EURL-AP Proficiency Test Microscopy 2014

Final version

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Table of content

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 31 of which 27 NRLs and 4 participants from third countries towards the European Union. A set of 9 blind samples consisting of compound feeds for mammals, fish feeds and fishmeals fortified or not with processed animal proteins (PAPs) was to be analysed. Overall results were very good with 78% of participating NRLs performing excellently according to the assigned performance criteria. The study allowed demonstrating that some muscular aggregates may sometimes be confused with feather fragments. Technical advice to avoid this by using staining and DIC or polarised light microscopy is given.

Keywords :

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

This report identified by an ISBN has been prepared from a draft version sent for revision to the network of NRLs on the 10th March 2015. After reception of the comments on the 25th March 2015, it was amended accordingly and approved by the signature of its authors.

Authors' signature for approval:

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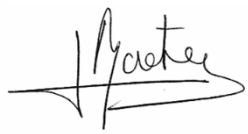

Wallonie

1. Foreword

European Union Reference Laboratories (EURL) were created in order to ensure a high level of quality and a uniformity of the results provided by European control laboratories. On 29th April 2004, the European Parliament and the Council adopted 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 by Commission Regulation EU/51/2013 [3] amending Annex VI of Commission Regulation EC/152/2009 [4]. The present study report is part of this activity scope.

2. Introduction

According to modified Annex VI of Commission Regulation EC/152/2009 [4] official controls for the detection of animal proteins in feed inside the EU are performed by light microscopy and/or PCR. 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 4 laboratories outside this EU network. A detailed list of the 31 participating labs is included in Annex 1.

Official announcement of the study was made on the 4th September 2014 to all participants.

On the 7th November 2014, the Excel report forms containing the instructions (Annex 2) were communicated to all participants – downloadable from the EURL-AP intranet for the NRLs or sent to the non-EU participants who do not have access to this intranet. On the same day, the 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 were 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 mail 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 28th November 2014. Notification has been done that this date was a deadline and that results arriving later would not be accepted.

26 NRL participants delivered their results on time. One NRL in agreement with the organiser was accepted to deliver its results on 2nd of December. Concerning the other non-EU participants two delivered their results on due time and one was accepted to deliver its results later due to mail problems. One non-EU participant, due to custom issues, did not receive its sample set and did not deliver its results. Thus only one participant had to be excluded. Results from NRLs and other participants were analysed separately in this report.

3.2. Material

3.2.1. Description of the samples

Nine different materials were prepared for the proficiency test.

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

- Target concentrations of mammalian PAPs around 0.1% considered for time being as the adulteration level that the method should be able to detect.
- Use of fishfeeds and fishmeals 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 [5].
- Adulteration with microscopically unusual or almost undetectable materials from terrestrial origin (milk powder, blood meal) but generating positive responses by PCR.
- Use of a bovine PAP without bone.

Each participating lab received about 50g 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 the blind sample set used.

Sample	Material	Nr of replicates	Expected results *	
			Terrestrial particles	Fish particles
1	feed I	1	-	-
2	feed I + 0.5% milk powder	1	-	-
3	feed II	1	-	-
4	feed II + 0.1% terrestrial PAP	1	+	-
5	fishmeal I	1	-	+
6	fishmeal I + 0.1% bovine PAP	1	-	+
7	fishfeed + 1% blood meal	1	-	+
8	fishfeed + 0.1% porcine PAP	1	+	+
9	fishmeal II	1	-	+
Total		**9**	**2**	**5**

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

3.2.2. Materials used in the preparation of the samples

Three main categories of matrices were used: compound feeds, fishmeals and fishfeeds.

- Feed I was a **compound feed for horses** bought from a local producer. This compound feed was used as blank in the EURL-AP microscopy proficiency test 2013 [6]. 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%. This compound feed was ground at 2 mm and used for preparing samples 1 and 2.
- Feed II was a **compound feed for mini pigs** bought from a local producer. It consisted of sugar beet pulp, wheat bran, sunflower cake, barley, palm cake, alfalfa pellets, molasse, wheat, rapeseed cake, corn gluten, calcium carbonate and feed complements (vitamins, salts, minerals). Its sediment content was about 1.3%. This compound feed was ground at 2 mm and used for preparing samples 3 and 4.
- Fishmeal I was a custom-made **mixture of two pure fishmeals** from Denmark and Iceland. The sediment content of the mixture was about 7.9%. It was used unground for preparing samples 5 and 6.
- The fishfeed was a custom-made **mix of two commercial compound feed for fry**. It consisted of fishmeal, fish oil, wheat flower and wheat gluten, soybean meal, corn gluten, dehulled horse beans, vitamin premix, yeasts, minerals and flavouring. The sediment content of the mixture was about 0.9%. It was ground at 2 mm and used for preparing samples 7 and 8.
- Fishmeal II was a commercial **pure fishmeal** from Peru. Its sediment was about 15.2%. It was used without grinding for preparing sample 9.

Adulterant material used:

- **Skimmed milk powder** was added to sample 2. Its purity was checked by microscopy and PCR
- A mixed porcine-bovine **terrestrial PAP** was used after grinding at 2 mm for preparing sample 4. Its final bone content was of about 49.3%. Its species composition was controlled by microscopy and PCR.
- A **pure bovine PAP** was used for preparing sample 6. This PAP was also produced by a pilot plant. This bovine PAP did not contain bones and had no sediment. Its purity was controlled by microscopy and PCR. By PCR the mean Ct values for ruminant PCR test was about 26-27 cycles

which is a late value compared to other pure PAPs. This is revealing a rather low charge of DNA for a PAP.

- A **pure porcine blood meal** was added to sample 7. This blood meal had no sediment. The sample was checked by microscopy and PCR.
- A **pure porcine PAP** was used for preparing sample 8. Its bone content was of about 37.5% and its purity was checked by microscopy and PCR.

3.2.3. Description of the mixing procedures

Adulteration of the different samples was performed by spiking, i.e. by adding to each separate sample the adequate amount of contaminant to the feed or feed material

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

3.3. Qualitative analysis

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

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

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

Considering the risk of false positive results, all results expressed as below LOD 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.

3.4. Performance criteria

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

Sample 2 (Feed I + 0.5% milk powder) is considered to be declared negative for terrestrial particles detection as milk powder is microscopically almost undetectable and is an authorized product.

Sample 6 (Fishmeal I + 0.1% bovine PAP) is also considered to be declared negative for terrestrial particles since the bovine PAP used does not present microscopically identifiable particles (e.g. bones, hairs) allowing to be categorised as from terrestrial origin.

Sample 7 (Fishfeed + 1% blood meal) is considered to be declared negative for terrestrial particles as blood meal particles do not present identifiable features or bone fragments allowing to classify this material as from terrestrial origin. Nevertheless participants that would be able to disclose this presence of blood meal and would therefore declare the sample as positive for terrestrial material (as this type of product is not known to be obtained from fish) should logically not be penalised, therefore such results have to be assimilated to a correct negative assignment.

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

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

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. Table 2 summarizes the results.

Table 2: Homogeneity study – Results.

Sample	Material	Light microscopy				NIRM		PCR			
		Nr of replicates	Terrestrial	Feathers	Fish	Nr of replicates	Animal	Nr of replicates	Ruminant	Porcine	Fish
1	feed I	10	-	-	-	5	-	3	-	nt	nt
2	feed I + 0.5% milk powder	10	-	-	-	5	-	3	+	nt	nt
3	feed II	10	-	-	-	5	-	3	+	nt	nt
4	feed II + 0.1% terrestrial PAP	10	+	-	-	5	+	3	+	nt	nt
5	fishmeal I	10	-	-	+	5	+	3	-	nt	+
6	fishmeal I + 0.1% bovine PAP	10	-	-	+	5	+	5	-	nt	+
7	fishfeed + 1% blood meal	10	-	-	+	5	+	3	-	+	+
8	fishfeed +0.1% porcine PAP	10	+	-	+	5	+	3	-	+	+
9	fishmeal II	10	-	-	+	5	+	3	+	nt	+

(Legend: + = systematically detected, - = systematically not detected, NIRM = near infrared microscopy, nt = not tested)

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 sediments of the samples and materials used for this study in complement to the official methods.

Sample 1 (Feed I) no animal material was detected by light microscopy. By PCR, no ruminant DNA was found.

Sample 2 (Feed I + 0.5% milk powder) did not show any animal particle by light microscopy. It was positive for the presence of ruminant DNA.

Sample 3 (Feed II) did not show any animal material by light microscopy. By PCR, it was tested positive for ruminant DNA although no indication from its composition could explain that.

Sample 4 (Feed II + 0.1% terrestrial PAP) was positive for terrestrial bones by light microscopy. No fish material could be detected. By PCR it was positive for ruminant DNA.

Sample 5 (Fishmeal I) presented fish particles by light microscopy. No terrestrial particles have been detected. PCR analyses revealed the sample only positive for fish DNA.

Sample 6 (Fishmeal I + 0.1% bovine PAP) did not reveal any terrestrial particle when observed by microscope but only fish particles. PCR analyses could not detect ruminant DNA while fish DNA was always detected.

Sample 7 (Fishfeed + 1% blood meal), on the exception of presence of blood meal particles (10/10), microscopy did not reveal any other terrestrial fragments. Fish particles were always observed. By PCR, results were positive for fish and pig DNA and negative for ruminant DNA.

Sample 8 (Fishfeed +0.1% porcine PAP) showed, aside fish particles, systematically terrestrial bones by microscopy. PCR analyses led to negative results for ruminant DNA but positive results for porcine and fish DNA.

Sample 9 (Fishmeal II) presented systematically fish particles by light microscopy. No terrestrial particles have been detected. PCR analyses revealed the sample positive for fish DNA and ruminant DNA, although no explanations accounts for this latter result.

Through the homogeneity study, feather fragments (including hydrolysed feathers) have not been detected.

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

Globally NRLs respected the instructions related to the proficiency test itself and to the current EU regulation.

From the NRL data it appeared that 67% of the results were obtained by one single determination, 20% obtained by two determinations and 9% required three determinations. Only one participant (lab 24) omitted to mention the number of determinations needed for its results.

From the pool of results based on either two or three determinations, some did not need to perform additional determinations at all as they concerned correctly identified negative results for both terrestrial and fish presence. This represents only 8% of superfluous repetitions over the total number of results. This might reflect the quest for perfection differing from routine situations from the participants rather than an erroneous interpretation of modified Annex VI of Commission Regulation EC/152/2009.

4.2.2. Overview of results and performance of the network

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

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

Sample	Material	n	AC	
			Terrestrial	Fish
1	feed I	27	1.000	0.963 (1)
2	feed I + 0.5% milk powder	27	0.963 (1)	0.963 (1)
3	feed II	27	1.000	1.000
4	feed II + 0.1% terrestrial PAP	27	0.963 (1)	0.963 (1)
5	fishmeal I	27	0.852 (4)	1.000
6	fishmeal I + 0.1% bovine PAP	27	0.852 (4)	1.000
7	fishfeed + 1% blood meal	27	0.963 (1)	1.000
8	fishfeed + 0.1% porcine PAP	27	0.926 (2)	1.000
9	fishmeal II	27	0.786 (6)	1.000

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

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

Problems of specificity for fish were limited to a background level, only 3 cases. Two cases of false positive results were observed for a same lab: one for sample 1 and one for sample 2.

Specificity issue for terrestrial animal presence was anecdotic for sample 2 and 7 with only one false positive result. More problems of specificity for terrestrial animal presence were depicted on sample 5 and 6; they will be analysed in another section. Finally sample 9 revealed also 6 false positive results for the presence of terrestrial fragments.

Sensitivity for fish was perfect.

For terrestrial animal presence, problems of sensitivity were limited to only one case for sample 4 and two cases for sample 8.

4.2.3. Detailed review of results per sample

Sample 1: Feed I

PD for fish particles:

- Lab 27: fishbones, scale (more than 5 as based on 1 determination only)

Aside this case of PD, two participants reported <LOD :

- Lab 19: with less than 10 feathers and muscles on 2 determinations
- Lab 20: with less than 10 fishbones on 2 determinations. No other types of animal particles detected.

Sample 2: Feed I + 0.5% milk powder

PD for terrestrial particles:

- Lab 23: particles of plasma

One case of <LOD by lab 19 that reported less than 10 feathers and muscles on 2 determinations.

One participant, lab 16, succeeded at detecting and identifying correctly the presence of milk powder. This participant nevertheless needed 3 determinations before declaring this presence.

PD for fish particles:

- Lab 27: scales (more than 5 as based on 1 determination only)

Aside this case of PD, three participants reported <LOD :

- Lab 8: 1 fishbone from 2 determinations
- Lab 23: 3 fishbones from 2 determinations
- Lab 26: in between 10-15 fishbones particles on 3 determinations

From the two first samples based on the same feed matrix, lab 27 erroneously reported fishbones. Whether this is based on erroneous identification or on a possible contamination issue is unclear as the situation is repeated. It has to be noted that this matrix was used previously [6] without any problem except for one participant that also erroneously identified fishbones. It would be interesting to determine the type of particle leading to confusion which probably also accounts for the <LOD cases.

Sample 3: Feed II

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

- Lab 8: one terrestrial bone fragment on 2 determinations
- Lab 28: 3 fishbones on 3 determinations

Sample 4: Feed II + 0.1% terrestrial PAP

ND for terrestrial particles:

- Lab 21

PD for fish particles:

- Lab 23: 6 fishbones detected and muscles (on 2 determinations)

One case of <LOD by lab 7 that reported less than 10 fishbones and muscles on 2 determinations.

The false positive result for fish reported by lab 23 should have been declared as <LOD as only a mean of 3 particles allowing correct categorisation for fish on 2 determinations was observed.

Sample 5: Fishmeal I

PD for terrestrial particles :

- Lab 21: bone fragments
- Labs 13 and 19: feathers
- Lab 17: hydrolysed feathers and one bone fragment

Sample 6: Fishmeal I + 0.1% bovine PAP

PD for terrestrial particles :

- Lab 21: bone fragments and a few feathers
- Labs 13 and 19: feathers
- Lab 17: hydrolysed feathers

Concerning the finding of feathers and hydrolysed feathers that conducted to false positive results for labs 13, 17, 19 and 21, the origin of this is probably linked to the matrix. The first reason for this hypothesis is that samples where blindly numbered and that no order in the reporting could be deduced. The second reason is that the participants that reported such structures did it for both sample 5 and 6. The sole exception is lab 21 that only reported feathers once. As the homogeneity study on these two sample types did not reveal potential problems for feather presence, it was decided to contact by email the participants to know if they would be able to send pictures supporting these findings, without mentioning them to be correct or wrong.

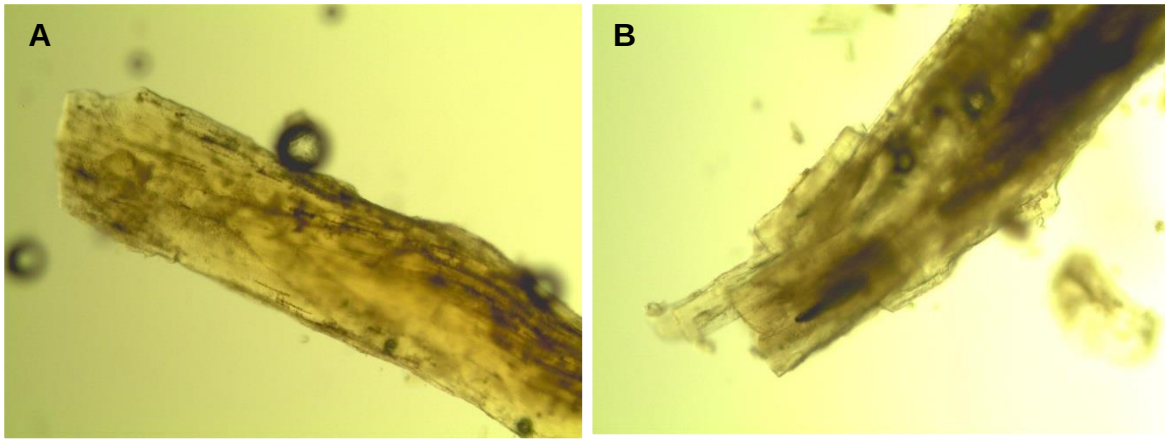

Figure 1: Structures erroneously identified as feathers into sample 5 (A) and 6 (B). Magnification unknown.

Among the explanations collected, a first laboratory mentioned not having taken pictures of the fragments and wrote to have been hesitating to characterise them as feathers. The same lab also mentioned to have found coagulated particles from the flotation possibly misinterpreted as hydrolysed feathers. The lab admitted to have been influenced by the finding of one feather fragment into sample 9 (see in that section). To summarize this case is clearly an example of confusion resulting from hesitations.

A second laboratory sent 11 pictures of structures identified as feathers. Nevertheless none of the structures illustrated could be certified as feathers. Some of these structures are shown in Figure 1.

A third lab sent pictures of the structures identified as feathers. On the 6 pictures sent, only one fragment could be identified as a feather fragment (Figure 2A) presenting nodes of barbules. All other fragments, such as the one illustrated on Figure 2B, could not be identified as feathers but more likely as miscellaneous fibres. However it seems that the pictures taken originate from the sediment fraction and not from the flotate or the raw material.

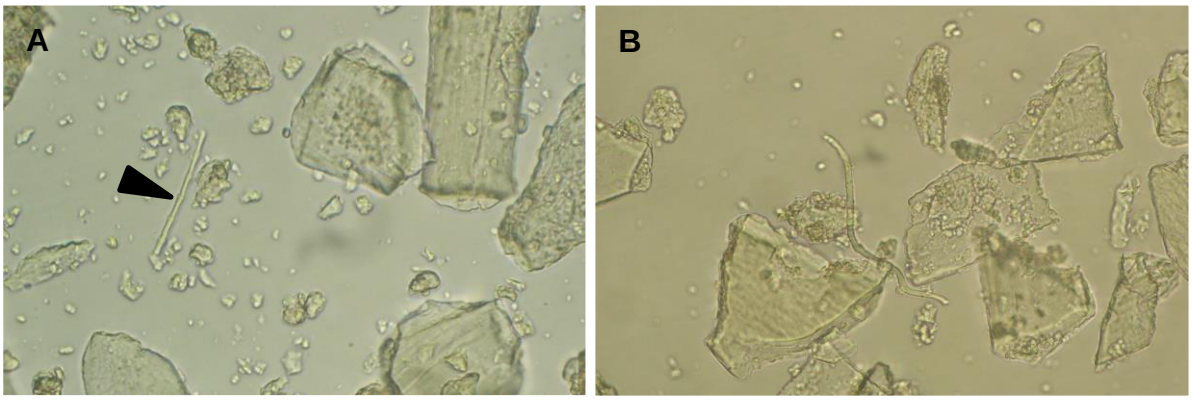

Figure 2: Feather (arrowhead) identified into sample 5 (A). Structure resembling a feather identified into sample 6 (B). Magnification unknown.

Thus from all the information collected from the participants for sample 5 and 6, only one single small feather fragment could be identified as such. From the pictures sent it also appeared that none of these participants were using the cystine reagent which is known to stain feathers brown and therefore offering a more reliable identification. The use of staining as a help can only be strongly encouraged as well as the use of polarised light microscopy allowing to better identify fibres from plant origin (such a cotton, linen,…) from fibres of animal origin (such as wool, feathers) which are barely birefringent. About the exact nature of the fragments misinterpreted as feathers, we made complementary observations on both flotate and raw material from sample 5 (Fishmeal I). Conclusions are as follows. The findings of similar structures occurred at a rate of only 2-3 per slide (Figure 3 A and B).

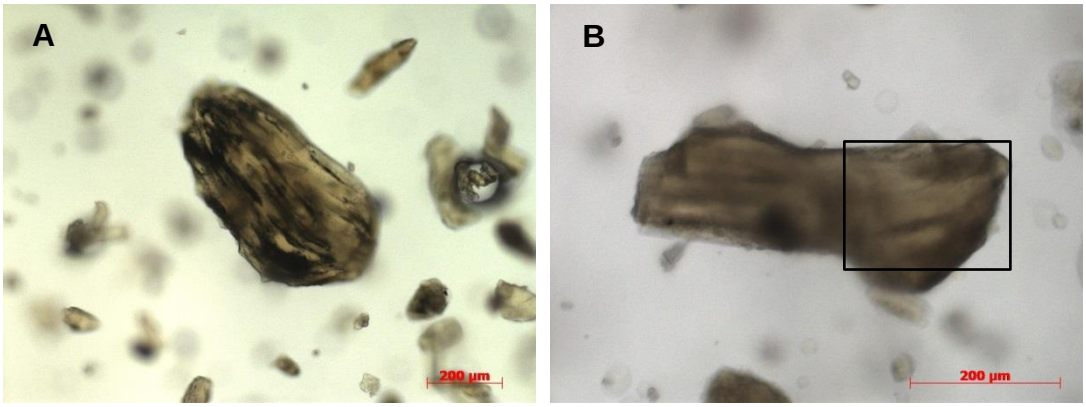

Figure 3: Structures that would erroneously be identified as feathers from sample 5 (A and B) and comparable to those from Figure 1. The square on figure 3B is detailed into Figure 5.

Although a straight identification of these structures was uncertain, staining with Fehling reagent clearly stained such structures in pinkish-violet as illustrated on Figure 4, thus revealing their muscular origin.

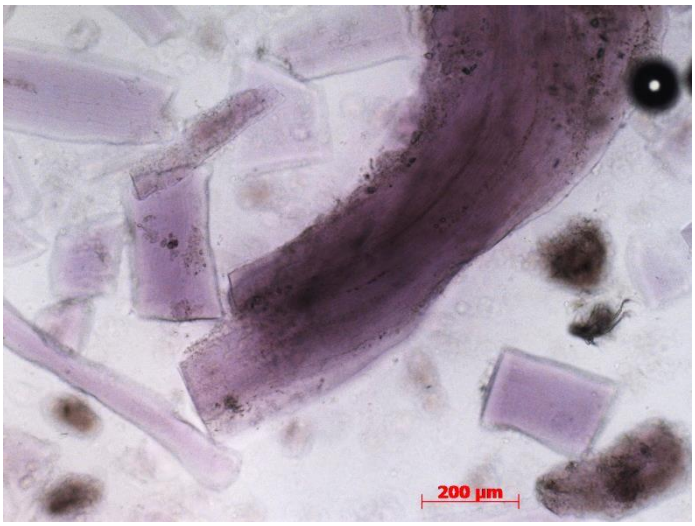

Figure 4: Fehling staining of structures that would erroneously be identified as feathers.

Observations at higher magnifications under bright field conditions allowed recognizing clearly the typical striated pattern from sarcomeres. This was even observable without Fehling staining but using DIC on glycerol mounted slides as shown by Figure 5.

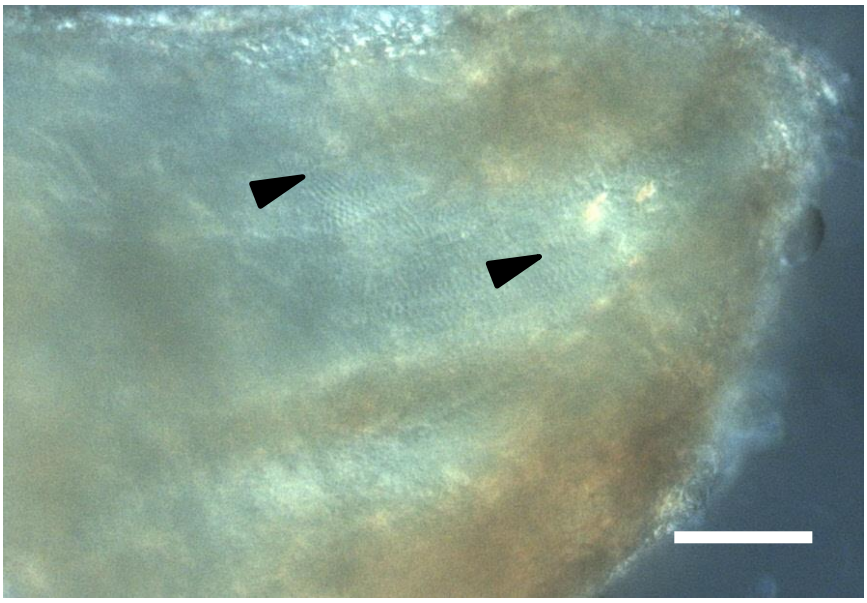

Figure 5: Enlargement of square from Fig 3B showing the striated pattern of the sarcomeres (arrowheads). DIC. Bar = 50 µm.

Sample 7: Fishfeed + 1% blood meal

PD for terrestrial particles:

- Lab 21: bone fragments

About the ability to properly identify the presence of blood meal, surprisingly 30% of the NRLs participants (8 on 27) were able to detect it. From these correct detections, at least 62% were supported by the TMB-H_2O_2 staining. In one case, suspicious particles were even isolated before staining. The immediate character of the reaction, the bluish colouring and the formation of air bubbles confirmed the blood presence as shown on Figure 6, received from a participant. This demonstrates the efficiency of this optional staining method which is recommended into the SOP.

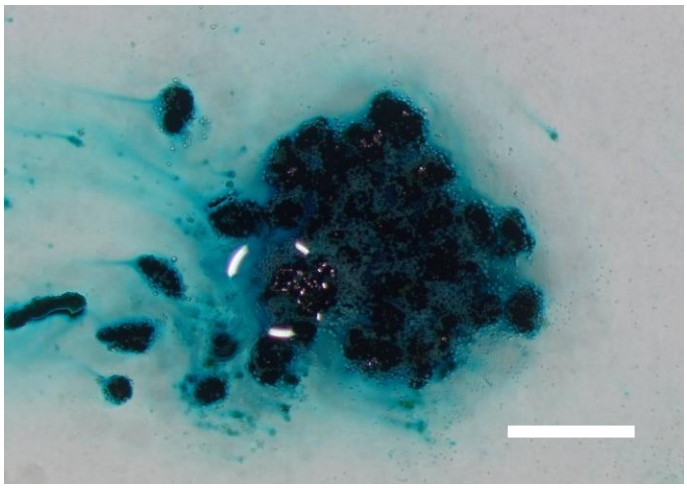

Figure 6: Isolated blood particles from sample 7 stained with TMB-H_2O_2. Bar = 1 mm.

Sample 8: Fishfeed + 0.1% porcine PAP

ND for terrestrial particles:

- Lab 12
- Lab 23: only found 2 bones based on 2 determinations.

Sample 9: Fishmeal II

PD for terrestrial particles :

- Lab 21: bone fragments
- Labs 2, 4, 13 and 19: feathers
- Lab 17: hydrolysed feather

As for fishmeal I (sample 5 and 6), in this second fishmeal a number of erroneous identifications for feathers or hydrolysed feathers occurred. As for samples 5 and 6, some requests for information were sent. On the eight pictures received from the laboratories, none allowed to categorize the found structures as feathers. Concerning lab 17, this participants should have declared his findings as <LOD for only one single feather fragment was truly detected.

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 (next page). A ranking of the labs was prepared based on the consolidated accuracy.

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 page 16).

21 labs out of 27 NRLs or in other words for 78% of the NRLs performed very well (2013 : 63%). 5 NRLs performed satisfyingly. Only one NRL was classified according the ranking criteria as underperforming for the present proficiency test. This lab requires improvement of proficiency. In agreement with the EURL-AP SOP for managing underperformances (available on the EURL-AP intranet since 18 January 2012), this underperforming participant is asked to report on the origin of his multiple errors as well as on the actions he will undertake in order to solve the problems.

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. Underlined lab codes refer to NRLs that were able to identify blood presence.

Terrestrial

lab code	AC	SE	SP
1	1.000	1.000	1.000
3	1.000	1.000	1.000
5	1.000	1.000	1.000
6	1.000	1.000	1.000
7	1.000	1.000	1.000
8	1.000	1.000	1.000
9	1.000	1.000	1.000
10	1.000	1.000	1.000
11	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
16	1.000	1.000	1.000
20	1.000	1.000	1.000
22	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
28	1.000	1.000	1.000
27	1.000	1.000	1.000
2	0.889	1.000	0.857
4	0.889	1.000	0.857
12	0.889	0.500	1.000
23	0.778	0.500	0.857
13	0.667	1.000	0.571
17	0.667	1.000	0.571
19	0.667	1.000	0.571
21	0.444	0.500	0.429

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
9	1.000	1.000	1.000
10	1.000	1.000	1.000
11	1.000	1.000	1.000
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
16	1.000	1.000	1.000
17	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
28	1.000	1.000	1.000
23	0.889	1.000	0.750
27	0.778	1.000	0.500

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

Consolidated			
lab code	AC	SE	SP
1	1.000	1.000	1.000
3	1.000	1.000	1.000
5	1.000	1.000	1.000
6	1.000	1.000	1.000
7	1.000	1.000	1.000
8	1.000	1.000	1.000
9	1.000	1.000	1.000
10	1.000	1.000	1.000
11	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
16	1.000	1.000	1.000
20	1.000	1.000	1.000
22	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
28	1.000	1.000	1.000
2	0.944	1.000	0.909
4	0.944	1.000	0.909
12	0.944	0.857	1.000
27	0.889	1.000	0.818
13	0.833	1.000	0.727
17	0.833	1.000	0.727
19	0.833	1.000	0.727
23	0.833	0.857	0.818
21	0.722	0.857	0.636

4.3. Qualitative analyses from the non-EU participants

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

For reminder, third countries participants were requested to perform the test by means of microscopic method.

Individual performances from the 3 participants outside the EU were assessed exactly as in previous section (4.2.4.). A ranking of those labs was prepared as well based on the consolidated accuracy.

Results are to be found in tables 7 and 8 (next page).

Tables 7 (left) and 8 (right): non-EU lab proficiencies regarding the detection of terrestrial and fish material. Ranking follows AC values for primary key and SE for second key.

Terrestrial			
lab code	AC	SE	SP
32	1.000	1.000	1.000
34	1.000	1.000	1.000
31	0.556	0.000	0.714

Fish			
lab code	AC	SE	SP
31	1.000	1.000	1.000
32	1.000	1.000	1.000
34	1.000	1.000	1.000

Two out of 3 non-EU labs performed faultless.

Lab 31 only presented difficulties at identifying terrestrial animal constituents. Errors concerned both sensitivity and specificity:

Sample 2: Feed I + 0.5% milk powder

No error but the sample was declared <LOD as less than 10 structures identified as animal hairs were found on 2 determinations.

Sample 4: Feed II + 0.1% terrestrial PAP

ND for terrestrial particles: <LOD but the only description given is the finding of animal hairs without other precision.

Sample 5: Fishmeal I

PD for terrestrial particles: mention of meat and bone meal is made.

Sample 8: Fishfeed +0.1% porcine PAP

ND for terrestrial particles

Sample 9: Fishmeal II

PD for terrestrial particles: mention of meat and bone meal is made.

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

Table 9: General non-EU lab proficiency regarding the detection of terrestrial and fish material. Ranking follows AC values as primary key and SE as second key.

Consolidated			
lab code	AC	SE	SP
32	1.000	1.000	1.000
34	1.000	1.000	1.000
31	0.778	0.714	0.818

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

5. Conclusions

Results demonstrated that the detection of fish particles was achieved without difficulties on both aspects: specificity and sensitivity.

For the detection of terrestrial particles problems of sensitivity were limited. This was not the case for specificity issues: results from the NRL network revealed still a lot of erroneous identification of feather fragments. They originated within pure fishmeal matrices (sample 5, 6 and 9) whether or not adulterated. Explanations for this confusion were found. Some muscular fibre aggregates were clearly responsible for this misinterpretation. Nevertheless in addition to that, past studies, focussing on hydrolysed feathers [5, 7] might have influenced the operators that over-focussed on a possible presence of feathers. Tools and techniques exist to prevent such confusion. The use of staining methods, such as the cystine and Fehling reagents, combined with other microscopic techniques of observations, such as polarization and DIC, are keys to a correct interpretation in case of doubt.

Some results related to samples that had to be considered as negative, consistent with the performance criteria because they were lacking or presenting unusual microscopic features, deserves some comments.

The study reserved some good unexpected identification capabilities for the presence of blood meal. About one third of the NRLs were able to identify this adulteration by blood meal at a level of 1%. The majority of these successful detections were supported by the use of TMB-H_2O_2, demonstrating the relevance of this staining method. On the opposite side and as expected, the addition of 0.5% milk powder revealed to be invisible by almost all participants. Only one NRL mentioned this presence of milk. Finally the use of a pure bovine PAP without bones also showed to be undetectable at least at the level of 0.1%. This pure bovine PAP is however an atypical processed animal protein. Aside the lack of microscopic features allowing its identification as terrestrial meal, its DNA content is also lower than that observed on classical PAPs. This explains the negative ruminant PCR response from the homogeneity study at the level used.

The global performance of the NRL network was very good. 78% out of them performed excellently. This percentage is the best ever obtained (as a simple comparison with the last two years: 63% in 2013 [6], 56% in 2012 [8]). It is also the first time since 2006 that only one single NRL was underperforming, this number has never been that low. Although this comparison is very positive, one has to think about the fact that it is only a comparison of studies involving different sample sets over a long period, thus only offering an impression which is not scientifically measurable.

Concerning the non-EU participants, two out of three performed excellently. All three non-EU participants showed the same difficulties at detecting milk powder, blood meal and a terrestrial PAP with an absence of microscopic markers.

Acknowledgment

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

References

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

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

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

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

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

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

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

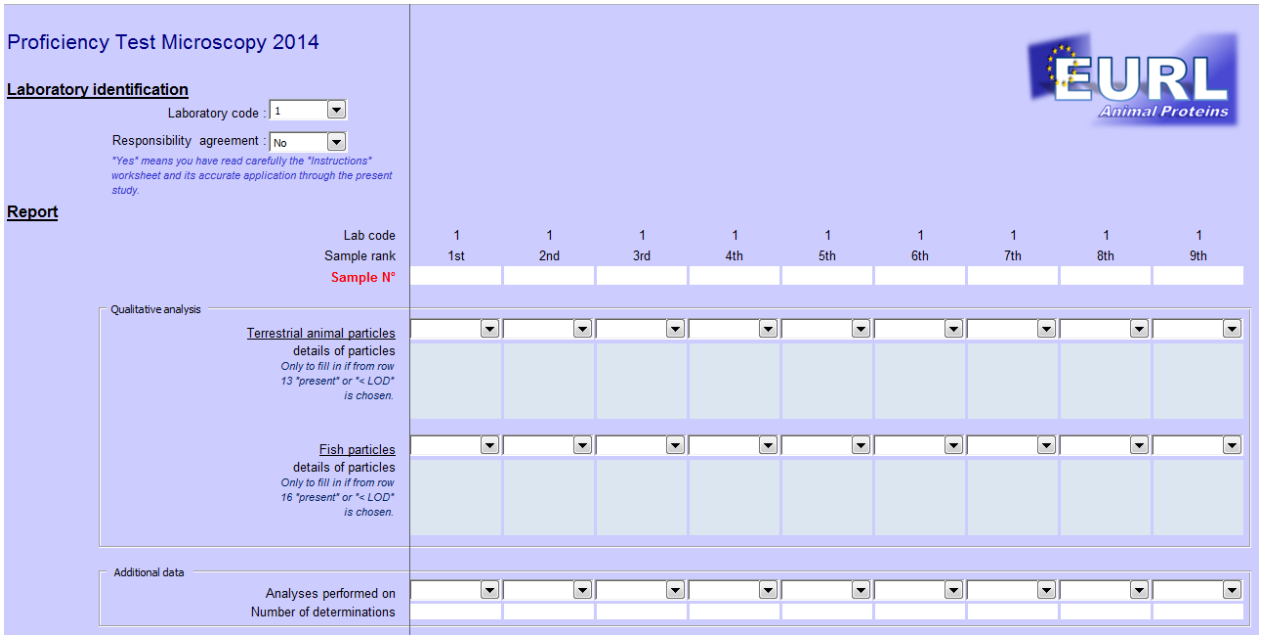
Annex 1

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

Country	Institute Name
Australia	*Biosecurity Sciences Laboratory*
Austria	Austrian Agency for Health and Food Safety
Belgium	Federal Agency for the Safety of the Food Chain
Bulgaria	National Diagnostic Research Veterinary Medical Institute
Croatia	Croatian Veterinary Institute
Cyprus	Cyprus Veterinary Services
Czech republic	Central Institute of sampling and testing in Agriculture
Denmark	The Danish Plant Directorate
Estonia	Veterinary and Food Laboratory
Finland	Finnish Food Safety Authority
France	DG for Fair Trading, Consumer Affairs and Fraud Control-Laboratory Directorate Rennes
Germany	Federal Institute for Risk Assessment
Greece	Feedstuffs Control Laboratory
Hungary	Central Agricultural Office-Directorate Food and Feed Safety-Central Feed Investigation Lab.
Ireland	Department of Agriculture and Food Microscopy Laboratory - Seed Testing Station
Italy	National Reference Centre for the Surveillance and Monitoring of Animal Feed
Japan	*Food and Agricultural Materials Inspection Center*
Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
Lithuania	National Food and Veterinary Risk Assessment Institute
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Norway	*LabNett AS*
Poland	National Veterinary Research Institute
Portugal	Laboratorio Nacional de Investigaçao Veterinaria
Romania	Hygiene Institute of Veterinary Health
Serbia	*Institute of Veterinary Medicine of Serbia*
Slovakia	State Veterinary and Food Institute
Slovenia	Veterinary Faculty-National Veterinary Institute-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 2014

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	1
Sample rank	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
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									

Wallonie

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
3	76	Absent		Absent		Sed. + Raw	2
1	103	Absent		Absent		Sed. + Raw	2
8	454	Present	bones, muscle fibres	Present	fisbones, muscle fibres, cartilage, gill	Sed. + Raw	2
5	643	Absent		Present	fisbones, muscle fibres, cartilage, scale, gill	Sed. + Raw	2
4	706	Present	bones, cartilage, hairs	Absent		Sed. + Raw	2
9	820	Absent		Present	fisbones, muscle fibres, cartilage, scale, gill	Sed. + Raw	2
6	1108	Absent		Present	fisbones, muscle fibres, cartilage, gill	Sed. + Raw	2
7	1243	Absent		Present	fisbones, muscle fibres, cartilage	Sed. + Raw	2
2	1591	Absent		Absent		Sed. + Raw	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
9	61	Present	feathers	Present	bones, gills, scales, muscle fibers	Sed. + Flot.	1
1	268	Absent		Absent		Sed. + Flot.	1
5	676	Absent		Present	bones, cartilage, scales, muscle fibers, otolites	Sed. + Flot.	1
7	748	Present	blood	Present	bones, cartilage	Sed. + Flot.	1
4	937	Present	bones	Absent		Sed. + Flot.	1
2	1525	Absent		Absent		Sed. + Flot.	1
3	1528	Absent		Absent		Sed. + Flot.	1
6	1537	Absent		Present	bones, scales, otolites	Sed. + Flot.	1
8	1906	Present	bones, muscle fibers	Present	bones, muscle fibers, cartilage	Sed. + Flot.	1

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
7	22	Absent		Present	muscles, bones, cartilage, gill, scales	Sed. + Flot.	1
3	109	Absent		Absent		Sed. + Flot.	1
1	136	Absent		Absent		Sed. + Flot.	1
9	391	Absent		Present	muscles, bones, cartilage, gill, scales	Sed. + Flot.	1
4	508	Present	bones	Absent		Sed. + Flot.	1
6	976	Absent		Present	muscles, bones, cartilage, gill, scales	Sed. + Flot.	1
8	982	Present	bones	Present	muscles, bones, cartilage, gill	Sed. + Flot.	1
5	1303	Absent		Present	muscles, bones, cartilage, gill, scales	Sed. + Flot.	1
2	1624	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **4**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
8	553	Present	Bones	Present	Fishbones, gills, cartilage	Sed. + Flot.	1
9	589	Present	Feathers	Present	Fishbones, gills, cartilage	Sed. + Flot.	1
7	715	Absent		Present	Fishbones, gills, cartilage	Sed. + Flot.	1
1	1291	Absent		Absent		Sed. + Flot.	1
5	1369	Absent		Present	Fishbones, gills, cartilage	Sed. + Flot.	1
2	1558	Absent		Absent		Sed. + Flot.	1
6	1702	Absent		Present	Fishbones, gills, cartilage	Sed. + Flot.	1
3	1891	Absent		Absent		Sed. + Flot.	1
4	1894	Present	Bones	Absent		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
9	655	Absent		Present	Muscle fibres, Fish Bone Scale, Gill, Cartilage, Teeth, Skin, Otolith	Sed. + Raw	1
1	664	Absent		Absent		Sed. + Raw	1
7	979	Absent		Present	Muscle fibres, Fish Bone, Scale, Gill, Cartilage, Skin	Sed. + Raw	1
5	1072	Absent		Present	Muscle Fibres, Fish Bone, Scale,Gill, Skin, Teeth, Otolith	Sed. + Raw	1
8	1114	Present	T bone	Present	Muscle Fibres, Fish bone, Scale, Gill, Skin, Cartilage.	Sed. + Raw	1
6	1240	Absent		Present	Muscle fibres, Fish bone, Scale, Gill, Skin, Cartilage,Teeth, Otolith	Sed. + Raw	1
2	1756	Absent		Absent		Sed. + Raw	1
4	1861	Present	T-bone	Absent		Sed. + Raw	1
3	1957	Absent		Absent		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
4	79	Present	bones	Absent		Sed. + Flot.	1
9	424	Absent		Present	bones, gils, scales	Sed. + Flot.	1
5	610	Absent		Present	bones, gils, scales	Sed. + Flot.	1
1	1225	Absent		Absent		Sed. + Flot.	1
6	1636	Absent		Present	bones, gils, scales	Sed. + Flot.	1
3	1792	Absent		Absent		Sed. + Flot.	1
2	1888	Absent		Absent		Sed. + Flot.	1
8	1972	Present	bones	Present	bones, gils, scales	Sed. + Flot.	2
7	2068	Absent		Present	bones, gils, scales	Sed. + Flot.	2

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
1	37	Absent		Absent		Sed. + Flot.	1
9	358	Absent		Present	bones, scales, cartilage, gill, skin, muscle fibres, teeth	Sed. + Flot.	1
7	385	Absent		Present	bones, scales, cartilage, otholith, gill, skin, blood, muscle fibres	Sed. + Flot.	1
4	574	Present	bones, muscle fibres	< LOD	fishbone, muscle fibres	Sed. + Flot.	2
6	778	Absent		Present	bones, scales, cartilage, gill, skin, muscle fibres, teeth, otholith	Sed. + Flot.	1
8	949	Present	bones, muscle fibres, cartilage	Present	bones, scales, cartilage, gill, skin, muscle fibres, otholith	Sed. + Flot.	1
5	1039	Absent		Present	bones, scales, cartilage, gill, skin, muscle fibres, teeth, otholith	Sed. + Flot.	1
3	1561	Absent		Absent		Sed. + Flot.	1
2	1855	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
6	745	Absent		Present	fish bone fragments, muscle fibres	Sed. + Raw	1
9	754	Absent		Present	fish bone fragments, muscle fibres	Sed. + Raw	1
2	799	Absent		< LOD	1 fish bone fragment, 2 muscle fibres	Sed. + Raw	2
7	1012	Present	blood meal	Present	fish bone fragments, muscle fibres	Sed. + Raw	1
5	1204	Absent		Present	fish bone fragments, muscle fibres	Sed. + Raw	1
4	1828	Present	bone fragments	Absent		Sed. + Raw	1
3	2023	< LOD	1 bone fragment, 4 muscle fibres	Absent		Sed. + Raw	2
8	2038	Present	bone fragments	Present	fish bone fragments, muscle fibres	Sed. + Raw	1
1	2050	Absent		Absent		Sed. + Raw	1

Laboratory identification code : **9**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
9	259	Absent		Present	Fish muscles, bone, cartilage, scales, tooth	Sed. + Flot.	1
1	301	Absent		Absent		Sed. + Flot.	1
4	772	Present	18 + 10 bone fragments	Absent		Sed. + Flot.	2
2	832	Absent		Absent		Sed. + Flot.	1
6	910	Absent		Present	>5 bone particles, scales, cartilage, muscles	Sed. + Flot.	1
5	1138	Absent		Present	'>5 bone particles, scales, cartilage, muscle	Sed. + Flot.	1
8	1345	Present	9 + 6 bone fragments	Present	>5 bone particles, scales, cartilage, muscle	Sed. + Flot.	2
7	1870	Absent		Present	>5 bone particles, scales, cartilage, muscle	Sed. + Flot.	2
3	2056	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **10**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	400	Absent		Absent		Sed. + Flot.	1
9	886	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	1036	Present	bones one particle of an insect may be a lab-contamination	Absent		Sed. + Flot.	1
6	1306	Absent		Present	fishbones, scales, muscle fibres, etc. it can't be excludet, that the muscle fibres found, only derive from FM	Sed. + Flot.	1
8	1312	Present	bones, muscle fibres, blood etc. no diff. between MBM- and FM-muscle fibres /blood possible	Present	fishbones, scales, muscle fibres, blood etc. no diff. Between MBM- and FM-muscle fibres /blood possible	Sed. + Flot.	1
5	1666	Absent		Present	fishbones, scales, muscle fibres, etc. it can't be excludet, that the muscle fibres found, only derive from FM	Sed. + Flot.	1
2	1822	Absent		Absent		Sed. + Flot.	1
7	1837	Absent		Present	fishbones, scales, muscle fibres, blood etc. it can't be excludet, that the muscle fibres / blood found, only derive from FM	Sed. + Flot.	1
3	1990	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **11**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	334	Absent		Absent		Sed. + Flot.	2
4	541	Present	bones	Absent		Sed. + Flot.	2
9	556	Absent		Present	Fishbones, scales, muscle, gills, cartilage	Sed. + Flot.	2
8	619	Present	bones	Present	Fishbones, scales, muscle, gills, cartilage	Sed. + Flot.	2
7	649	Present	blood	Present	Fishbones, scales, muscle, gills, cartilage	Sed. + Flot.	2
5	874	Absent		Present	Fishbones, scales, muscle, gills, cartilage	Sed. + Flot.	2
6	1042	Absent		Present	Fishbones, scales, muscle, gills, cartilage	Sed. + Flot.	2
3	1825	Absent		Absent		Sed. + Flot.	2
2	2020	Absent		Absent		Sed. + Flot.	2

Laboratory identification code : **12**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	4	Absent		Absent		Sed. + Flot.	2
4	13	Present	bones	Absent		Sed. + Flot.	1
9	160	Absent		Present	fishbones, gills, scales, muscle fibers	Sed. + Flot.	1
8	586	Absent		Present	fishbones, cartilages, muscle fibers	Sed. + Flot.	2
6	679	Absent		Present	fishbones, otoliths, gills, muscle fibers	Sed. + Flot.	1
7	1078	Absent		Present	fishbones, cartilages, muscle fibers	Sed. + Flot.	2
5	1171	Absent		Present	fishbones, otoliths, cartilages, muscle fibers	Sed. + Flot.	1
2	1657	Absent		Absent		Sed. + Flot.	1
3	1693	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **13**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	112	Present	bones	Absent		Sed. + Raw	1
1	367	Absent		Absent		Sed. + Raw	1
9	490	Present	feathers	Present	bones, cartilages	Sed. + Flot.	3
8	883	Present	bones, cartilages, muscles	Present	bones, cartilages, muscles	Sed. + Raw	2
7	1111	Absent		Present	bones, cartilages, muscles	Sed. + Raw	2
5	1336	Present	feathers	Present	bones, cartilages	Sed. + Flot.	3
6	1438	Present	feathers	Present	bones, cartilages	Sed. + Flot.	3
3	1660	Absent		Absent		Sed. + Raw	1
2	1789	Absent		Absent		Sed. + Raw	1

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	202	Absent		Absent		Sed. + Raw	1
9	226	Absent		Present	Bone, Gills, Cartilage, Muscle, scales	Sed. + Raw	1
5	478	Absent		Present	Bone, Gills, Cartilage, Muscle, scales	Sed. + Raw	1
8	652	Present	Bone, Muscle	Present	Bone, Gills, Cartilage, Muscle, scales	Sed. + Raw	1
6	1339	Absent		Present	Bone, Gills, Cartilage, Muscle, scales	Sed. + Raw	1
7	1342	Absent		Present	Bone, Gills, Cartilage, Muscle, scales	Sed. + Raw	1
3	1726	Absent		Absent		Sed. + Raw	1
2	1987	Absent		Absent		Sed. + Raw	1
4	1993	Present	Bone	Absent		Sed. + Raw	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
3	43	Absent		Absent		Sed. + Flot.	1
9	457	Absent		Present	Bone, muscle	Sed. + Flot.	1
1	598	Absent		Absent		Sed. + Flot.	1
5	775	Absent		Present	Bone, muscle	Sed. + Flot.	1
7	814	Absent		Present	Bone, muscle	Sed. + Flot.	1
4	1201	Present	Bone	Absent		Sed. + Flot.	1
2	1459	Absent		Absent		Sed. + Flot.	1
8	1873	Present	Bone	Present	Bone, muscle	Sed. + Flot.	1
6	1999	Absent		Present	Bone, muscle	Sed. + Flot.	1

Laboratory identification code : **16**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	142	Absent		Absent		Sed. + Raw	3
4	145	Present	bones, few feathers	Absent		Sed. + Raw	3
9	193	Absent		Present	bones,meatfiber,gills, cartilage,scales,teeth	Sed. + Raw	3
6	712	Absent		Present	bones,meatfiber,gills,cartilage, scales, teeth, otoliths.	Sed. + Raw	3
2	898	Present	milkpowder	Absent		Sed. + Raw	3
5	907	Absent		Present	bones,meatfiber, gills, cartilage,scales,teeth, otoliths	Sed. + Raw	3
8	1939	Present	bones,(meatfiber), blood	Present	bones, meatfiber cartilage, scales.	Sed. + Flot.	3
7	1969	Present	blood,	Present	bones, meatfiber, cartilage, scales.	Sed. + Flot.	3
1	2017	Absent		Absent		Sed. + Raw	3

Laboratory identification code : **17**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	46	Present	bones	Absent		Sed. + Flot.	1
9	292	Present	hydrolised feathers	Present	bones, cartilage muscles	Sed. + Flot.	1
1	433	Absent		Absent		Sed. + Flot.	1
2	700	Absent		Absent		Sed. + Flot.	1
8	1147	Present	bones	Present	bones, cartilage, muscles	Sed. + Flot.	1
5	1237	Present	hydrolised feathers, 1bone	Present	bones, cartilage, muscles	Sed. + Flot.	1
6	1372	Present	hydrolised feathers		bones, cartilage, muscles	Sed. + Flot.	1
3	1858	Absent		Absent		Sed. + Flot.	1
7	2002	Present	blood	Present	bones, cartilage, muscles	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
9	**28**	Present	feathers	Present	bones, cartilage,scales,otoliths, gills	Sed. + Flot.	1
1	**532**	< LOD	feathers,muscles	Absent		Sed. + Flot.	2
2	**865**	< LOD	feathers,muscles	Absent		Sed. + Flot.	2
8	**1015**	Present	bones	Present	bones, cartilage,scales,otoliths, gills	Sed. + Flot.	1
4	**1135**	Present	bones	Absent		Sed. + Flot.	1
6	**1405**	Present	feathers	Present	bones, cartilage,scales,otoliths, gills	Sed. + Flot.	1
5	**1567**	Present	feathers	Present	bones, cartilage,scales,otoliths, gills	Sed. + Flot.	1
3	**1759**	Absent		Absent		Sed. + Flot.	1
7	**1903**	Present	blood	Present	bones, cartilage,scales,otoliths, gills	Sed. + Flot.	1

Laboratory identification code :	20

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
7	88	Present	Bloodmeal, 2 terrestrial bone particles	Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bone, fish scales, otholits and gills.	Sed. + Raw	1
1	169	Absent		< LOD	No more than 5 particles derived from fish were detected on average per determination. The particles were identified as fish 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.	Sed. + Raw	2
3	637	Absent		Absent		Sed. + Raw	1
4	640	Present	More than 5 particles derived from terrestrial animals were detected on average per determination. The particles were identified as bone.	Absent		Sed. + Raw	1
8	685	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 bone, fish scales, otholits and gills.	Sed. + Raw	1
5	742	Absent		Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bone, fish scales, otholits and gills.	Sed. + Raw	1
9	787	Absent		Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bone, fish scales, otholits and gills.	Sed. + Raw	1
6	1009	Absent		Present	More than 5 particles derived from fish were detected on average per determination. The particles were identified as fish bone, fish scales, otholits and gills.	Sed. + Raw	1
2	2053	Absent		Absent		Sed. + Raw	1

Laboratory identification code : **21**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
9	919	Present	Bone fragments, muscle fibers	Present	gills, bone fragments, muscle fibers, scales	Sed. + Flot.	1
7	1210	Present	Bone fragments, muscle fibers	Present	'Bone fragments, muscle fibers, scales, gills	Sed. + Flot.	1
2	1954	Absent		Absent		Sed. + Flot.	1
4	2026	Absent		Absent		Sed. + Flot.	1
8	1840	Present	'Bone fragments, muscle fibers	Present	Bone fragments, scales	Sed. + Flot.	1
1	1159	Absent		Absent		Sed. + Flot.	1
5	1105	Present	Bone fragments, muscle fibers	Present	Bone fragments, muscle fibers, scales, gills	Sed. + Flot.	1
6	1174	Present	bone fragments, muscle fibers, a few feathers	Present	Bone fragments, muscle fibers, scales, gills	Sed. + Flot.	1
3	472	Absent		Absent		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
8	289	Present	bones	Present	bones, scales,gills,muscles,cartilages	Sed. + Flot.	1
3	538	Absent		Absent		Sed. + Flot.	2
5	577	Absent		Present	bones, gills,cartilages, muscles, scales	Sed. + Flot.	1
1	631	Absent		Absent		Sed. + Flot.	2
2	1294	Absent		Absent		Sed. + Flot.	2
4	1300	Present	bones,feather	Absent		Sed. + Flot.	1
7	1375	Present	blood	Present	bones, gills, cartilages, scales, muscles	Sed. + Flot.	1
6	1504	Absent		Present	bones, gills,cartilages,muscles	Sed. + Flot.	1
9	1876	Absent		Present	bones, cartilages, scales, gills, muscles	Sed. + Flot.	1

Laboratory identification code : **23**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
8	157	< LOD	2 bones	Present	fish bones, gills ,scales and muscles	Sed. + Flot.	2
4	178	Present	Bones and muscles	Present	6 fishbones and muscles	Sed. + Flot.	2
3	373	Absent		Absent		Sed. + Flot.	2
2	436	Present	> 5 particlesof plasma	< LOD	3 fish bones	Sed. + Flot.	2
9	1150	Absent		Present	fishbones, scales, gils, cartilago and muscles	Sed. + Flot.	2
7	1276	Absent		Present	fishbones,scales,gill,otholite and muscles	Sed. + Flot.	2
1	1324	Absent		Absent		Sed. + Flot.	2
5	1633	Absent		Present	fishbones,scale,gill and muscles	Sed. + Flot.	2
6	1834	Absent		Present	fishbones,scale,gill and muscles	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
8	91	Present	bones	Present	scales, gills, muscles	Sed. + Flot.	
2	601	Absent		Absent		Sed. + Flot.	
4	739	Present	bones	Absent		Sed. + Flot.	
1	1192	Absent		Absent		Sed. + Flot.	
3	1330	Absent		Absent		Sed. + Flot.	
9	1381	Absent		Present	scales, gills, muscles	Sed. + Flot.	
6	1669	Absent		Present	scales, gills, muscles	Sed. + Flot.	
7	1936	Absent		Present	scales, gills, muscles	Sed. + Flot.	
5	1996	Absent		Present	scales, gills, muscles	Sed. + Flot.	

Laboratory identification code : **25**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	1165	Absent		Absent		Sed. + Flot.	1
1	1885	Absent		Absent		Sed. + Flot.	1
8	421	Present	bone, hair	Present	bone, cartil., muscle	Sed. + Flot.	1
2	1162	Absent		Absent		Sed. + Flot.	1
6	547	Absent		Present	bone, cartil., muscle	Sed. + Flot.	1
5	1864	Absent		Present	bone, cartil., muscle	Sed. + Flot.	1
4	442	Present	bone, hair, muscle	Absent		Sed. + Flot.	1
7	187	Absent		Present	bone, cartil., muscle	Sed. + Flot.	1
9	1348	Absent		Present	bone, cartil., muscle	Sed. + Flot.	1

Laboratory identification code : **26**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
8	388	Present	bones	Present	fishbones, splinters, scale, otholit	Sed. + Flot.	3
3	571	Absent		Absent		Sed. + Flot.	3
2	1063	Absent		< LOD	fishbones	Sed. + Flot.	3
4	1069	Present	bones	Absent		Sed. + Flot.	3
9	1084	Absent		Present	fishbones, splinters, scale, otholit, gills	Sed. + Flot.	3
7	1144	Absent		Present	fishbones, splinters, gills	Sed. + Flot.	3
6	1207	Absent		Present	fishbones, splinters, gills, otholit	Sed. + Flot.	3
5	1600	Absent		Present	fishbones, splinters, gills, otholit	Sed. + Flot.	3
1	1918	Absent		Absent		Sed. + Flot.	3

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
7	484	Absent		Present	muscles fibers, cartilage, blood (ruminant négative)	Sed. + Raw	1
8	487	Present	bones, muscles fibers	Present	cartilage , muscles fibers, scales, fishbones	Sed. + Raw	1
2	535	Absent		Present	muscle fibers , scales	Sed. + Raw	1
3	604	Absent		Absent		Sed. + Raw	1
4	838	Present	bones	Absent		Sed. + Raw	1
9	853	Absent		Present	scales , fishbones , lens, vertebra , unidentifiable fragments	Sed. + Raw	1
6	877	Absent		Present	scales, fishbones, cartilage, unidentifiable fragments	Sed. + Raw	1
1	1357	Absent		Present	scale, fishbones, muscle fibre	Sed. + Raw	1
5	1930	Absent		Present	cartilage, scales, muscle fibers, unidentifiable fragments	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
7	121	Absent		Present	fish bones, scales, cartlages, gills	Sed. + Flot.	1
2	667	Absent		Absent		Sed. + Flot.	2
8	850	Present	bones	Present	fish bones, scales, gills, cartilages	Sed. + Flot.	1
1	928	Absent		Absent		Sed. + Flot.	2
9	1183	Absent		Present	fish bones, scales, gills, cartilages	Sed. + Flot.	1
3	1396	Absent		< LOD	3 fish bones	Sed. + Flot.	3
5	1831	Absent		Present	fish bones, scales, gills, cartiles	Sed. + Flot.	1
4	1927	Present	bones	Absent		Sed. + Flot.	1
6	1966	Absent		Present	fish bones, scales, gills, cartilages	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
7	55	Absent		Present	gills fishbones	Sed. + Raw	2
8	124	Absent		Present	gills fishbones crustacean	Sed. + Raw	2
3	505	Absent		Absent		Sed. + Raw	2
1	730	Absent		Absent		Sed. + Raw	2
6	943	Absent		Present	gills fishbones feathers	Sed. + Raw	2
9	1117	Present	meatbonemeal	Present	gills fishbones	Sed. + Raw	2
4	1168	< LOD	animalhair	Absent		Sed. + Raw	2
2	1261	< LOD	animalhair	Absent		Sed. + Raw	2
5	1798	Present	meatbonemeal	Present	gills fishbones	Sed. + Raw	2

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
8	256	Present	bones	Present	fishbones, gills, cartilage, scale	Sed. + Flot.	1
3	340	Absent		Absent		Sed. + Flot.	1
4	409	Present	bones, muscles	Absent		Sed. + Flot.	1
6	448	Absent		Present	fishbones, gills, cartilage, scale, muscles	Sed. + Flot.	1
5	709	Absent		Present	fishbones, gills, cartilage, scale, muscles	Sed. + Flot.	1
7	946	Absent		Present	fishbones, gills, cartilage, scale, muscles	Sed. + Flot.	1
1	1060	Absent		Absent		Sed. + Flot.	1
2	1228	Absent		Absent		Sed. + Flot.	1
9	1282	Absent		Present	fishbones, gills, cartilage, scale, muscles	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
4	376	Present		Absent		Sed. + Flot.	2
7	847	Absent		Present		Sed. + Flot.	3
2	931	Absent		Absent		Sed. + Flot.	2
1	961	Absent		Absent		Sed. + Flot.	2
9	1018	Absent		Present		Sed. + Flot.	3
8	1180	Present		Present		Sed. + Flot.	2
3	1429	Absent		Absent		Sed. + Flot.	2
5	1501	Absent		Present		Sed. + Flot.	2
6	2032	Absent		Present		Sed. + Flot.	2