EURL-AP Proficiency Test Microscopy 2013

Final version

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Summary

This study, organised by the European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP), was designed for evaluating the proficiency levels of the NRL network to detect the presence of animal remains in feed by applying light microscopy, one of the official methods as described in Annex VI of regulation EC/152/2009 modified by regulation EU/51/2013 . In addition to the NRLs, the study was also open to some non-EU participants which had to apply also microscopic methods. The total number of participants was 33 of which 27 NRLs and 6 foreign participants. A set of 8 blind samples consisting of compound feeds and various fish feeds fortified or not with processed animal proteins (PAPs) was to be analysed. Adulteration levels by PAPs were all performed at 0.1% w/w. Overall results were satisfying although a major problem of specificity was noted for the identification of hydrolysed feather meal with merely 22% of correct detection. The present study also demonstrated that new interpretation rules of Annex VI of regulation EC/152/2009 regarding the presence of low levels of animal particles were reducing the number of total errors by 18% compared to the former ones.

Keywords :

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

1. Foreword

European Union Reference Laboratories (EURL) were created in order to ensure a high level of quality and a uniformity of the results provided by European control laboratories. On 29th April 2004, the European Parliament and the Council adopted 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://eurl.craw.eu). It has to develop the following priority axes:

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

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

(iii) To develop new analytical methods for the detection of animal proteins in feedingstuffs (light microscopy, near infrared microscopy, PCR, immunology …);

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

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

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

A working document version of this report was diffused to the NRL network for comments on the 17th March 2013. Comments and remarks had to be communicated to the EURL-AP by the 14th April. The present final version of the report was prepared based on the NRL inputs.

2. Introduction

According to recently modified Annex VI of Commission Regulation EC/152/2009 [3] official controls for the detection of animal proteins in feed inside the EU are performed by light microscopy and/or PCR. The objective of the present proficiency test is strictly to evaluate the performance of the network of 27 NRLs to detect the presence of processed animal proteins in feed only by light microscopy.

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

3. Material and methods

3.1. Study organisation

Participants were the 27 NRLs and 6 laboratories outside this EU network. These six foreign participants were the Servicio Nacional de Sanidad y Calidad Agroalimentaria from Argentina, the Biosecurity Sciences Laboratory from Australia, the China Agricultural University, the Food and Agricultural Materials Inspection Center from Japan, the Centro Nacional de Servicios de Constatación en Salud Animal from Mexico and LabNett AS from Norway. A detailed list of the 33 participating labs is included in Annex 1.

Official announcement of the study was made on the 6th September 2013 to all participants.

On the 8th November 2013, 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 that same day, sets of blind samples were sent by express shipment to the participants.

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

- Laboratories participating to the proficiency test are themselves 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.
- Participants were asked to sign the summarized results sheet that is automatically generated when filling the form and to return it by email to the EURL-AP. Only when both the Excel file and the fax were received by EURL-AP were results taken into consideration.
- Deadline for providing results in the ad hoc forms to the EURL-AP was fixed at 29th November 2013. Notification has been done that this date was a deadline and that results arriving later would not be accepted.

All NRL participants delivered their results on time. Concerning non-EU participants, two out of them were accepted to deliver their results later mainly due to shipment transport delays and custom issues. One non-EU participant did not deliver its results. Thus only one 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

Eight different materials were prepared for the proficiency test.

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

- Feed matrix conditioning (pelletized) that requires grinding before analysis as requested by Commission Regulation EC/152/2009 [3].
- Target concentrations of mammalian PAPs around 0.1% considered for the time being as the adulteration level that the method should be able to detect.

- Use of fishfeeds as matrices for assessing the detection capabilities of PAPs as because since the 1st June 2013 non-ruminant PAPS are authorized in aquafeed according to Commission Regulation EU/56/2013 [4].
- Adulteration with microscopically undetectable materials from terrestrial origin (porcine mucoproteins powder and ruminant whey powder) but generating positive responses by PCR.
- As used in a former study, in 2011[5], the presence of feather meal presenting a certain degree of hydrolysis which leads to an alteration of the morphological criteria of typical feather fragments.

Each participating lab received about 55g of 8 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.

Sample	Material	Nr of replicates
1	fishfeed I	1
2	fishfeed II + 0.1% pork PAP	1
3	fishfeed III+ 0.1% pork mucoproteins + 0.1% whey	1
4	fishfeed IV+ 0.1% hydrolysed feather	1
5	fishfeed I + 0.1% bovine PAP	1
6	fishfeed V	1
7	blank I	1
8	blank II	1
Total		**8**

3.2.2. Materials used in the preparation of the samples

Two main categories of matrices were used: compound feeds and fishfeeds.

Compound feeds:

- A **compound feed for rabbits** served as blank (sample 7). The feed was bought from a local producer. It consisted of wheat bran, alfalfa pellets, sunflower cake, sugar beet pulp, molasses, wheat, rapeseed cake, palm cake, cooked soybean cake, barley, calcium carbonate, monocalcium phosphate and feed complements (vitamins, salts, minerals). Its sediment content was about 0.9%.
- A **compound feed for horses** served as blank (sample 8). The feed was bought from a local producer. It was composed of wheat bran, soybean hulls, wheat gluten, molasses, corn, calcium carbonate, corn gluten and feed complements (salts, vitamins, minerals). Its sediment content was about 1.6%.

Fishfeeds:

- Fishfeed I was a commercial **complete feed for salmons** that was used as such (sample 1) and for preparing spiked sample 5. It consisted of fish oil, fishmeal, wheat, sunflower, soybean, wheat gluten, beans, vitamins and minerals. Its sediment content was about 1.1%.
- Fishfeed II a commercial **broodstock fishfeed** was used to prepare spiked sample 2. Its composition included soybean cake, groundnut cake, wheat and wheat gluten, tuna and fishmeals, rapeseed oil and cake, fish oil, monocalcium phosphate, salts, vitamins and amino acids. It had a sediment of about 1.8%.

- Fishfeed III was a commercial **complete fishfeed** was used to prepare spiked sample 3. It contained fishmeal, fish oil, wheat gluten, hydrolysed fish proteins, beans, soya protein concentrate, sunflower meal and wheat, vitamins and salts. Its sediment was about 1.2%.
- Fishfeed IV was a commercial **complete fishfeed** was used for preparing spiked sample 4. It consisted of soya protein concentrate, rapeseed oil, wheat gluten, wheat, fishmeal and fish oil, beans, sunflower seed meal, vitamins and minerals. Its sediment content was about 0.5%.
- Fishfeed V was a commercial **complete feed for trout** that was used as such (sample 6). It contained fishmeal, wheat gluten, fish oil, soybean, corn gluten, wheat, beans, hydrolysed fish proteins, vitamins and minerals. Its sediment content was about 3.3%.

Adulterant material used:

- A pure **porcine PAP** was used for sample 2. This PAP was produced by a pilot plant. Its final bone content was of about 14%. Its purity was controlled by microscopy and PCR.
- A **bovine PAP** was used for preparing sample 5. This PAP was also produced by a pilot plant. Its final bone content was very high about 85%. Its purity was controlled by microscopy and PCR.
- A **hydrolysed feather meal** was used for preparing sample 4. This meal was used and tested in a former EURL-AP proficiency test [5]. Tested by PCR, it was positive for poultry (chicken/turkey) DNA only.
- Commercial **porcine mucoprotein powder** was used for sample 3. Such powder is spray dried and do not present microscopic features comparable to PAPs. This was verified by microscopy. By PCR it was only positive for porcine DNA.
- **Whey powder** coming from cow milk was also added to sample 3. Microscopic analysis of the material did not allow detecting particles as found in PAPs.

3.2.3. Description of the mixing procedures

Adulteration of the different samples was performed by spiking.

Prior to sample preparation, mixing of the materials and filling of the sample containing vials the rooms where those activities were performed were cleaned and secured from any presence of possibly interfering material.

3.3. Qualitative analysis

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

According to the change of result expression as stated by regulation EU/51/2013 [6] amending regulation EC/152/2009 results are expressed in three formulations:

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

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

These binary results were analysed by classical statistics: accuracy, sensitivity and specificity. All those statistics were expressed as fractions.

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

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

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

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

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

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

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

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

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	Fish		Nr of replicates	Ruminant	Porcine
1	fishfeed I	10	-*	+	1.13	5	-	3/5+
2	fishfeed II+ 0.1% porcine PAP	5	+	+	1.89	5	1/5+	+
3	fishfeed III + 0.1% porcine mucoproteins + 0.1% whey	5	-	+	1.23	5	+	+
4	fishfeed IV + 0.1% hydrolysed feather	5	+	+	0.5	5	-	-
5	fishfeed I + 0.1% bovine PAP	5	+	+	1.17	5	+	-
6	fishfeed V	10	-	+	3.26	5	-	-
7	blank I	10	-	-	0.89	5	-	-
8	blank II	10	-*	-	1.56	5	1/5+	-

(Legend: sed = sediment, + = present, - = not present, * = one single bone particle detected)

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

Fishfeed I (**sample 1**) was negative for any presence of animal material by light microscopy for 9 replicates. The 10th replicate showed the presence of a single terrestrial bone particle (~100µm in diameter) at the outside of the coverslip. By PCR it revealed to be negative for ruminant DNA presence but 3 replicates out of 5 were positive for porcine DNA presence, this remains unexplained as referring to the absence of porcine signal for sample 5 based on the same fishfeed.

Fishfeed V (**sample 6**) did not present any terrestrial particle and was negative for the presence of ruminant and porcine DNA.

Blank I (**sample 7**) was microscopically free from any presence of animal material which was confirmed through detection of ruminant and pig targets by PCR.

Blank II (**sample 8**) revealed to be negative for microscopic animal traces except for one out of 10 replicates where a single bone particle, classified as terrestrial, was detected. By PCR it was only positive for ruminant DNA for one replicate out of 5.

The adulterated samples presented the following results.

Fishfeed II + 0.1% pork PAP (**sample 2**) presented systematically terrestrial bones (average of 9 bones per replicates on the minimum of 3 slides of sediment) aside the expected fish ones. PCR analyses revealed the sample as positive for porcine DNA and, on the exception of one replicate, negative for ruminant DNA.

Fishfeed III + 0.1% porcine mucoprotein powder + 0.1% whey powder (**sample 3**) did not reveal any terrestrial particle when observed by microscope. PCR analyses showed presence of both ruminant and porcine DNA.

Fishfeed IV + 0.1% hydrolysed feather meal (**sample 4**) systematically showed few terrestrial bone fragments (average of 3 bones per replicate on the minimum of 3 slides of sediment) as well as numerous feather fragments in the flotate.

Fishfeed I + 0.1% bovine PAP (**sample 5**) showed, aside fish particles, systematically terrestrial bones by microscopy (average of 12 bones on per replicate on the minimum of 3 slides of sediment). PCR analyses revealed the samples as positive for ruminant but negative for pork.

Near infrared microscopy analyses did not reveal inconsistencies in the materials used and the samples prepared.

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

4.2. Qualitative analyses from the NRLs

4.2.1. On the respect of the instructions

Annex VI of EC regulation 152/2009 [3] as modified by EU regulation 51/2013 [6] imposes to give detailed information on the type of animal particles being found if any (e.g. bones, feathers, scales, hairs,…) As stated in the instructions to the present study this was also mandatory. Some participants from the NRL network did not respect those instructions (labs 1, 3, 8 and 15). As reporting is a topic that must be integrated in the scope of a proficiency test, those NRLs were declared as underperforming, at least administratively.

In agreement with the EURL-AP SOP for managing underperformances (available on the EURL-AP intranet since 18 January 2012), these 4 participants are asked to report on the explanation of this non-compliance with the legal requirements.

4.2.2. Overview of results and performance of the network

Table 3, on next page, summarizes the results submitted by the 27 NRLs for the eight sample types submitted to qualitative analysis.

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

Problems of sensitivity for fish were limited to a background level. Two cases of false negative results were observed for a same lab: one for sample 1 and one for sample 3 only. After diffusion of the working document version of the report this lab explained this sensitivity issue by a transcription error in the

reporting. For terrestrial animal presence, problems of sensitivity were more frequent and need to consider attention as all of those case occurs at adulteration level of 0.1%, and basically on comparable matrices – fishfeeds. This inability to detect terrestrial particles accounted for 7% of the bovine PAP adulteration, for 15% of the porcine PAP adulteration and finally for 78% of the hydrolysed feather adulteration. The latter result being abnormally high, it will be scrutinized with details in the present report.

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

Sample	Material	n	AC	
			Terrestrial	Fish
1	fishfeed I	27	0.852 (4)	0.963 (1)
2	fishfeed II + 0.1% porcine PAP	27	0.852 (4)	1.000
3	fishfeed III+ 0.1% porcine mucoproteins + 0.1% whey	27	0.963 (1)	0.963 (1)
4	fishfeed IV+ 0.1% hydrolysed feather	27	0.222 (21)	1.000
5	fishfeed I + 0.1% bovine PAP	27	0.926 (2)	1.000
6	fishfeed V	27	1.000	1.000
7	blank I	27	1.000	0.926 (2)
8	blank II	27	0.963 (1)	0.926 (2)

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

Problems of specificity also arose. Erroneous detection of fish (7%) was noted on sample 7 and 8, both blank compound feed matrices. False positive terrestrial particles detection occurred also on sample 1 (15%), sample 3 and sample 8 (both 4%).

4.2.3. Detailed review of results per sample

Sample 1: Fishfeed I

PD for terrestrial particles:

- Lab 11 : feathers (more than 5 as based on 1 repetition only)
- Lab 17 : 1 bone, feathers (nr not specified) on 2 repetitions
- Lab 18 : bones, muscle fibres (nr not specified) on 2 repetitions
- Lab 22 : bones (more than 5 as based on 1 repetition only)

Aside those PD, a number of participants reported <LOD :

- Labs 5 and 10 : with less than 10 bones on 2 repetitions
- Lab 14 and 20 : in between 10-15 bones on 3 repetitions
- Lab 15 : 3 bones on 3 repetitions
- Lab 23 : 5 bones on 2 repetitions
- Lab 28 : less than 5 bones on 1 repetition

From those details, it appears that Lab 17 did not respect the rules on the expression of results (point 2.1.5.2. of Annex VI of EC/152/2009) with as a consequence of being penalized. Lab 28 also failed to follow the rules on the number of repetitions (point 2.1.4.3. of Annex VI of EC/152/2009) as it should have at least one additional determination.

Considering the relative high number of <LOD cases, one should raise the question of cross-contamination. The homogeneity study classified the sample as fit for purpose although the single atypical bone particle detection. As this sample had to be ground before sedimentation, it cannot be excluded that a cross-contamination occurred during the grinding process. However years of prevention of this risk should counterbalance this possibility.

ND for fish particles:

- Lab 16 was unsuccessful in detecting fish material*

Sample 2: Fishfeed II + 0.1% porcine PAP

ND for terrestrial particles:

- Lab 12
- Lab 8 : in between 10-15 unspecified particles on 3 repetitions
- Lab 14 : in between 10-15 bone fragments on 3 repetitions
- Lab 27 : 4 particles of bone on 3 repetitions

From the details, the 3 laboratories that reported <LOD for terrestrial, followed the rules on the number of repetitions (point 2.1.4.3. of Annex VI of EC/152/2009). In this respect, the problem they encountered is either that of incorrect identification of other terrestrial bones, either a recovery issue during the sedimentation or an imperfect (non-representative) mass reduction process when sub-portions are taken for the repetitions.

Sample 3: Fishfeed III + 0.1% porcine mucoproteins + 0.1% whey powder

PD for terrestrial particles:

- Lab 22 : bones (more than 5 as based on 1 repetition only)

Some <LOD were also reported:

  - Lab 20 : in between 10-15 bones on 3 repetitions
  - Lab 28 : less than 10 bones on 2 repetitions
  - Lab 15 : 3 bone particles on 3 repetitions
  - Lab 23 : a few (less than 10 on 2 repetitions) particles difficult to recognize (*sic*) but classified as terrestrial

Here again it has to be noted that Lab 15 did probably not exactly comply with the number of repetitions (point 2.1.4.3. of Annex VI of EC/152/2009) as it could have stopped after one single determination.

ND for fish particles:

- Lab 16 failed in detecting fish particles*

Sample 4: Fishfeed IV + 0.1% hydrolysed feather meal

ND for terrestrial particles :

- Labs 1, 3, 4, 5, 8, 10, 14, 16*, 17, 19, 20, 23, 25, 26, 27 and 28
- Lab 21 : less than 10 hairs on 2 repetitions

* *After diffusion of the working document version of the report, Lab 16 notified the organizer that a general inversion of the sample numbers occurred. This error of transcription generated an underperforming situation which is not linked to scientific competences but to an issue of traceability. As reporting is part of the proficiency assessment, the participant was not excluded but considered as underperforming.*

- Lab 7 : in between 10-15 feathers on 3 repetitions
- Lab 22 : in between 10-15 bones on 3 repetitions
- Lab 15: 2 bone particles on 3 repetitions
- Lab 18 : in between 10-15 particles of bones and muscle fibres on 3 repetitions

Among the laboratories that reported particles <LOD, Lab 7 is the only one that detected feathers but in too low numbers. All others did not report on the presence of feathers. On the contrary the majority of the laboratories that reported the sample as truly positive mentioned the presence of feathers (5 out of 6) – hydrolysed or not.

Lab 21 detected structures identified as hairs and not as feathers. It has to be mentioned that the same lab, although probably using the cystine reagent, identified the presence of feathers in other samples where those where not supposed to be i.e. in samples 2, 5 and 7.

Sample 5: Fishfeed I + 0.1% bovine PAP

ND for terrestrial particles :

- Labs 4 and 14: in between 10-15 bone fragments on 3 repetitions

This number of terrestrial particles is too low when considering the bone proportion of the PAP used (0.85) as well as the data from the homogeneity study.

Sample 6: Fishfeed V

No errors were noted. However some <LOD cases were reported for terrestrial particles:

- Labs 16*, 20 and 22 : in between 10-15 bones on 3 repetitions
- Lab 28 : less than 10 bones on 2 repetitions

Lab 20 made this proficiency test by repeating systematically 3 times the determinations for each sample, doing so it found bone particles identified as terrestrial at a level <LOD too frequently (for sample 1, 3 and 6) whereas they were not supposed to be present. Acting in that way multiplies the risk of false positive results while the condition for the number of determinations precisely tends to avoid such situations.

Sample 7: Blank I

No errors were noted for terrestrial particle detection, although two cases of <LOD occurred:

- Lab 12 : less than 10 bones on 2 repetitions
- Lab 21 : less than 10 thin brown hairs or feathers on 2 repetitions

PD for fish particles:

- Labs 11 and 16* : fishbones, gills, scales

Sample 8: Blank II

PD for terrestrial particles:

- Lab 16*

* *After diffusion of the working document version of the report, Lab 16 notified the organizer that a general inversion of the sample numbers occurred. This error of transcription generated an underperforming situation which is not linked to scientific competences but to an issue of traceability. As reporting is part of the proficiency assessment, the participant was not excluded but considered as underperforming.*

PD for fish particles:

- Lab 16*
- Lab 23 : 8 fishbone fragments, but no muscle fibres

4.2.4. Individual performances of NRLs in qualitative analysis

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

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

Terrestrial

lab code	AC	SE	SP
2	1.000	1.000	1.000
6	1.000	1.000	1.000
13	1.000	1.000	1.000
24	1.000	1.000	1.000
11	0.875	1.000	0.800
1	0.875	0.667	1.000
3	0.875	0.667	1.000
7	0.875	0.667	1.000
19	0.875	0.667	1.000
25	0.875	0.667	1.000
26	0.875	0.667	1.000
5	0.875	0.667	1.000
10	0.875	0.667	1.000
12	0.875	0.667	1.000
15	0.875	0.667	1.000
20	0.875	0.667	1.000
28	0.875	0.667	1.000
21	0.875	0.667	1.000
23	0.875	0.667	1.000
16	0.750	0.667	0.800
17	0.750	0.667	0.800
18	0.750	0.667	0.800
8	0.750	0.333	1.000
27	0.750	0.333	1.000
4	0.750	0.333	1.000
22	0.625	0.667	0.600
14	0.625	0.000	1.000

Fish

lab code	AC	SE	SP
1	1.000	1.000	1.000
2	1.000	1.000	1.000
3	1.000	1.000	1.000
4	1.000	1.000	1.000
5	1.000	1.000	1.000
6	1.000	1.000	1.000
7	1.000	1.000	1.000
8	1.000	1.000	1.000
10	1.000	1.000	1.000
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
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
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
27	1.000	1.000	1.000
28	1.000	1.000	1.000
11	0.875	1.000	0.500
23	0.875	1.000	0.500
16	0.500	0.667	0,000

* *After diffusion of the working document version of the report, Lab 16 notified the organizer that a general inversion of the sample numbers occurred. This error of transcription generated an underperforming situation which is not linked to scientific competences but to an issue of traceability. As reporting is part of the proficiency assessment, the participant was not excluded but considered as underperforming.*

Details of the results were commented in section 4.2.3.

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

Considering the composition of the sample set, ranking criteria for the present test were decided as follows:

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

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

Consolidated			
lab code	AC	SE	SP
2	1.000	1.000	1.000
6	1.000	1.000	1.000
13	1.000	1.000	1.000
24	1.000	1.000	1.000
1	0.938	0.889	1.000
3	0.938	0.889	1.000
5	0.938	0.889	1.000
7	0.938	0.889	1.000
10	0.938	0.889	1.000
12	0.938	0.889	1.000
15	0.938	0.889	1.000
19	0.938	0.889	1.000
20	0.938	0.889	1.000
21	0.938	0.889	1.000
25	0.938	0.889	1.000
26	0.938	0.889	1.000
28	0.938	0.889	1.000
11	0.875	1.000	0.714
17	0.875	0.889	0.857
18	0.875	0.889	0.857
23	0.875	0.889	0.857
4	0.875	0.778	1.000
8	0.875	0.778	1.000
27	0.875	0.778	1.000
22	0.813	0.889	0.714
14	0.813	0.667	1.000
16	0.625	0.667	0.571

17 labs out of 27 NRLs or in other words for 63% of the NRLs performed very well. 4 NRLs performed satisfyingly. Finally 6 NRLs were classified according the ranking criteria as underperforming for the present proficiency test. Those labs require improvement of proficiency. In agreement with the EURL-AP SOP for managing underperformances (available on the EURL-AP intranet since 18 January 2012), these 6 underperforming participants are asked to report on the origin of their multiple errors as well as on the actions they will undertake in order to solve their problems.

Aside the strictly scientific issues, 4 NRLs presenting non-compliance with legal requirements (details in section 4.2.1.) are also asked to deliver explanations on their underperformance.

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 to.

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.

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

Terrestrial			
lab code	**AC**	**SE**	**SP**
33	0.750	1.000	0.600
32	0.750	0.333	1.000
35	0.750	0.333	1.000
34	0.625	1.000	0.400
31	0.625	0.000	1.000

Fish			
lab code	**AC**	**SE**	**SP**
31	1.000	1.000	1.000
32	1.000	1.000	1.000
33	1.000	1.000	1.000
35	1.000	1.000	1.000
34	*n.a.*	*n.a.*	*n.a.*

Lab 34 only performed the detection for terrestrial animal remains and did not deliver results for fish detection. For the 4 other participants the detection of fish, for both sensitivity and specificity, occurred successfully.

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

Sample 1: Fishfeed I

PD for terrestrial particles:

- Lab 34 : bones

Sample 2: Fishfeed II + 0.1% porcine PAP

ND for terrestrial particles:

- Labs 31
- Labs 32 and 35 : bones, buts mentioned as <LOD

Sample 3: Fishfeed III + 0.1% porcine mucoproteins + 0.1% whey powder

PD for terrestrial particles:

- Labs 33 and 34 : bones

Sample 4: Fishfeed IV + 0.1% hydrolysed feather meal

ND for terrestrial:

- Labs 31, 32 and 35

It has to be noted that none of these 5 participants detected the presence of hydrolysed feather fragments. Labs 33 and 34 only reported having detected bones.

Sample 5: Fishfeed I + 0.1% bovine PAP

ND for terrestrial particles :

- Lab 31

Sample 6: Fishfeed V

PD for terrestrial particles:

- Labs 33 and 34 reported terrestrial bones

As for the NRL participants, an indicative ranking of the non-EU participants was also realized on a consolidated evaluation including their proficiency in detecting both terrestrial and fish materials based on the same criteria (page 12) 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. (*n.a.* = not applicable)

Consolidated			
lab code	**AC**	**SE**	**SP**
33	0,875	1,000	0,714
32	0,875	0,778	1,000
35	0,875	0,778	1,000
31	0,813	0,667	1,000
34	*n.a.*	*n.a.*	*n.a.*

One participant obtained a satisfying result (line in blue in table 9). Three participants were classified as underperforming (lines in red in table 9) according to the applied criteria.

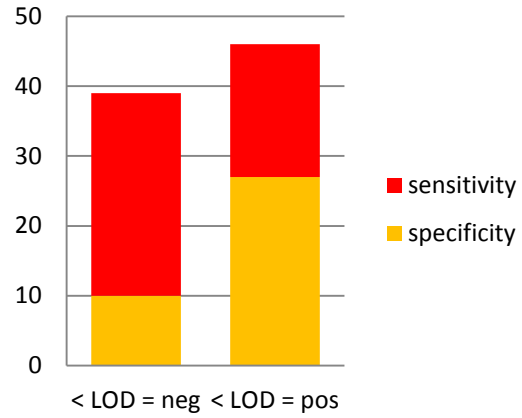
Lab 34 was not classified.

5. Conclusions

The study aimed at evaluating the proficiency levels of the participants to detect the presence of animal remains in feed with dedicated focus on fishfeed matrices. This choice was in line with the recent reauthorisation of utilisation of PAPs from non-ruminants to aquaculture [4]. EU participants were required to apply the official method as described in revised Annex VI of regulation EC/152/2009 [3].

Results demonstrated that the detection of fish particles was achieved without difficulties, except for some few erroneous fish detection in blank matrices. A more worrying situation was noted for the detection of terrestrial particles. Effectively the study showed that problems of sensitivity for terrestrial particles were still present although through the sample set a contamination level of 0.1% was used. The most striking issue is that related to the failure at identifying and hence detecting hydrolysed feather fragments (78% of errors). In 2011 [5] a study conducted by the EURL-AP used the same hydrolysed feather meal at 0.5% within a fishfeed. At that time the percentage of lack of detection for feather fragments was reaching 70%. Considering the lower percentage of adulteration in the present study, the difficulty of detecting such animal PAP remains probably unchanged. In other words no significant improvement since 2011 could be noticed. As from past study, the use of cystine reagent for differentiating such structures from others can only be advised.

Further analysis of the global results (Graph 1) showed that sensitivity issues accounted for 74% of the total number of 39 errors considering all <LOD results as negative. By comparing those data with the application of past interpretation rules, i.e. before the introduction of the "<LOD" rule by Commission Regulation EU/51/2013 [6], the total number of errors on this test set would have been increased up to 46 thus an increase of 18% of errors. By applying past interpretation rules, the percentage of sensitivity issues would have been decreased to 59% and the percentage of specificity issues would have been increased to 41%.



Graph 1: Comparative error graph between current interpretation rules (< LOD=neg) and former interpretation rules, the so-called zero tolerance rules (< LOD=pos).

This would have represented 17 false positive results more all of them linked to terrestrial particle detection. Concerning the sensitivity, the present legal result expression accounted for 10 more cases of false negative results, when compared to those which would have been obtained by past result expression rules. Those additional false negative results were distributed as follows: 3 ND for sample 2 (fishfeed + 0.1% pork PAP), 5 ND for sample 4 (fishfeed + 0.1% hydrolysed feather) and 2 ND for sample 5 (fishfeed + 0.1% bovine PAP).

Even by applying a “zero tolerance” rule, it has to be noted that the detection of hydrolysed feather meal would still have been underperforming with a sensitivity score of 0.41 which is closer to that observed from past study [5]. The issue of disclosing and identifying hydrolysed feather fragments seems not linked to the interpretation rules, but to the operator’s capabilities which in this case were not improved as discussed.

An advantage of the current interpretation rule is unexpectedly, but clearly, illustrated by the number of additional false negative results originating by a too low number of terrestrial particles being recognised as such. This reflects either a problem of recovery rate during the sedimentation process or confusion with other types of particles (masking effect of fish material which is predominantly present in the test). Considering both the improvement of the method standardisation of the revised EC/152/2009 (number of slides, size of coverslip, recommended amount of material to be used for slide preparation, etc) and the share of bones of the PAPs used, it is probable that the recovery rate is a candidate for explaining this increase of false negative results for sample 2 and 5. Data from the homogeneity study also supported this explanation. However the fish masking effect is also proven to account for some false negative results. Effectively nonetheless the optimisations brought to the method, as well as future ones, skills of operators in correct identification still need to be questioned for any erroneous result. As an illustration of this, permanent slides from sample 2 received from a participant after diffusion of the draft report who was declaring the sample as free from terrestrial bones were analysed by EURL-AP microscopists. All slides were found to contain terrestrial bones, in comparable number to that from the homogeneity study. Pictures were taken and communicated to the participant.

The global performance of the NRL network was very good for 63% out of them –this percentage was of 56% in 2012 [7]. A satisfying global performance was achieved by an additional 15% of the NRLs. The remaining 22% of the NRLs were classified as underperforming.

Concerning the non-EU participants, and on the exception of one participant that did not deliver results for fish detection, only one out of four performed satisfyingly. Among those participants, only terrestrial detection caused some problems of both sensitivity and specificity. None of them notified the presence of feather fragments in sample 4.

As outcome of this study the EURL-AP makes some recommendations:

1. The implementation of the official method described in the Annex VI of Regulation EC/1852/2009 and the related SOPs shall be respected strictly. This is the first prerequisite to any further improvement related to the microscopic detection of animal proteins in feedingstuffs.
2. The use of cystine reagent should be used for investigations on the possible presence of feather meals (hydrolysed or not)

Acknowledgment

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

References

[1] EU. 2004. Commission Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L 165, 30/04/2004: 1-141.

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

[3] EU. 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.

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

[5] Veys P, Berben G and Baeten V. 2012. EURL-AP Proficiency Test Microscopy 2011 : Final version. CRA-W, Gembloux, Belgium

[6] EU. 2013. Commission Regulation (EU) No 51/2013 of 16 January 2013 amending regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed. Official Journal of the European Union L 20, 23/1/2013: 33-43.

[7] Veys P and Berben G. 2013. EURL-AP Proficiency Test Microscopy 2012 : Final version. CRA-W, Gembloux, Belgium

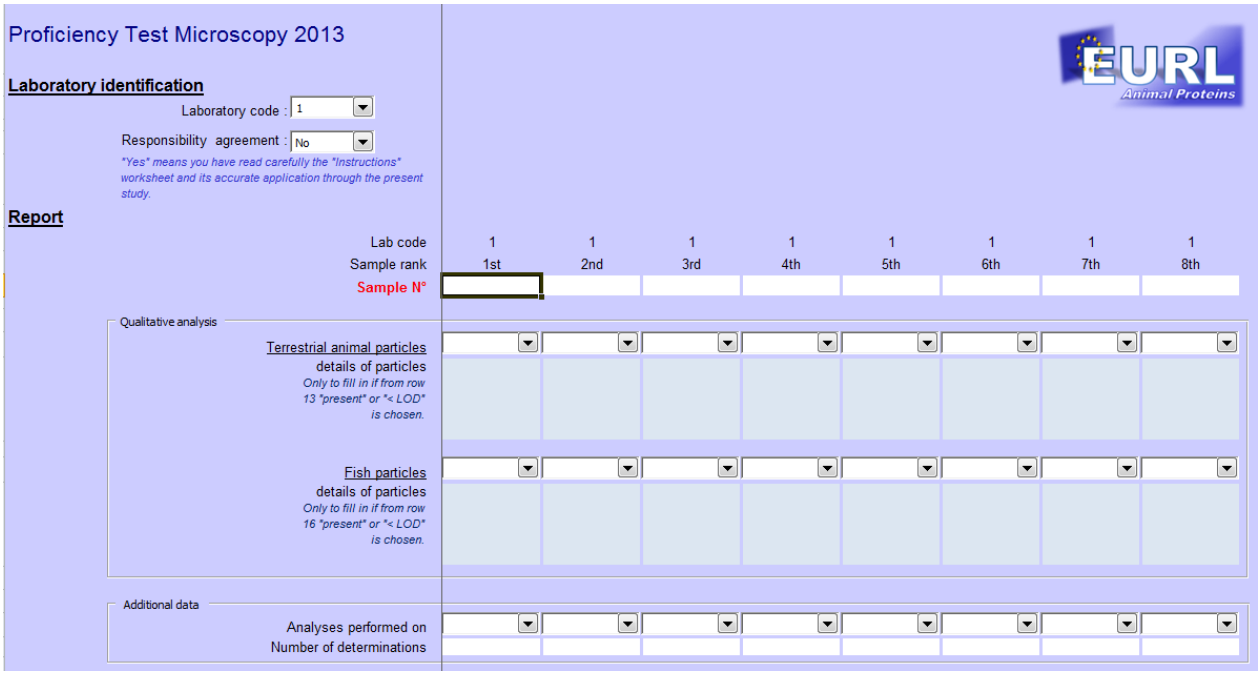
Annex 1

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

Country	Institute Name
Argentina	*Servicio Nacional de Sanidad y Calidad Agroalimentaria*
Australia	*Biosecurity Sciences Laboratory*
Austria	Austrian Agency for Health and Food Safety
Belgium	Federal Agency for the Safety of the Food Chain
Bulgaria	National Diagnostic Research Veterinary Medical Institute
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 Food and Veterinary Risk Assessment Institute
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Norway	*LabNett AS*
Mexico	*Centro Nacional de Servicios de Constatación en Salud Animal*
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 Microscopy 2013

Laboratory identification

Laboratory code : 1

Responsibility agreement : No

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

Report

Lab code	1	1	1	1	1	1	1	1
Sample rank	1st	2nd	3rd	4th	5th	6th	7th	8th
Sample N°								
Qualitative analysis								
Terrestrial animal particles								
details of particles (Only to fill in if from row 13 "present" or "< LOD" is chosen.)								
Fish particles								
details of particles (Only to fill in if from row 16 "present" or "< LOD" is chosen.)								
Additional data								
Analyses performed on								
Number of determinations								

Annex 3

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

Laboratory identification code : **1**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
7	391	Absent		Absent		Sed. + Flot.	2
2	673	Present		Present		Sed. + Flot.	2
6	2170	Absent		Present		Sed. + Flot.	2
5	2200	Present		Present		Sed. + Flot.	2
3	2557	Absent		Present		Sed. + Flot.	2
1	3013	Absent		Present		Sed. + Flot.	2
4	3055	Absent		Present		Sed. + Flot.	2
8	3067	Absent		Absent		Sed. + Flot.	2

Laboratory identification code : **2**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
7	424	Absent		Absent		Sed. + Flot.	2
5	484	Present	bones, (muscle)	Present	bones, muscle	Sed. + Flot.	2
1	967	Absent		Present	bones, muscle	Sed. + Flot.	2
2	1399	Present	bones, (muscle)	Present	bones, muscle	Sed. + Flot.	2
6	1444	Absent		Present	bones, muscle	Sed. + Flot.	2
8	2737	Absent		Absent		Sed. + Flot.	2
4	3022	Present	traces of hydrolised feathers (?)	Present	bones, scales, muscle	Sed. + Flot.	2
3	3052	Absent		Present	bones, muscle	Sed. + Flot.	2

Laboratory identification code : **3**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	76	Absent		Present		Sed. + Flot.	1
7	226	Absent		Absent		Sed. + Flot.	1
5	352	Present		Present		Sed. + Flot.	1
2	541	Present		Present		Sed. + Flot.	2
8	625	Absent		Absent		Sed. + Flot.	1
6	2005	Absent		Present		Sed. + Flot.	1
4	2395	Absent		Present		Sed. + Flot.	1
3	2689	Absent		Present		Sed. + Flot.	1

Laboratory identification code :	4						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	61	Absent		Absent		Sed. + Flot.	1
6	289	Absent		Present	Fishbone, cartilage, gill, scale, muscle, fishskin, blood	Sed. + Flot.	1
1	538	Absent		Present	Fishbone, cartilage, gill, scale, muscle, fishskin	Sed. + Flot.	1
4	547	Absent		Present	Fishbone, cartilage, gill, scale, muscle, fishskin	Sed. + Flot.	1
5	2035	< LOD	Bone (average 5), muscle (differentiation not possible), cartilage (differentiation not possible)	Present	Fishbone, gill, scale, fishskin, cartilage(differentiation not possible), muscle(differentiation not possible)	Sed. + Flot.	3
3	2590	Absent		Present	Fishbone, cartilage, gill, scale, muscle, fishskin, blood	Sed. + Flot.	1
8	2671	Absent		Absent		Sed. + Flot.	1
2	2719	Present	Bone (> 6), muscle (differentiation not possible), cartilage (differentiation not possible), blood (differentiation not possible)	Present	Fishbone, gill, scale, fishskin, cartilage(differentiation not possible), muscle(differentiation not possible), blood (differentiation not possible)	Sed. + Flot.	1

Laboratory identification code :	5						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
1	10	< LOD	bones	Present	fishbones, cartilage	Sed. + Raw	2
7	325	Absent		Absent		Sed. + Raw	1
8	427	Absent		Absent		Sed. + Raw	2
5	1012	Present	bones	Present	fishbones, cartilage	Sed. + Flot.	2
2	1465	Present	bones	Present	fishbones, cartilage	Sed. + Flot.	2
6	2038	Absent		Present	fishbones, cartilage	Sed. + Raw	1
3	2194	Absent		Present	fishbones, cartilage	Sed. + Raw	1
4	2494	Absent		Present	fishbones, cartilage	Sed. + Raw	1

Laboratory identification code :	6						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
5	682	Present	bones	Present	fishbones,cartilages,scales,gills,otoliths, muscles	Sed. + Flot.	1
7	1018	Absent		Absent		Sed. + Flot.	1
1	1165	Absent		Present	fishbones,cartilages,scales,gills,otoliths,muscles	Sed. + Flot.	1
6	2137	Absent		Present	fishbones,cartilages,scales,gills,otoliths,muscles	Sed. + Flot.	1
3	2227	Absent		Present	fishbones,cartilages,scales,gills,otoliths,muscles	Sed. + Flot.	1
2	2554	Present	bones	Present	fishbones,cartilages,scales,gills,otoliths,muscles	Sed. + Flot.	1
4	2824	Present	3 bones, feathers	Present	fishbones,cartilages,scales,gills,otoliths,muscles	Sed. + Flot.	1
8	2836	Absent		Absent		Sed. + Flot.	1

Laboratory identification code :	7						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
4	481	< LOD	feather	Present	fishbones, gills, cartilages, scales	Sed. + Flot.	3
1	571	Absent		Present	fishbones, gills, cartilages, scales	Sed. + Flot.	1
7	1117	Absent		Absent		Sed. + Flot.	1
6	1477	Absent		Present	fishbones, gills, cartilages, scales	Sed. + Flot.	1
5	2134	Present	bones	Present	fishbones, gills, cartilages, scales	Sed. + Flot.	1
2	2521	Present	bones	Present	fishbones, gills, cartilages, scales	Sed. + Flot.	1
3	2656	Absent		Present	fishbones, gills, cartilages, scales	Sed. + Flot.	1
8	2770	Absent		Absent		Sed. + Flot.	1

Laboratory identification code :	8						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
1	109	Absent		Present		Sed. + Flot.	2
6	1411	Absent		Present		Sed. + Flot.	2
7	1447	Absent		Absent		Sed. + Flot.	2
5	2233	Present		Present		Sed. + Flot.	2
2	2686	< LOD		Present		Sed. + Flot.	3
4	2725	Absent		Present		Sed. + Flot.	2
8	2902	Absent		Absent		Sed. + Flot.	2
3	2920	Absent		Present		Sed. + Flot.	2

Laboratory identification code :	10						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
1	274	< LOD	No more than 5 particles derived from terrestrial animals were detected on average per determination, the particles were identified as bone. This low level presence, being below the limit of detection of the microscopic method means that a risk of false positive result cannot be excluded.	Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bones and scales.	Sed. + Raw	2
7	1348	Absent		Absent		Sed. + Raw	1
5	1705	Present	More than 5 particles derived from terrestrial animals were detected on average per determination. The particles were identified as bone.	Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bones and scales.	Sed. + Raw	1
8	2011	Absent		Absent		Sed. + Raw	1
3	2260	Absent		Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bones and scales.	Sed. + Raw	1
6	2269	Absent		Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bones, teeth and scales.	Sed. + Raw	1
2	2488	Present	More than 5 particles derived from terrestrial animals were detected on average per determination. The particles were identified as bone.	Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bones and scales.	Sed. + Raw	1
4	2923	Absent		Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bones and scales.	Sed. + Raw	1

Laboratory identification code :	11						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
6	1180	Absent		Present	bones, gills, scale	Sed. + Flot.	1
7	1216	Absent		Present	bones, gills, scale	Sed. + Flot.	1
1	1462	Present	feathers	Present	bones, gills, scale	Sed. + Flot.	1
5	1969	Present	bones	Present	bones, gills, scale	Sed. + Flot.	1
8	1978	Absent		Absent		Sed. + Flot.	1
4	2263	Present	fetahers	Present	bones, gills, scale	Sed. + Flot.	2
2	2752	Present	bones	Present	bones, gills, scale	Sed. + Flot.	1
3	2788	Absent		Present	bones, gills, scale	Sed. + Flot.	1

Laboratory identification code :	12						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	490	< LOD	bones	Absent		Sed. + Raw	2
1	868	Absent		Present	fish bones, scales, muscles fibers	Sed. + Raw	1
5	913	Present	bones, muscles fibers	Present	fish bones, scales, muscles fibers	Sed. + Raw	1
6	1114	Absent		Present	fish bones, scales, muscles fibers	Sed. + Raw	1
3	2524	Absent		Present	fish bones, scales, muscles fibers	Sed. + Raw	1
2	2620	Absent		Present	fish bones, scales, muscles fibers	Sed. + Raw	1
4	2989	Present	bones	Present	fish bones, scales, muscles fibers	Sed. + Raw	1
8	3001	Absent		Absent		Sed. + Raw	1

Laboratory identification code :	13						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	94	Absent		Absent		Sed. + Flot.	2
1	175	Absent		Present	Fishbones, gills, scales, otholits, muscles fibers	Sed. + Flot.	2
4	349	Present	hydrolysed feathers	Present	Fishbones, gills, scales, otholits, cartilages, muscles fibers	Sed. + Flot.	2
5	847	Present	bones	Present	Fishbones, gills, scales, muscles fibers	Sed. + Flot.	1
6	2104	Absent		Present	Fishbones, gills, scales, cartilages, muscles fibers	Sed. + Flot.	2
2	2389	Present	bones	Present	Fishbones, gills, scales,cartilages, muscles fibers	Sed. + Flot.	1
8	2803	Absent		Absent		Sed. + Flot.	2
3	2854	Absent		Present	Fishbones, gills, scales, otholits, muscles fibers	Sed. + Flot.	2

Laboratory identification code :	14						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
1	142	< LOD	bone fragments	Present	'Bone fragments, cartilage scales, gills, muscles	Sed. + Flot.	3
6	190	Absent		Present	Bone fragments, cartilage scales, gills, muscles	Sed. + Flot.	2
7	292	Absent		Absent		Sed. + Flot.	1
5	319	< LOD	bone fragments	Present	'Bone fragments, cartilage scales, gills, muscles	Sed. + Flot.	3
2	574	< LOD	'bone fragments	Present	"Bone fragments, cartilage scales, gills, muscles	Sed. + Flot.	3
3	2623	Absent		Present	Bone fragments, cartilage scales, gills, muscles	Sed. + Flot.	2
4	2659	Absent		Present	Bone fragments, cartilage scales, gills, muscles	Sed. + Flot.	2
8	3034	Absent		Absent		Sed. + Flot.	1

Laboratory identification code :	15						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
2	475	Present		Present		Sed. + Flot.	1
1	505	< LOD	3 bones particles	Present		Sed. + Flot.	3
8	658	Absent		Absent		Sed. + Flot.	1
6	1279	Absent		Present		Sed. + Flot.	1
7	1414	Absent		Absent		Sed. + Flot.	1
5	1738	Present		Present		Sed. + Flot.	1
3	2392	< LOD	3 bones particles	Present		Sed. + Flot.	3
4	2461	< LOD	2 bones particles	Present		Sed. + Flot.	3

Laboratory identification code :	16						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	193	Absent		Present	Fishbones, Gills, Scales, Cartilage & Muscle	Sed. + Raw	1
1	307	Absent		Absent		Sed. + Raw	1
2	442	Present	Bone & Muscle	Present	Fishbones, Gills, Cartilage, Scales & Muscle	Sed. + Raw	1
4	646	Absent		Present	Fishbones, Gills, Scales & Muscle	Sed. + Raw	1
6	1147	< LOD	Bone	Present	Bone, Gills, Scales, Muscle	Sed. + Raw	3
5	1804	Present	Bone & Muscle	Present	Fishbones, Scales, Gills,Cartilage & Muscle	Sed. + Raw	1
8	2638	Present	Bone & Muscle	Present	Fishbones, Gills, Scales, Teeth & Muscle	Sed. + Raw	1
3	2755	Absent		Absent		Sed. + Raw	1

Laboratory identification code :	17						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
6	157	Absent		Present	fishbones,scales,otholites,gills,muscle	Sed. + Flot.	1
7	160	Absent		Absent		Sed. + Flot.	2
1	472	Present	1 bone,feather	Present	fishbones,scales,otholites,gills,muscle	Sed. + Flot.	2
2	640	Present	bones,muscle	Present	fishbones,scales,otholites,gills,muscle	Sed. + Flot.	1
5	1903	Present	bones muscle	Present	fishbones,scales,otholites,gills,muscle	Sed. + Flot.	1
3	2425	Absent		Present	fishbones,scales,otholites,gills,muscle	Sed. + Flot.	1
4	2956	Absent		Present	fishbones,scales,otholites,gills,muscle	Sed. + Flot.	1
8	2968	Absent		Absent		Sed. + Flot.	2

Laboratory identification code :	18						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
1	340	Present	bones, musclefibres no diff.between MBM- and FM-musclefibres possible	Present	fishbones, scales, musclefibres, etc. no diff.between MBM- and FM-musclefibres possible	Sed. + Flot.	2
2	409	Present	bones, musclefibres no diff.between MBM- and FM-musclefibres possible	Present	fishbones, scales, musclefibres, etc. no diff.between MBM- and FM-musclefibres possible	Sed. + Flot.	2
5	649	Present	bones, musclefibres no diff.between MBM- and FM-musclefibres possible	Present	fishbones, scales, musclefibres, etc. no diff.between MBM- and FM-musclefibres possible	Sed. + Flot.	1
8	691	Absent		Absent		Sed. + Flot.	1
7	1315	Absent		Absent		Sed. + Flot.	1
6	1345	Absent		Present	fishbones, scales, muscle fibres, etc. it can't be excludet, that the muscle fibres found, only derive from FM	Sed. + Flot.	1
4	2428	< LOD	bones, musclefibres no diff.between MBM- and FM-musclefibres possible	Present	fishbones, scales, musclefibres, etc. no diff.between MBM- and FM-musclefibres possible	Sed. + Flot.	3
3	2722	Absent		Present	fishbones, scales, muscle fibres, etc. it can't be excludet, that the muscle fibres found, only derive from FM	Sed. + Flot.	1

Laboratory identification code :	19						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
1	43	Absent		Present	Muscle fibres, Fish Bone Gill Scale Cartilage	Sed. + Raw	1
7	259	Absent		Absent		Sed. + Raw	1
2	607	Present	T bone, Muscle fibres	Present	Muscle fibres, Fish Bone, Gill, Scale, Cartilage, Mollusc	Sed. + Raw	1
6	1213	Absent		Present	Muscle Fibres, Fish Bone, Scale, Gill,Cartilage	Sed. + Raw	1
4	2164	Absent		Present	Muscle Fibres, fish bone, Scale, Gill Cartilage.	Sed. + Raw	1
5	2266	Present	T Bone, Muscle Fibres	Present	Muscle Fibres Fish Bone, Scale, Gill	Sed. + Raw	1
3	2491	Absent		Present	Muscle Fibres, Fish Bone Scale, Gill	Sed. + Raw	1
8	2605	Absent		Absent		Sed. + Raw	1

Laboratory identification code :	20						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
4	514	Absent		Present	fishbones, splinters, scales	Sed. + Flot.	3
1	637	< LOD	bones	Present	fishbones, splinters, gills	Sed. + Flot.	3
5	880	Present	bones	Present	fishbones, splinters	Sed. + Flot.	3
7	919	Absent		Absent		Sed. + Flot.	3
6	2236	< LOD	bones	Present	fishbones, splinters	Sed. + Flot.	3
8	2407	Absent		Absent		Sed. + Flot.	3
2	2422	Present	bones	Present	fishbones, splinters, scales, gills	Sed. + Flot.	3
3	2458	< LOD	bones	Present	fishbones, splinters, scales, gills	Sed. + Flot.	3

Laboratory identification code :	21						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	457	< LOD	Thin brown hair/feather	Absent		Sed. + Flot.	2
4	679	< LOD	hair	Present	meatfiber, fishbone, cartilage,scale	Sed. + Flot.	2
1	802	Absent		Present	meatfiber, fishbone, cartilage,scale	Sed. + Flot.	2
6	1246	Absent		Present	meatfiber, fishbone, cartilage,scale	Sed. + Flot.	2
2	1432	Present	bones, feather	Present	meatfiber, fishbone, cartilage,scale	Sed. + Flot.	2
5	1606	Present	bones,feather	Present	meatfiber, fishbone, cartilage,scale	Sed. + Flot.	2
8	2539	Absent		Absent		Sed. + Flot.	2
3	2821	Absent		Present	meatfiber, fishbone, cartilage,scale	Sed. + Flot.	2

Laboratory identification code :	22						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	688	Absent		Absent		Sed. + Raw	1
5	946	Present	bones	Present	bones, cartilage, gills, scale	Sed. + Raw	1
6	1312	< LOD	bones	Present	bones, cartilage, gills, scale	Sed. + Raw	3
1	1330	Present	bones	Present	bones, cartilage, gills, scale	Sed. + Raw	1
3	1666	Present	bones	Present	bones, cartilage, gills, scale	Sed. + Raw	1
4	2098	< LOD	bones	Present	bones, cartilage, gills, scale	Sed. + Raw	3
8	2572	Absent		Absent		Sed. + Raw	1
2	2653	Present	bones	Present	bones, cartilage, gills, scale	Sed. + Raw	1

Laboratory identification code :	23						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
6	91	Absent		Present	high number of fish bone particles and muscle fibres	Sed. + Flot.	2
1	604	< LOD	5 bone fragments; unknown number of muscle fibres	Present	high number of fish bone particles and muscle fibres	Sed. + Flot.	2
7	853	Absent		Absent		Sed. + Flot.	1
5	1144	Present	10 bone fragments; unknown number of muscle fibres	Present	high number of fish bone particles and muscle fibres	Sed. + Flot.	1
2	1300	Present	10 bone fragments; unknown number of muscle fibres	Present	fish bone particles and muscle fibres	Sed. + Flot.	1
4	1867	Absent		Present	high number of fish bone particles and muscle fibres	Sed. + Flot.	2
8	2506	Absent		Present	8 bone fragments; no muscle fibres	Sed. + Flot.	1
3	3019	< LOD	a few particles difficult to recognise	Present	high number of fish bone particles and muscle fibres	Sed. + Flot.	2

Laboratory identification code :	24						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
6	388	Absent		Present	fishbones, cartilage, scales, muscles	Sed. + Flot.	1
2	1036	Present	bones	Present	fishbones, cartilage	Sed. + Flot.	1
1	1264	Absent		Present	fishbones, cartilage, scales	Sed. + Flot.	1
5	1276	Present	bones	Present	fishbones, cartilage, scales	Sed. + Flot.	1
7	1282	Absent		Absent		Sed. + Flot.	1
3	1303	Absent		Present	fishbones, cartilage, scales, otolith, muscles	Sed. + Flot.	1
4	1735	Present	1 bone, feathers	Present	fishbones, shrimp, cartilage, scales,muscles	Sed. + Flot.	1
8	1780	Absent		Absent		Sed. + Flot.	1

Laboratory identification code :	25						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
2	277	Present	bones, muscle fibres	Present	fisbones, muscle fibres, cartilage	Sed. + Raw	2
5	418	Present	bones, muscle fibres	Present	fisbones, muscle fibres, cartilage, scale	Sed. + Raw	2
6	982	Absent		Present	fisbones, muscle fibres, cartilage	Sed. + Raw	2
1	1363	Absent		Present	fisbones, muscle fibres, cartilage	Sed. + Raw	2
7	1381	Absent		Absent		Sed. + Raw	2
3	1831	Absent		Present	fisbones, muscle fibres, cartilage, gill	Sed. + Raw	2
8	1846	Absent		Absent		Sed. + Raw	2
4	2560	Absent		Present	fisbones, muscle fibres, cartilage, scale	Sed. + Raw	2

Laboratory identification code :	26						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
6	454	Absent		Present	fish bones, scales, muscle fibers	Sed. + Raw	1
5	616	Present	bones	Present	fish bones, scales, muscle fibers	Sed. + Raw	1
1	1066	Absent		Present	fish bones, scales, muscle fibers	Sed. + Raw	1
2	1069	Present	bones	Present	fish bones, scales, muscle fibers	Sed. + Raw	2
7	1183	Absent		Absent		Sed. + Raw	1
3	1402	Absent		Present	fish bones, scales, muscle fibers	Sed. + Raw	1
4	1801	Absent		Present	fish bones, scales, muscle fibers	Sed. + Raw	1
8	2110	Absent		Absent		Sed. + Raw	1

Laboratory identification code :	27						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
6	421	Absent		Present	gills, fishbones, scales, muscle fibres	Sed. + Raw	2
5	583	Present	bones; muscle fibres	Present	fishbones, muscle fibres (impossible to discern between muscle fibres from fish and from T.A.)	Sed. + Raw	1
2	1366	< LOD	bones (4 particles on three determinations)	Present	fishbones, gills, muscle fibres	Sed. + Flot.	3
4	1669	Absent		Present	fishbones, gills, scales, muscle fibres	Sed. + Raw	1
7	1744	Absent		Absent		Sed. + Raw	1
8	1912	Absent		Absent		Sed. + Raw	1
3	2161	Absent		Present	fishbones, gills, scales, muscle fibres	Sed. + Raw	1
1	2254	Absent		Present	fishbones, scales, muscle fibres	Sed. + Raw	1

Laboratory identification code :	28						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
2	310	Present	Bones	Present	Fishbones, Cartilages, Gills, scales.	Sed. + Flot.	1
6	520	< LOD	Bones	Present	Fishbones, Cartilages, Gills, scales.	Sed. + Flot.	2
5	1375	Present	Bones	Present	Fishbones, Cartilages, Gills, scales.	Sed. + Flot.	1
7	1645	Absent		Absent		Sed. + Flot.	1
8	1813	Absent		Absent		Sed. + Flot.	1
3	1963	< LOD	Bones	Present	Fishbones, Cartilages, Gills, scales.	Sed. + Flot.	2
4	2230	Absent		Present	Fishbones, Cartilages.	Sed. + Flot.	1
1	2947	< LOD	Bones	Present	Fishbones, Cartilages, Gills, scales.	Sed. + Flot.	1

Laboratory identification code :	31						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
2	244	Absent		Present	fish bone	Sed. + Flot.	1
6	256	Absent		Present	fish bone, fish scales, fish muscle	Sed. + Flot.	1
1	1099	Absent		Present	fish bone, fish muscle	Sed. + Flot.	1
3	1105	Absent		Present	fish bone, fish muscle	Sed. + Flot.	1
5	1210	Absent		Present	fish bone	Sed. + Flot.	1
7	1843	Absent		Absent		Sed. + Flot.	1
8	2143	Absent		Absent		Sed. + Flot.	1
4	2527	Absent		Present	fish bone, fish muscle	Sed. + Flot.	1

Laboratory identification code :	32						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	655	Absent		Absent			3
6	1081	Absent		Present	bones		3
5	1177	Present	bones	Present	bones		3
2	1267	< LOD	bones	Present	bones		3
3	1600	Absent		Present	bones		3
4	1768	Absent		Present	bones		3
8	1945	Absent		Absent			3
1	2980	Absent		Present	bones		3

Laboratory identification code :	33						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
2	13	Present	bones	Present	fishbones	Sed. + Flot.	1
6	322	Present	bones	Present	fishbones	Sed. + Flot.	1
7	523	Absent		Absent		Sed. + Flot.	1
1	934	Absent		Present	fishbones	Sed. + Flot.	1
3	1369	Present	bones	Present	fishbones	Sed. + Flot.	1
5	1441	Present	bones	Present	fishbones	Sed. + Flot.	1
4	1966	Present	bones	Present	fishbones	Sed. + Flot.	1
8	2176	Absent		Absent		Sed. + Flot.	1

Laboratory identification code :	34						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
6	25	Present	Bone				2
2	376	Present	Bone				2
4	448	Present	Bone				2
5	550	Present	Bone				2
7	589	Absent					2
1	901	Present	Bone				2
3	1336	Present	Bone				2
8	2275	Absent					2

Laboratory identification code :	35						
Sample type	**Sample N°**	**Terrestrial animal part.**	**Details of terrestrial part.**	**Fish part.**	**Details of fish part.**	**Fractions used**	**Number of determinations**
7	556	Absent		Absent		Sed. + Flot.	1
2	1333	< LOD	bones	Present	fishbones	Sed. + Flot.	1
3	1897	Absent		Present	fishbones, cartilage, mustle	Sed. + Flot.	1
8	2044	Absent		Absent		Sed. + Flot.	1
5	451	Present	bones,mustle	Present	fishbones,mustle	Sed. + Flot.	1
4	613	Absent		Present	fishbones, mustle	Sed. + Flot.	1
6	619	Absent		Present	fishbones, cartilage, mustle	Sed. + Flot.	1
1	835	Absent		Present	fishbones, mustle	Sed. + Flot.	1