.07	0.055	> 10			

Laboratory identification code : **34**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Absent	Absent	Yes	49.27	0.000				
7	Present	Present	No	10.09	0.378	5	bone	> 10	
2	Absent	Absent	No	10.10	0.073				
6	Present	Present	No	10.07	0.382	2	bone	> 10	
3	Present	Absent	No	10.03	0.256	4	bone		
1	Absent	Absent	No	10.09	0.241				
5	Absent	Present	Yes	50.57	0.000			8	

Laboratory identification code : **35**

Sample N°	Terrestrial animal part.	Fish part.	Use of .9 optional protocol	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Absent	Absent	No	5.05	0.026				
6	Absent	Present	No	5.06	0.120			> 10	
3	Present	Absent	No	5.04	0.155	> 10			
4	Absent	Absent	Yes	0.00	0.000				
7	Absent	Present	No	5.06	0.159			> 10	
1	Absent	Absent	No	5.05	0.144				
5	Present	Absent	Yes	0.00	0.000	> 10			