CRL-AP Proficiency Test 2009

Final report

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Summary

On behalf of DG Sanco, the Community Reference Laboratory for animal proteins in feedingstuffs (CRL-AP) organized in 2009 the present proficiency test for all National Reference Laboratories (NRLs) having in charge the analyses of animal proteins in feedingstuffs in each of the Member States of the European Union (EU). The goal of the study was to evaluate the performance of the NRLs to apply the light microscopic method for qualitative detection of animal constituents in feedingstuffs as stated by Commission Regulation 2009/152/EC. This study also included some official control labs from countries outside EU.

The number of participants was of 33 (26 NRLs and 7 labs outside EU). A set of 9 blind samples was sent to each participant. Different feed matrices were used for the sample preparation. Some samples from the set were adulterated with terrestrial meat and bone meal or fish meal. Blank samples were sent in order to check for possible cross-contaminations. The homogeneity of the sample materials was tested by the organiser and considered as sufficient.

The study showed that a majority of participants obtained a very good level of global performance comparable to previous CRL-AP studies. The score of 69% NRL having excellent result is the best ever achieved for this EU network. Nevertheless some participants were still underperforming. The organisers asked for action plans in order to remediate to those underperformances.

The results showed that the limit of detection (LOD) for the method is of 0.0025% of MBM in feed. This value is in line with previous in-house test obtained by a join team from the CRL-AP and China Agricultural University. This indicates a need for reassessing the classically admitted 0.1% mentioned in the official method.

Keywords :

Meat and bone meals – Processed animal proteins – Light microscopy – Qualitative analysis

1. Foreword and aim of the study

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

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

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

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

(iii)   To develop new analytical methods for the detection of animal proteins in feedingstuffs (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 CRL-AP organized in 2009 a proficiency test aiming to evaluate the performance of the NRLs to detect the presence of processed animal proteins by the light microscopic method as stated in the Annex VI of Commission Regulation 2009/152/EC. On proposal of the Commission, invitations to participate the proficiency test were also sent to some official control labs outside the EU.

The final version of this report has been prepared according to a first draft version submitted for comments and revision to the NRL network. Results of this report were discussed during the 4th CRL-AP Annual Workshop, held in Turin, Italy on the 28th and 29th of April 2010.

2. Introduction

On the 9th November 2009, a set of 9 blind samples for the CRL-AP Proficiency Test 2009 have been sent by express shipment to the 26 NRLs and to 7 laboratories outside this EU network. Those seven foreign participants were the Canadian Food Inspection Agency, the US Food and Drug Administration, the Croatian Veterinary Institute, the Stellenbosh University from South Africa, the Servicio Nacional de Sanidad y Calidad Agroalimentaria from Argentina, the China Agricultural University, the Food and Agricultural Materials Inspection Center from Japan. The detailed list of the 33 participating labs is included in Annex 1.

The following instructions have been given to each participating lab:

- Analysis of the 9 blind samples by applying the protocol described in Annex VI of Commission Regulation 2009/152/EC [1].

- Mention has been 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.

- Qualitative analyses have been requested for each of the 9 samples. Participants were asked to provide additional data such as the number of slides observed, whether or not they observed the whole slides, the sample and sediment weights, the number of particles they had detected to support their conclusions and to further specify the exact nature of the particles when their number were less or equal to 5.

- The results had to be encoded by way of an Excel report form -downloadable from the CRL-AP intranet (Annex 2) or sent to the foreign participants who do not have access to this intranet. Participants were asked to carefully read the instructions on how to fill in the result form and to testify they did it prior to encoding their results. No other support for communicating the results was accepted.

- A summarized results sheet was automatically generated without the need for the participant of re-encoding the data. Participants were asked to sign the summarized results sheet and to send it by fax to the CRL-AP. Results were taken into consideration only when both the Excel file and the fax were received.

- The results had to be sent in both forms concomitantly to the CRL-AP by the 8th December 2009. Notification has been done that this date was a deadline and that results arriving later would not be accepted. A shift of the deadline was nevertheless proposed for participants outside EU due to custom related delays in delivery of the samples.

Results from 32 labs were accepted on a total of 33 participating laboratories. One participant of a country outside the EU did not report its results.

Results from NRLs or from participants outside the NRL network were analysed separately in this report.

3. Material and methods

3.1. Material

3.1.1. Description of the samples

Eight different samples containing typical feed ingredients and/or processed animal proteins (PAPs) from various animal origin at different concentration levels have been prepared as shown in table 1.

The composition was established taking into account the following features:

- o Target concentrations of mammalian meat and bone meal (referred to as MBM through the text) inferior to the classical 0.1% considered for the time being as the adulteration level that the method should be able to detect.

- o Presence of fishmeal that could interfere with the detection of constituents from terrestrial animals when using light microscopy [2] (the so-called "masking effect").

- o Feed matrix conditioning (milled or pelleted) that requires grinding before analysis as requested by Commission Regulation 2009/152/EC.

Each participating lab received about 55g of 9 blind samples to which a unique random number was assigned. Details of the samples are indicated in table 1.

Table 1: Composition of blind samples set used in the CRL-AP Proficiency Test 2009.

Sample	Material	Nr of replicates
1	Blank A	1
2	Blank B (Pellets)	2
3	0.01% MBM	1
4	0.005% MBM	1
5	0.0025% MBM	1
6	Pure fish	1
7	Pure fish + 0.5% MBM	1
8	Pellets + 0.1% MBM	1
Total		**9**

3.1.2. Materials used in the preparation of the samples

The **first feed matrix** used for Blank A was a classical compound feed produced by a local plant. The matrix is composed of wheat, corn and corn glutenfeed, soya, rapeseed, palm kernel and coconut meal, beet pulp, citrus pulp, molasse, minerals and vitamins. Sediment content of this compound feed was about 0.6% (STD 0.08%).

A **second feed matrix** was a pelleted feed supplement for bovines from a producer. It is composed of rapeseed and palm cattle cake, wheat and wheat glutenfeed, corn, soya bean, barley beat pulp, salts, minerals and vitamins. Its sediment content was about 0.6% (STD 0.06%). This feed matrix was used for the Blank B and the Pellets + 0.1% MBM. It was also used but after grinding for the preparation of the 0.01%, 0.005% and 0.0025% MBM materials.

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

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

- The **MBM** used was a mix of 50% ovine-porcine meat and bone meal and 50% pure bovine meat and bone meal treated at least at 133°C, 3 bars fo r 20 min. Its final bone content was of about 48%. Its purity was controlled by PCR.

- The **fish meal** used for sample 6 was originating from Scotland. The fishbone content was of about 13%. Purity of the fish meal was controlled by PCR.

- The **fish meal** used for sample 7 came from Chili. The fishbone content was of about 14%. Purity of the fish meals was investigated by PCR.

3.1.3. Description of the mixing procedures

The **stepwise dilution procedure** developed by CRA-W and JRC-IRMM was used to produce the following samples: 3, 4, 5 and 7. This procedure has been successfully used in numerous former European interlaboratory studies aiming to evaluate different light microscopy protocols.

The **spiking procedure** was used for the production of sample 8.

3.2. Qualitative analysis

Qualitative analysis concerned the presence or absence of terrestrial (MBM) and/or fish. 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 – Results of the detection of terrestrial and fish particles by light microscopy

Material	Nr of replicates analysed (Nr of total slides)	Terrestrial	Mean nr of terrestrial particles / slide	Fish	Remark
Blank A	5 (13)	-	ND	-	
Blank B (Pellets)	5 (15)	-	ND	-	ground samples
0.01% MBM	5 (15)	+	10.7	-	
0.005% MBM	5 (15)	+	5.2	-	
0.0025% MBM	5 (15)	+	2.9	- (1)	
Pure fish	3 (11)	-	ND	+	
Pure fish + 0.5% MBM	5 (15)	+	4.7	+	
Pellets + 0.1% MBM	5 (15)	+	>10	-	ground samples

Legend: + = present, - = not present, ND = not determined

The homogeneity was studied on 10g of sample material for each replicate. Only 3 g were used for the fish containing samples. For the homogeneity study the sediment fraction was analysed, flotate was examined only when needed (e.g. for the blanks).

Blank A and **Blank B** were negative for any presence of animal material.

In the **0.01% MBM** the presence of terrestrial bones was systematically observed (i.e. on each slide particles were found). No fish particles were ever noted on the total of 15 slides. The average number of terrestrial particles per slide was of 10.7.

In the **0.005% MBM** the presence of terrestrial bones was systematically observed (i.e. on each slide particles were found). No fish particles were ever noted on the total of 15 slides. The average number of terrestrial particles per slide was of 5.2.

In the **0.0025% MBM** the presence of terrestrial bones was observed in all replicates (only 3 slides out of 15 did not present terrestrial bones). On the exception of a single particle, no fish particles were noted on the total of 15 slides. The average number of terrestrial particles per slide was of 2.9.

The **pure fish** used did not present terrestrial bones.

For the **pure fish + 0.5% MBM** sample, the presence of terrestrial bones was reported for each of the 5 sediments analysed. Only one slide on the total of 15 was free from any terrestrial bones.

In the **pellets + 0.1% MBM** sample, terrestrial bones were systematically reported.

4.2. Qualitative analyses from the NRLs

4.2.1. <u>Preliminary remark</u>

On the respect of the legislation:

- Lab 3 did not respect the Commission Regulation 2009/152/EC instructions stating that at least 5g of the sample are required for analyses (on the sieve fractions or sediment). Amounts of 2g were taken for the sedimentation by this lab, but only for samples 6 and 7 based on pure fish meals.

- Labs 1, 27 and 32 did not respect the Commission Regulation 2009/152/EC instructions indicating that when fish is detected at least 3 slides have to be observed: "… at least two additional slides […] and the total sediment fraction shall be examined."

- Lab 1 reported some results based on a single slide observation.

4.2.2. <u>Overview of results and performance of the method</u>

Table 3 summarizes the results submitted by the 26 NRLs for the eight types of materials submitted to qualitative analysis.

Table 3: Global results expressed as accuracy (AC) for the eight samples

Sample	Material	n	AC	
			Terrestrial	**Fish**
1	blank A	26	0.846 (4)	0.885 (3)
2	blank B (Pellets)	52	0.885 (6)	0.846 (8)
3	0.01% MBM	26	1.000	0.923 (2)
4	0.005% MBM	26	0.962 (1)	0.885 (3)
5	0.0025% MBM	26	0.962 (1)	0.885 (3)
6	pure fish	26	0.923 (2)	1.000
7	pure fish + 0.5% MBM	26	0.923 (2)	1.000
8	pellets + 0.1% MBM	26	0.923 (2)	0.885 (3)

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

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

The ratio of false positive results reported for the blank materials A (sample 1) and B (sample 2) is of 13% (or 10/78) and for terrestrial particles of 14% (or 11/78). These percentages are relatively higher than those observed from the last CRL-AP PT 2008 study [3].

The correct detection of 0.01% MBM (sample 3) in a ground matrix is faultless. Nevertheless two NRLs have false positive results for the presence of fish (2/26 or 8%).

The detection of MBM at lower levels, 0.005% (sample 4) and 0.0025% (sample 5), in the same ground matrix is almost faultless too with only one false negative result for the presence of terrestrial particles (1/26 or 4%) for both concentrations. Three NRLs falsely detected fish (3/26 or 12%) for both adulteration levels.

Fish particles are perfectly identified in a pure fish meal (sample 6). There are however 8% (2/26) of false positive detection of terrestrial particles in this pure fish meal by two NRLs.

When a pure fish meal is adulterated at 0.5% MBM (sample 7), 8% of false negative results were recorded for the detection of terrestrial animal particles.

Finally the detection of 0.1% MBM added to a pelleted matrix (sample 8) generated only 8% (2/26) of false negative for terrestrial particles but produced also some false positive results for fish: 12% (3/26).

There are some few cases of "no results" reports. For reminder this was one of the possible statements in case of inconclusive results on the presence or the absence of relevant material or in case of inability of the laboratory to provide a result. The details of those "no results" are:

- Lab 23 for blank B sample (1 for fish). It observed only 1 particle possibly identified as fish bone.

- Lab 19 for blank B sample (2 for terrestrial, 2 for fish). It observed 2 blood cells and 1 fish bone.

- Lab 22 for pellets + 0.1% MBM (1 for terrestrial, 1 for fish). It only detected a large number (11 to 50) of muscle fibres but no bones on a total of 13 slides.

4.2.3. Detailed review of results for each sample material

Blank A :

Some terrestrial particles were detected:

- Lab 6 reported 1 terrestrial bone on a total of 3 slides

- Labs 18, 22 and 26 detected particles identified as feathers accordingly following details: feather fragments on a total of 3 slides, 6-10 feathers on a total of 10 slides, unspecified feather particles on a total of 15 slides.

- Lab 19 reported the presence of 2 blood cells from terrestrial animal out of 12 slides (but considered it as negative for terrestrial)

Fish particles were detected:

- Lab 1 detected 2 fish bones on a total of 2 slides (but considered it as negative for fish)

- Lab 17 detected 6-10 fish particles on a total of 4 slides.

- Lab 19 and 23 reported respectively 3 fish bones on 12 slides, and 1 fish scale on 5 slides

Blank B :

Terrestrial particles were reported as follows:

- Labs 3, 9 and 13 detected for one replicate 2 terrestrial bones on a total of respectively 5, 6 and 4 slides

- Lab 18 reported for one replicate the presence of feather meal on a total of 3 slides.

- Lab 19 reported for one replicate the presence of 2 blood cells of terrestrial animal on a total of 10 slides.

Presence of fish was also reported as follows:

- Lab 1 observed for one replicate 5 fishbones on 2 slides (but considered it as negative for fish)

- Lab 12 reported for one replicate 3 fishbones on 12 slides.

- Lab 17 detected for one replicate 6-10 fish particles on 4 slides.

- Lab 19 reported for one replicate the presence of 1 fishbone on a total of 11 slides.

- Lab 23 observed for one replicate1 particle possibly identified as fishbone on a total of 5 slides.

- Lab 26 detected on both replicates fish particles (6-10 and 11-50 each time on a total of 15 slides.

0.01% MBM:

Some presence of fish was reported:

- Lab 4 reported the presence of 5 fish bones on a total of 3 slides.

- Lab 11 detected 3 fish bones on a total of 5 slides.

In addition some other labs identified fish particles but considered the sample as negative for fish:

  - Lab 1 detected 4 fish bones on a total of 2 slides.

  - Lab 19 detected 1 fishbone on a total of 13 slides.

0.005% MBM:

Only one lab failed to detect the presence of terrestrial particles (Lab 19).

Few labs reported fish particles:

- Lab 11 detected 3 fish bones on a total of 8 slides.

- Lab 19 detected 5 fish bones on a total of 10 slides.

- Lab 26 reported 6-10 particles of fish origin on a total of 15 slides.

In addition some other labs identified fish particles but considered the sample as negative for fish:

  - Lab 1 detected 5 fish bones on a total of 2 slides.

0.0025% MBM:

Only one lab declared this sample as negative for terrestrial although having detected 2 terrestrial bones (Lab 19).

Few labs reported fish particles:

- Lab 11 detected 2 fish bones on a total of 13 slides.

- Lab 12 detected 2 atypical fish bones on a total of 15 slides.

- Lab 26 reported 6-10 particles of fish origin on a total of 15 slides.

In addition some other labs identified fish particles but considered the sample as negative for fish:

  - Lab 1 detected 4 fish bones on a total of 2 slides.

  - Lab 19 detected 2 fish bones on a total of 10 slides.

Pure fish

Two labs reported the presence of terrestrial particles:

- Lab 18 and 29 detected 6-8 terrestrial particles on a total of respectively 3 and 4 slides.

Pure fish + 0.5% MBM :

Lab 23 failed to detect the presence of terrestrial particles.

Labs 19 considered the sample as negative although having found 2 particles (feather and bone) from terrestrial origin on a total of 11 slides.

Pellets + 0.1% MBM :

Only Lab 18 did not detect terrestrial particles from this sample.

Two labs reported fish particles:

- Lab 1 detected over 50 fish bones and scales on 1 slide
- Lab 11 found 2 fish bones on a total of 5 slides

Lab 22 only detected muscle and no other type of animal particles through a total of 13 slides.

4.2.4. Detection of MBM at low concentration levels

During the 3rd CRL-AP Annual Workshop, held in Gembloux in March 2009, results on the estimation of the limit of detection (LOD) were presented. Results, obtained by the collaborative team from the CRL-AP and Chinese Agricultural University, showed that the LOD of the light microscopic method for the detection of animal proteins in feed was, for the samples analysed, far below the classically admitted 0.1% adulteration. The values for LOD obtained were systematically found to be around the 0.0025% of contamination (with a β-error < 5%). The average number of animal particles detected was of 2-3.

By using samples 0.01%, 0.005% and 0.0025% MBM, the organiser of the present proficiency test wanted to outline the ability of the participants to detect low contamination levels and to validate by large scale testing an LOD value for the microscopic method.

Results (table 3) showed that even at the lowest MBM concentration, the sensitivity was of 0.962, or in other terms the β-error (1 – sensitivity) was of 0.038 or equals to 3.8%. This is perfectly in line with the previous proposals for setting the LOD value around 0.0025% for the method on basis of the experiments conducted at the CRL-AP.

Interestingly, data collected on the details of the particles found allow fine-tuning of expression of results in term of number of particles being detected.

When calculating the average number of MBM particles detected per slide (minimum and maximum numbers) through the 26 participants the following values where found:

Table 4: Averages of numbers of particles per slide at low concentration levels

	MBM concentration (in %)		
	0.0025	0.005	0.01
av. min nb of particles per slide	0.7	1.1	1.5
av. max nb of particles per slide	1.3	3.3	5.1

From these experimental values (Table 4) a modelling for the numbers of animal particles from the observation of 6 slides, which is the mean slide number used by the participants for the three samples, delivers the following information (Fig. 1). The minimum number of animal particles detectable around the LOD value is 4.4 with a β-error of 0.038. In order to guarantee a reproducible result it is needed to go up to the next entire particle number, what will furthermore reduce somehow the β-error. 5 particles from 6 slides is thus the smallest experimentally proved unit of observation for a truly reliable positive agreement on the presence of MBM in feed.
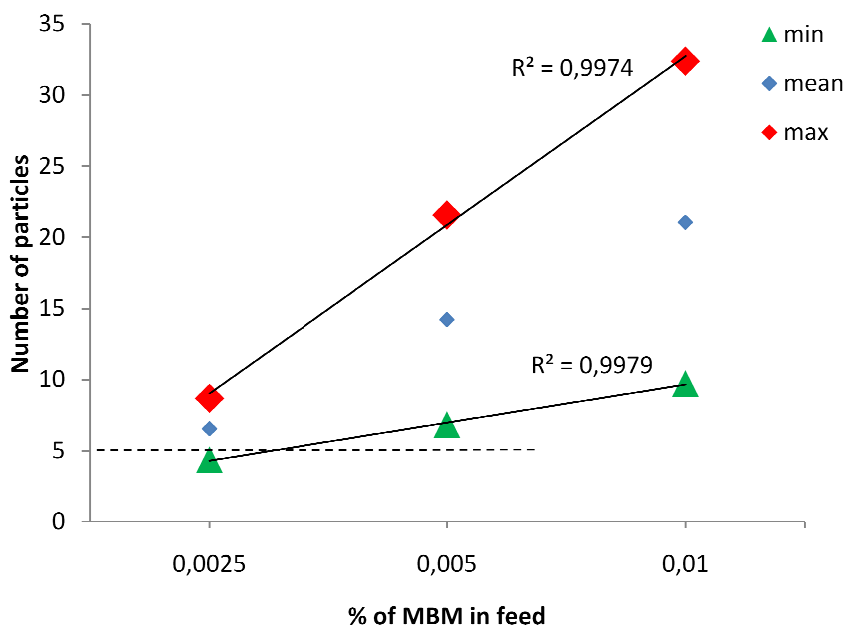
Figure 1: Number of MBM particles detected on 6 slides. (min = average minimum number, max = average maximum number, mean = theoretical mean, R^2 = coefficients of determination)

4.2.5. 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 accuracy.

Results are to be found in tables 5 and 6 (next page).

Concerning the ability to detect terrestrial animal constituents, 10 labs provided incorrect results according to the following details (table 5):

- PD for MBM in blank A : labs 6, 18, 22, 26
- PD for MBM in blank B : labs 3, 9, 13, 18, 19
- ND for MBM in 0.005% MBM : lab 19
- ND for MBM in 0.0025% MBM : lab 19
- PD for MBM in pure fish : labs 18 and 29

- ND for MBM in pure fish + 0.5% MBM : labs 19 and 23

- ND for MBM in the pellets + 0.1% MBM : labs 18, 22

Concerning the ability to detect fish material, 9 labs encountered problems (table 6):

- PD for fish in blank A : labs 17, 19, 23

- PD for fish in blank B : labs 11, 12, 17, 19, 23, 26

- PD for fish in 0.01% MBM : labs 4 and 11

- PD for fish in 0.005% MBM : labs 11, 19 and 26

- PD for fish in 0.0025% MBM : labs 11, 12 and 26

- PD for fish in the pellets + 0.1% MBM : labs 1, 11 and 22

**Tables 5 (left) and 6 (right): NRL proficiencies regarding the detection
of terrestrial and fish material. Ranking follows AC values.**

Terrestrial				Fish			
lab code	AC	SE	SP	lab code	AC	SE	SP
1	1.000	1.000	1.000	2	1.000	1.000	1.000
2	1.000	1.000	1.000	3	1.000	1.000	1.000
4	1.000	1.000	1.000	5	1.000	1.000	1.000
5	1.000	1.000	1.000	6	1.000	1.000	1.000
10	1.000	1.000	1.000	9	1.000	1.000	1.000
11	1.000	1.000	1.000	10	1.000	1.000	1.000
12	1.000	1.000	1.000	13	1.000	1.000	1.000
14	1.000	1.000	1.000	14	1.000	1.000	1.000
16	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
20	1.000	1.000	1.000	20	1.000	1.000	1.000
24	1.000	1.000	1.000	24	1.000	1.000	1.000
25	1.000	1.000	1.000	25	1.000	1.000	1.000
27	1.000	1.000	1.000	27	1.000	1.000	1.000
32	1.000	1.000	1.000	29	1.000	1.000	1.000
34	1.000	1.000	1.000	32	1.000	1.000	1.000
3	0.889	1.000	0.750	34	1.000	1.000	1.000
6	0.889	1.000	0.750	1	0.889	1.000	0.857
9	0.889	1.000	0.750	4	0.889	1.000	0.857
13	0.889	1.000	0.750	22	0.889	1.000	0.857
26	0.889	1.000	0.750	12	0.778	1.000	0.714
29	0.889	1.000	0.750	17	0.778	1.000	0.714
23	0.889	0.800	1.000	23	0.778	1.000	0.714
22	0.778	0.800	0.750	19	0.556	1.000	0.429
18	0.556	0.800	0.250	26	0.556	1.000	0.429
19	0.444	0.400	0.500	11	0.444	1.000	0.286

A general ranking of the participants was performed on a consolidated evaluation; including their proficiency in detecting both terrestrial and fish materials through the 10 blind samples (table 7):

Table 7: 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
5	1.000	1.000	1.000
10	1.000	1.000	1.000
14	1.000	1.000	1.000
16	1.000	1.000	1.000
20	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
27	1.000	1.000	1.000
32	1.000	1.000	1.000
34	1.000	1.000	1.000
1	0.944	1.000	0.909
3	0.944	1.000	0.909
4	0.944	1.000	0.909
6	0.944	1.000	0.909
9	0.944	1.000	0.909
13	0.944	1.000	0.909
29	0.944	1.000	0.909
12	0.889	1.000	0.818
17	0.889	1.000	0.818
22	0.833	0.857	0.818
23	0.833	0.857	0.818
18	0.778	0.857	0.727
11	0.722	1.000	0.545
26	0.722	1.000	0.545
19	0.500	0.571	0.455

The table illustrates the very good level of global performance (= consolidated AC superior to 0.90, *i.e.* having just one false result) for 18 labs out of 26 NRLs or in other words for 69% of the NRLs.

A second category (cells in blue in table 7) of NRLs having a satisfying global performance is defined (= consolidated AC below 0.90 <u>and</u> having no more than three false results including a maximum of two ND for terrestrial material). NRLs included in this category are nevertheless asked to report to the CRL-AP on the possible source of these deviations. Attention has to be paid by the lab 22 that had missed two times the detection of terrestrial material (cells in blue underlined).

A third category (cells in red in table 7) includes participants that are underperforming (= consolidated AC below 0.90 <u>and</u> having either at least four false results or three ND for terrestrial). Those participants require improvement of proficiency. These participants are asked to report on the origin of those multiple errors as well as on the actions they will undertake in order to solve this critical issue.

4.3. Qualitative analyses from the non-EU participants

4.3.1. Individual performances of other participants in qualitative analysis

Individual performances from the 6 participants outside the EU were assessed exactly as in previous section (4.2.4.). A ranking of those labs was prepared based on the accuracy.

Results are to be found in tables 8 and 9.

Tables 8 (left) and 9 (right): Lab proficiencies regarding the detection of terrestrial and fish material. Ranking follows AC values.

Terrestrial				Fish			
lab code	AC	SE	SP	lab code	AC	SE	SP
33	1.000	1.000	1.000	15	1.000	1.000	1.000
28	0.889	1.000	0.750	28	1.000	1.000	1.000
7	0.778	0.600	1.000	33	1.000	1.000	1.000
15	0.778	0.600	1.000	30	0.667	1.000	0.571
31	0.556	1.000	0.000	7	0.556	1.000	0.429
30	0.444	0.200	0.750	31	0.222	1.000	0.000

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

- PD for MBM in blank A : labs 28 and 31
- PD for MBM in blank B : lab 31
- ND for MBM in 0.005% MBM : labs 7 and 30
- ND for MBM in 0.0025% MBM : labs 7, 15 and 30
- PD for MBM in Pure fish : labs 30 and 31
- ND for MBM in Pure fish + 0.5% MBM : lab 30
- ND for MBM in the pellets + 0.1% MBM : lab 15

Concerning the ability to detect fish material, 9 labs encountered problems:

- PD for fish in blank A : labs 7 and 31
- PD for fish in blank B : labs 30 and 31
- PD for fish in 0.01% MBM : labs 7 and 31
- PD for fish in 0.005% MBM : labs 7, 30 and 31
- PD for fish in 0.0025% MBM : labs 7 and 31
- PD for fish in the pellets + 0.1% MBM : lab 31

Ranking of the non-EU participants was also realized on a consolidated evaluation; including their proficiency in detecting both terrestrial and fish materials through the 9 blind samples based on the same criteria as defined in the above section (table 10):

Two participants (labs 33 and 28) obtained an excellent level of global performance with respectively a faultless set of answers, and a single PD.

Lab 15 obtained a satisfying result (cells in blue in table 10).

The other three participants were underperforming (cells in red in table 10) according to EU standards.

Table 10: General lab proficiency regarding the detection of terrestrial and fish material. Ranking follows AC values as primary key and SE as second key.

Consolidated lab code	AC	SE	SP
33	1.000	1.000	1.000
28	0.944	1.000	0.909
15	0.889	0.714	1.000
7	0.667	0.714	0.636
30	0.556	0.429	0.636
31	0.389	1.000	0.000

5. Conclusions

The study aiming at evaluating the proficiency of the participants to detect prohibited animal proteins delivered good results. The performance of the NRL network is kept at an excellent level. The false detections on blank materials are nevertheless slightly higher when compared to the CRL-AP PT 2008 study [3]. However the number of false positive on the blanks occurring from the observation of barely 1-3 particles of animal origin (either terrestrial of fish) accounts for a large proportion of those erroneous decisions. Such situation will be improved in the future when considering the data collected on the samples adulterated at very low levels of MBM concentration.

Effectively the design of the study enables to experimentally test the proposal for a limit of detection (LOD) made by the CRL-AP during the 2008 CRL-AP Annual Workshop. The ability of the NRL network to detect MBM at levels that low as 0.01%, 0.005% and 0.0025% revealed to be above the organiser's expectations. The detection of MBM had an optimal sensitivity at 0.01%. The sensitivity score obtained for the disclosure of MBM at 0.0025% authorises to reconsider the LOD value of 0.1% from the Commission Regulation 2009/152/EC text.

This study also made a proposal on a minimum number of particles that needs to be taken into account before taking a decision with an acceptable β-error (< 5%). A minimum of 5 particles based on 6 slides is required to obtain a reliable result. Results based on lower numbers of particles must be considered as non conclusive.

Among the NRLs, 69% of them obtained a very good level of global performance. This is the best score achieved since proficiency test are organised for this network. However four NRLs are considered as underperforming and are asked to take actions in order to improve their proficiency.

The global results table on the method performance reveals once again a small problem of specificity for the detection of fish. This was already noted from past studies [3, 4, 5] and is related to the diversity of fishmeal particles that can easily be confused with particles from the used matrices [3]. Once more the utilisation of Alizarin Red for a better distinction between atypical plant and fish particle is recommended.

Concerning the participants outside EU, two performed successfully and three of them failed. Although the instructions specified to use the official EU method, the organiser however cannot state on the respect of this clause. From the NRL network experience, coordinated by the CRL-AP since 2006, it is clearly established that training is absolutely required for the improvement of the detection capabilities. So participation to organised training sessions dedicated to PAPs detection in feed can only be recommended for laboratories that are performing less well.

Acknowledgment

6. References

[1] 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.

[2] Gizzi G, von Holst C, Baeten V, Berben G and van Raamsdonk L. 2003. Intercomparison study for the determination of processed animal proteins including meat and bone meal in animal feed. Joint Research Centre IHCP, Ispra, Italy.

[3] Veys P, Berben G and Baeten V. 2008. CRL-AP Proficiency Test 2008: Final report. CRA-W, Gembloux, Belgium

[4] Veys P and Baeten V. 2007. CRL-AP Interlaboratory Study 2006 : Final report. CRA-W, Gembloux, Belgium

[5] Veys P, Berben G and Baeten V. 2007. CRL-AP Proficiency Test 2007: Final report. CRA-W, Gembloux, Belgium

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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
Canada	*Canadian Food Inspection Agency*
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	National Diagnostic Centre of Food and Veterinary Service
Lithuania	National Veterinary Laboratory
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Poland	National Veterinary Research Institute
Portugal	Laboratorio Nacional de Investigaçao Veterinaria
Romania	Hygiene Institute of Veterinary Health
Slovakia	State Veterinary and Food Institute
Slovenia	Veterinary Faculty-National Veterinary Institute-Unit for pathology of animal nutrition and environmental hygiene
South Africa	*Stellenbosch University*
Spain	Laboratorio Arbitral Agroalimentario
Sweden	National Veterinary Institute, Department of Animal Feed
United Kingdom	Veterinary Laboratories Agency
USA	*US Food and Drug Administration*

Annex 2

Excel result report form.

Annex 3

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

Laboratory identification code :   1

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
5	Present	Not present	2	Yes	10	0.043	6 to 10	4 bone fragments in a first extract and 6 bone fragments in a second extract	4	2 fish-bone fragments in a first extract and 2 fish-bone fragments in a second extract
6	Not present	Present	3	Yes	10	0.864			> 50	fish-bone and scale fragments, muscular fibers in sieved fraction
8	Present	Present	1	Yes	10	0.04	> 50	bone fragments	> 50	fish-bone and scale fragments
2	Not present	Not present	2	Yes	10	0.383			5	3 fish-bone fragments in a first extract and 2 fish-bone fragments in a second extract
1	Not present	Not present	2	Yes	10	0.026			4	2 fish-bone fragments in a first extract and 2 fish-bone fragments in a second extract
4	Present	Not present	2	Yes	10	0.034	11 to 50	6 bone fragments in a first extract and 9 bone fragments in a second extract	5	4 fish-bone fragments in a first extract and 1 fish-bone fragment in a second extract
2	Not present	Not present	1	Yes	10	0.03				
7	Present	Present	3	Yes	10	0.608	11 to 50	bone fragments	> 50	fish-bone and scale fragments, muscular fibers in sieved fraction
3	Present	Not present	2	Yes	10	0.034	11 to 50	16 bone fragments in a first extract and 8 bone fragments in a second extract	4	2 fish-bone fragments in a first extract and 2 fish-bone fragments in a second extract

Laboratory identification code :   2

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
6	Not present	Present	20	Yes	5.03	0.772			> 50	
5	Present	Not present	10	Yes	5.333	0.054	6 to 10			
8	Present	Not present	10	Yes	5.819	0.03	> 50			
2	Not present	Not present	10	Yes	5.218	0.032				
1	Not present	Not present	10	Yes	5.091	0.039				
7	Present	Present	20	Yes	5.056	0.761	11 to 50		> 50	
2	Not present	Not present	5	Yes	5.16	0.019				
4	Present	Not present	10	Yes	5.42	0.05	6 to 10			
3	Present	Not present	10	Yes	5.327	0.059	6 to 10			

Laboratory identification code :   3

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Present	Not present	3	Yes	10.04	0.057	11 to 50			
6	Not present	Present	3	Yes	2.01	0.198			> 50	
1	Not present	Not present	2	Yes	10	0.052				
7	Present	Present	3	Yes	2.02	0.224	> 50		> 50	
8	Present	Not present	3	Yes	10	0.076	> 50			
2	Not present	Not present	4	Yes	10.02	0.07				
5	Present	Not present	3	Yes	10.02	0.055	11 to 50			
3	Present	Not present	3	Yes	10	0.058	> 50			
2	Present	Not present	5	Yes	9.99	0.074	2	bone particles		

Laboratory identification code :   4

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
6	Not present	Present	10	Yes	5	0.694			> 50	
5	Present	Not present	2	Yes	10	0.08	2	bones		
2	Not present	Not present	3	Yes	10	0.087				
8	Present	Not present	6	Yes	10	0.094	> 50			
3	Present	Present	3	Yes	10	0.09	6 to 10		5	fishbone
1	Not present	Not present	2	Yes	10	0.05				
7	Present	Present	8	Yes	5	0.68	11 to 50		> 50	
2	Not present	Not present	3	Yes	10	0.076				
4	Present	Not present	3	Yes	10	0.073	11 to 50			

Laboratory identification code :   5

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
1	Not present	Not present	7	Yes	10	0.075				
5	Present	Not present	8	Yes	10	0.085	6 to 10			
8	Present	Not present	7	Yes	10	0.079	> 50			
2	Not present	Not present	8	Yes	10	0.082				
6	Not present	Present	20	Yes	10	1.588			> 50	
2	Not present	Not present	9	Yes	10	0.077				
3	Present	Not present	8	Yes	10	0.093	11 to 50			
4	Present	Not present	9	Yes	10	0.084	11 to 50			
7	Present	Present	9	Yes	10	1.532	> 50		> 50	

Laboratory identification code :　6

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
5	Present	Not present	6	Yes	10	0.07	1	land animal bone		
6	Not present	Present	8	Yes	10.06	1.61			> 50	
1	Present	Not present	3	Yes	10.02	0.04	1	land animal bone		
8	Present	Not present	2	Yes	10.03	0.1	> 50	land animal bone and cartilage		
7	Present	Present	9	Yes	10.03	1.49	11 to 50	land animal bones	> 50	
4	Present	Not present	6	Yes	10.05	0.06	6 to 10	land animal bones		
2	Not present	Not present	3	Yes	10.03	0.1				
2	Not present	Not present	3	Yes	10.03	0.1				
3	Present	Not present	3	Yes	10.04	0.09	11 to 50	land animal bone		

Laboratory identification code :　7

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
6	Not present	Present		No	1	0			> 50	
8	Present	Not present	1	Yes	1	0	6 to 10			
2	Not present	Not present	1	Yes	1	0				
1	Not present	Present		No	1	0			11 to 50	
5	Not present	Present	1	Yes	1	0			2	fishbone
2	Not present	Not present		No	1	0				
3	Present	Present		No	1	0	2	bone	3	fishbone
7	Present	Present		No	1	0	6 to 10		11 to 50	
4	Not present	Present		No	1	0			11 to 50	

Laboratory identification code :　9

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
6	Not present	Present	12	Yes	5.0056	0.748			> 50	
8	Present	Not present	6	Yes	10.077	0.11	> 50			
1	Not present	Not present	3	Yes	10.12	0.07				
7	Present	Present	12	Yes	5.0983	0.729	> 50		> 50	
2	Not present	Not present	4	Yes	10.044	0.071				
2	Present	Not present	6	Yes	10.081	0.09	2	bone particles		
3	Present	Not present	5	Yes	10.054	0.073	11 to 50			
4	Present	Not present	6	Yes	10.074	0.084	6 to 10			
5	Present	Not present	6	Yes	10.063	0.077	5	bone particles		

Laboratory identification code : 10

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
6	Not present	Present	5	Yes	5.0019	0.805			> 50	fishbones, scales, muscles
1	Not present	Not present	5	Yes	5.0016	0.032				
8	Present	Not present	5	Yes	5.0016	0.048	6 to 10	bones, muscle		
2	Not present	Not present	5	Yes	5.0006	0.043				
5	Present	Not present	5	Yes	5.0015	0.044	5	bones		
2	Not present	Not present	5	Yes	5.0007	0.045				
3	Present	Not present	5	Yes	5.0018	0.047	11 to 50	bones		
4	Present	Not present	5	Yes	5.0005	0.047	11 to 50	bones		
7	Present	Present	5	Yes	5.0007	0.795	11 to 50	bones, muscles	> 50	fishbones, scales, muscles

Laboratory identification code : 11

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Present	Present	8	Yes	10	0.08	6 to 10	bones	3	bones
5	Present	Present	13	Yes	10	0.081	5	bones	2	bones
1	Not present	Not present	6	Yes	10	0.074				
2	Not present	Not present	9	Yes	10	0.085				
6	Not present	Present	20	Yes	10	1.596			> 50	bones, scale, muscles, cartilage, teeth, skin
8	Present	Present	5	Yes	10	0.097	11 to 50	bones	2	bones
3	Present	Present	5	Yes	10	0.083	11 to 50	bones	3	bones
7	Present	Present	8	Yes	10	1.452	6 to 10	bones	> 50	bones, scale, muscles, cartilage, teeth, skin
2	Not present	Present	4	Yes	10	0.063			1	bones

Laboratory identification code : 12

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Present	Not present	18	Yes	5.01	0.152	5	T-bone, low contamination		
5	Present	Present	15	Yes	5.23	0.12	2	T- bone, low contamination	2	Fish bone, morphology is not very clear (not classical fish bone)
2	Not present	Not present	15	Yes	5.1	0.11				
1	Not present	Not present	15	Yes	5.4	0.095				
2	Not present	Present	12	Yes	5.34	0.164			3	Fish bone, very low contamination
6	Not present	Present	8	Yes	5.1	2.05			> 50	Fish bone, Muscle fibres, Fish scales, Skin
7	Present	Present	8	Yes	5.2	2.45	6 to 10	T-bone	> 50	Fish bone, Muscle fibres, Fish scales
8	Present	Not present	8	Yes	5.23	0.154	6 to 10	Feathers		
3	Present	Not present	15	Yes	5.34	0.102	2	T-bone, low contamination		

Laboratory identification code : 13

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
6	Not present	Present	5	Yes	10.02	1.58			> 50	fishbone,gill,scale,teeth,muscle,otolth
1	Not present	Not present	3	Yes	10.12	0.06				
8	Present	Not present	4	Yes	9.64	0.08	> 50	bones		
2	Present	Not present	4	Yes	10.12	0.07	2	bones		
5	Present	Not present	4	Yes	9.99	0.11	2	bones		
2	Not present	Not present	3	Yes	9.94	0.07				
4	Present	Not present	4	Yes	9.79	0.08	4	bones		
3	Present	Not present	3	Yes	10	0.06	2	bones		
7	Present	Present	5	Yes	9.97	1.43	6 to 10	bones	> 50	scales,gill,otolits,fishbone,muscle,teeth

Laboratory identification code : 14

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
1	Not present	Not present	4	Yes	10.46	0.067				
2	Not present	Not present	4	Yes	10.214	0.112				
4	Present	Not present	5	Yes	10.484	0.115	6 to 10	Bone and Muscle		
5	Present	Not present	6	Yes	10.431	0.099	1	Bone and Muscle		
8	Present	Not present	5	Yes	10.031	0.105	> 50			
2	Not present	Not present	5	Yes	10.443	0.078				
6	Not present	Present	5	Yes	10.277	2.763			> 50	
7	Present	Present	5	Yes	10.214	1.823	6 to 10		> 50	
3	Present	Not present	5	Yes	10.441	0.146	5	Bone and Muscle		

Laboratory identification code : 15

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Not present	Not present	2	Yes	5	0.008				
1	Not present	Not present	2	Yes	5	0.012				
6	Not present	Present	2	Yes	5	0.289			> 50	
2	Not present	Not present	2	Yes	5	0.01				
8	Not present	Not present	2	Yes	5	0.013				
3	Present	Not present	2	Yes	5	0.019	6 to 10			
5	Not present	Not present	2	Yes	5	0.023				
4	Present	Not present	2	Yes	5	0.026	2	bones		
7	Present	Present	2	Yes	5	0.57	11 to 50		> 50	

Laboratory identification code :   16

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
8	Present	Not present	7	Yes	10.008	0.048	6 to 10	bone		
6	Not present	Present	7	Yes	10.006	1.072			> 50	
5	Present	Not present	7	Yes	10.006	0.056	6 to 10	bone		
1	Not present	Not present	7	Yes	10.002	0.042				
4	Present	Not present	7	Yes	10.009	0.046	4	bone		
7	Present	Present	7	Yes	10.003	1.173	6 to 10	bone	> 50	
2	Not present	Not present	7	Yes	10.008	0.027				
3	Present	Not present	7	Yes	10.007	0.048	11 to 50	bone		
2	Not present	Not present	7	Yes	10.004	0.064				

Laboratory identification code :   17

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
1	Not present	Present	4	Yes	10	0.143			6 to 10	
4	Present	Not present	5	Yes	10	0.242	11 to 50			
2	Not present	Not present	6	Yes	10	0.216				
8	Present	Not present	5	Yes	10	0.269	11 to 50			
6	Not present	Present	4	Yes	10	1.505			> 50	
5	Present	Not present	5	Yes	10	0.196	4	1 muscle, 2 bones, 1 cartilage		
7	Present	Present	5	Yes	10	1.302	6 to 10		> 50	
2	Not present	Present	4	Yes	10	0.178			6 to 10	
3	Present	Not present	4	Yes	10	0.152	6 to 10			

Laboratory identification code :   18

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
8	Not present	Not present	3	Yes	10	0.068				
2	Not present	Not present	3	Yes	10	0.059				
6	Present	Present	3	Yes	10	1.427	6 to 10		> 50	
2	Present	Not present	3	Yes	10	0.057		feather meal		
1	Present	Not present	3	Yes	10	0.048		feather fragments		
5	Present	Not present	3	Yes	10	0.047		muscle fibres		
7	Present	Present	3	Yes	10	1.485	6 to 10		> 50	
4	Present	Not present	3	Yes	10	0.052	2	2 bones and muscle fibres		
3	Present	Not present	3	Yes	10	0.125	6 to 10			

Laboratory identification code :   19

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Not present	Present	10	Yes	10	0.125			5	fishbones
7	Not present	Present	11	Yes	10.01	1.484	2	1 feather, 1 bone	> 50	
8	Present	Not present	10	Yes	10	0.125	11 to 50			
1	Not present	Present	12	Yes	10	0.229	2	blood cells	3	fishbones
3	Present	Not present	13	Yes	10	0.08	6 to 10		1	fishbone
2	No results	No results	10	Yes	10	0.152	2	blood cells		
6	Not present	Present	10	Yes	10	1.443			> 50	
2	No results	No results	11	Yes	10.01	0.135			1	fishbone
5	Not present	Not present	10	Yes	10	0.085	2	bones	2	fishbones

Laboratory identification code :   20

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Present	Not present	8	No	10.19	0.05	6 to 10			
1	Not present	Not present	10	No	10.18	0.041				
7	Present	Present	8	No	10.16	1.171	3	bone	> 50	
6	Not present	Present	11	No	10.09	1.068			> 50	
8	Present	Not present	8	No	10.07	0.038	> 50			
3	Present	Not present	8	No	10.12	0.044	11 to 50			
2	Not present	Not present	8	No	10.07	0.046				
5	Present	Not present	9	No	10.18	0.042	4	bone		
2	Not present	Not present	8	No	10.18	0.035				

Laboratory identification code :   22

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Present	Present	7	No	10	1.613	5	bones	> 50	
4	Present	Not present	11	Yes	10	0.124	5	bones		
2	Not present	Not present	8	Yes	10	0.068				
6	Not present	Present	20	No	10	1.669			> 50	
1	Present	Not present	10	Yes	10	0.081	6 to 10	feather		
3	Present	Not present	15	Yes	10	0.117	6 to 10	bones		
2	Not present	Not present	11	Yes	10	0.068				
5	Present	Not present	11	Yes	10	0.105	4	bones		
8	No results	No results	13	Yes	10	0.161	11 to 50	muscle	11 to 50	muscle

Laboratory identification code :   23

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Not present	Present	5	Yes	10	1.414			> 50	fishmeal l.
1	Not present	Present	5	Yes	10	0.048			1	fish scale
2	Not present	No results	5	Yes	10	0.053			1	fish bone?
6	Not present	Present	5	Yes	10	1.255			> 50	fishmeal II.
2	Not present	Not present	5	Yes	10	0.065				
3	Present	Not present	5	Yes	10	0.062	3	bones		
8	Present	Not present	5	Yes	10	0.065	> 50	in refuse, bones, mu, fea musclefiber, and 2 feather		
4	Present	Not present	5	Yes	10	0.06	2	bones		
5	Present	Not present	5	Yes	10	0.091	1	bone		

Laboratory identification code :   24

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Not present	Not present	4	Yes	10.001	0.294				
4	Present	Not present	4	Yes	10.001	0.198	6 to 10			
6	Not present	Present	4	Yes	10.002	1.536			> 50	
7	Present	Present	4	Yes	10	1.366	11 to 50		> 50	
2	Not present	Not present	4	Yes	10.001	0.175				
1	Not present	Not present	4	Yes	10.001	0.013				
8	Present	Not present	4	Yes	10	0.072	11 to 50			
3	Present	Not present	4	Yes	10.001	0.073	11 to 50			
5	Present	Not present	4	Yes	10.002	0.12	6 to 10			

Laboratory identification code :   25

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Present	Not present	2	Yes	10.05	0.62	4	3 bone fragments & one piece of muscle tissue		
8	Present	Not present	2	Yes	10.29	0.23	> 50			
2	Not present	Not present	2	Yes	10.51	0.175				
1	Not present	Not present	2	Yes	10.49	0.103				
2	Not present	Not present	2	Yes	10.52	0.141				
7	Present	Present	20	Yes	10.16	2.182	11 to 50	Actually 50 slides were observed; mucle tissue present (could be fish and/or terrestial)	> 50	mucle tissue present (could be fish and/or terrestial)
3	Present	Not present	2	Yes	10.56	0.137	11 to 50			
6	Not present	Present	20	Yes	10.1	1.819		Actually 50 slides were observed; mucle tissue present (could be fish and/or terrestial)	> 50	mucle tissue present (could be fish and/or terrestial)
5	Present	Not present	2	Yes	10.56	0.136	2	2 bone fragments		

Laboratory identification code : 26

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
1	Present	Not present	15	Yes	20.16	0.13		feather		
7	Present	Present	15	Yes	10.01	1.63	5	bones	> 50	
8	Present	Not present	15	Yes	13.41	0.2	6 to 10			
2	Not present	Present	15	Yes	14.7	0.18			11 to 50	
6	Not present	Present	15	Yes	11.4	2.01			> 50	
2	Not present	Present	15	Yes	23.64	0.29			6 to 10	
5	Present	Present	15	Yes	20.09	0.23	6 to 10		6 to 10	
3	Present	Not present	15	Yes	21.08	0.28	6 to 10			
4	Present	Present	15	Yes	20.01	0.24	6 to 10		6 to 10	

Laboratory identification code : 27

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
5	Present	Not present	5	Yes	10	0.067	6 to 10			
6	Not present	Present	11	Yes	10	1.49			> 50	
8	Present	Not present	7	Yes	10.01	0.078	11 to 50			
2	Not present	Not present	7	Yes	10	0.07				
1	Not present	Not present	5	Yes	10	0.064				
3	Present	Not present	11	Yes	10	0.095	11 to 50			
4	Present	Not present	9	Yes	10	0.072	11 to 50			
7	Present	Present	2	Yes	10	1.456	6 to 10		> 50	
2	Not present	Not present	8	Yes	10	0.072				

Laboratory identification code : 28

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
5	Present	Not present	4	Yes	6.64	0.051	6 to 10			
7	Present	Present	4	Yes	6.89	1.226	6 to 10		> 50	
1	Present	Not present	4	Yes	6.08	0.041	3	Bone fragments		
6	Not present	Present	4	Yes	6.44	0.823			> 50	
8	Present	Not present	4	Yes	6.74	0.052	> 50			
2	Not present	Not present	4	Yes	7.1	0.048				
3	Present	Not present	4	Yes	6.21	0.055	> 50			
2	Not present	Not present	4	Yes	7.71	0.059				
4	Present	Not present	4	Yes	6.62	0.056	> 50			

Laboratory identification code : 29

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
7	Present	Present	4	Yes	10.069	0.923	6 to 10		> 50	
2	Not present	Not present	4	Yes	10.026	0.034				
3	Present	Not present	4	Yes	10.163	0.038	6 to 10			
6	Present	Present	4	Yes	10.083	1.096	6 to 10		> 50	
5	Present	Not present	4	Yes	10.025	0.034	5	bones		
1	Not present	Not present	4	Yes	10.125	0.039				
8	Present	Not present	4	Yes	10.019	0.026	11 to 50			
2	Not present	Not present	4	Yes	10.641	0.043				
4	Present	Not present	4	Yes	10.121	0.042	5	bones		

Laboratory identification code : 30

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
4	Not present	Present	10	Yes	10.03	0.048			3	fish teeth, scale
7	Not present	Present	10	Yes	10	1.247			> 50	
1	Not present	Not present	10	Yes	10.05	0.052				
6	No results	Present	10	Yes	10.04	1.1			> 50	
2	Not present	Present	10	Yes	10.02	0.05			2	tooth, scale
2	Not present	Present	10	Yes	10.01	0.061			5	fish tooth, scale, bones
8	Present	Not present	10	Yes	10.03	0.038	6 to 10	bones		
5	Not present	Not present	10	Yes	10.04	0.041				
3	Not present	Not present	10	Yes	10.04	0.035				

Laboratory identification code : 31

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
6	Present	Present	3	No	#REF!	#REF!	3	2 bone, 1 hair	> 50	
1	Present	Present	2	Yes	3.113	0.424	4	2 bone, 2 feather	6 to 10	
7	Present	Present	3	Yes	3.193	0.041	3	Bone	> 50	
2	Present	Present	2	No	3.665	0.556	6 to 10		11 to 50	
8	Present	Present	2	No	3.55	0.039	11 to 50		11 to 50	
2	Present	Present	1	No	3.189	0.024	6 to 10	Bone	11 to 50	Bone
3	Present	Present	2	No	4.5	0.038	4	Bone	4	Bone
5	Present	Present	1	No	4.343	0.043	11 to 50		11 to 50	
4	Present	Present	1	No	4.62	0.038	6 to 10		6 to 10	

Laboratory identification code : 32

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
5	Present	Not present	7	Yes	10	0.074	6 to 10			
2	Not present	Not present	10	Yes	10	0.098				
6	Not present	Present	5	Yes	5	0.714			> 50	
8	Present	Not present	5	Yes	10	0.086	> 50			
1	Not present	Not present	7	Yes	10	0.091				
3	Present	Not present	4	Yes	10	0.064	11 to 50			
2	Not present	Not present	10	Yes	10	0.086				
7	Present	Present	2	Yes	5	0.708	11 to 50		> 50	
4	Present	Not present	3	Yes	10	0.079	11 to 50			

Laboratory identification code : 33

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Not present	Not present	7	Yes	10.05	0.099				
5	Present	Not present	7	Yes	10.02	0.084	3	3 bones		
4	Present	Not present	4	Yes	10.02	0.072	6 to 10			
7	Present	Present	4	Yes	10.05	1.451	6 to 10		> 50	
8	Present	Not present	4	Yes	10.07	0.104	> 50			
1	Not present	Not present	7	Yes	10.07	0.066				
3	Present	Not present	4	Yes	10.03	0.1	6 to 10			
6	Not present	Present	4	Yes	10.03	1.534			> 50	
2	Not present	Not present	7	Yes	10.03	0.092				

Laboratory identification code : 34

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ≤5	Number of fish part. detected	Comment if number of fish part. ≤5
2	Not present	Not present	4	Yes	10	0.098				
2	Not present	Not present	4	Yes	10	0.08				
8	Present	Not present	4	Yes	10	0.09	> 50	bone,1 piece musclefiber,		few fishbonelike pieces estimatet as terrestrial particles - poultry
7	Present	Present	3	Yes	10	1.527	> 50	bone	> 50	fishbone, scale, teeht, meatfiber
5	Present	Not present	2	Yes	10	0.084	11 to 50	bone		
6	Not present	Present	4	Yes	10	1.598			> 50	Fishbone, gill, teeth, scale, meatfiber,
1	Not present	Not present	4	Yes	10	0.066				
4	Present	Not present	4	Yes	10	0.079	11 to 50	bone,cartilage		
3	Present	Not present	4	Yes	10	0.104	11 to 50	bone, meatfiber (1 piece)		