EURL-AP Proficiency Test Microscopy 2012

Final version

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July 2013

ISBN 978-2-87286-084-5
Dépôt légal D/2013/1463/4

Editor :

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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 the official method as described in Annex VI of regulation EC/152/2009. 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 7 blind samples consisting of blanks and feed matrices fortified with poultry processed animal proteins was to analyse. Fish derived material was not used in the test. Results indicated a lack of specificity higher than that observed during past studies. Cases of erroneous presence of terrestrial particles and fish particles reached 10% of all responses. Analyses of those cases show that it is due to a few underperforming participants. Follow up of the underperformances is asked for the NRL participants involved.

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 29 April 2004, the European Parliament and the Council adopted the Regulation EC/882/2004 [1], improving the effectiveness of the official food and feed controls while redefining the obligations of the relevant authorities and their obligations in the organization of these controls.

On March 2011, the Commission Regulation EC/208/2011 [2] renewed the Walloon Agricultural Research Centre as European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP, http://eurl.craw.eu). It has to develop the following priority tasks:

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

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

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

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

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

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

This final report was prepared based on a working document version diffused through the NRL network and discussed during the 7th EURL-AP Annual Workshop held in Ljubljana, Slovenia from 17th to 18th April 2013.

2. Introduction

According to 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. The objective of the present proficiency test is strictly to evaluate performances of the network of 27 NRLs (already including the Croatian NRL) to detect the presence of processed animal proteins in feed for the year 2012.

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

3. Material and methods

3.1. Study organisation

Official announcement of the study was made on the 4th September 2012 to all participants.

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 China Agricultural University, the Food and Agricultural Materials Inspection Center from Japan, LabNett AS from Norway, the Institute of Veterinary Medicine of Serbia and Biosecurity Sciences Laboratory from Australia. A detailed list of the 33 participating labs is included in Annex 1.

On the 5th November, 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.

Some general recommendations were delivered to the participants:

- Mention was done in the instructions that each participating laboratory was itself responsible to reach appropriate homogeneity of the sample sub-portions that had to be taken from the whole sample vial for analysis.
- Results had to be encoded by way of an Excel report form (Annex 2). Participants were asked to carefully read the instructions on how to fill in the result form and to testify they did it prior to encoding their results. No other support for communicating the results was accepted.
- A summarized results sheet was automatically generated. Participants were asked to sign the summarized results sheet and to return it by fax and email to the EURL-AP. Only when both the Excel file and the fax were received by EURL-AP were results taken into consideration.
- The results had to be sent in both forms concomitantly to the EURL-AP by the 26th November 2012. Notification has been done that this date was a deadline and that results arriving later would not be accepted.

On the exception of one NRL that asked the EURL-AP to deliver its results later for sound reasons, 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. Thus no participant had to be excluded. Results from NRLs and non-EU participants were analysed separately in this report.

3.2. Material

3.2.1. Description of the samples

Five 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 PAP largely inferior to the classical 0.1% considered for the time being as the adulteration level that the method should be able to detect.

- Total absence of any fishmeal or fish containing ingredient, in order to investigate on the problems of specificity for fish.

Each participating lab received about 55g of 7 blind samples to which a unique random number was assigned. Details of the samples are indicated in table 1.

Table 1: Composition of the blind sample set used.

Sample	Material	Nr of replicates
1	blank I (pellets)	1
2	blank II	2
3	blank III	2
4	blank III + 0.05% poultry PAP	1
5	poultry PAP	1
Total		**7**

3.2.2. Materials used in the preparation of the samples

The different feed matrices used for the preparation of the sample set were:

- A **compound feed for rabbits (blank I).** This matrix is an external reference material bought from a proficiency tests organisation. Its composition was determined by IAG members as such: wheat bran, alfalfa dehydrated, sunflower cake, barley, oat, beet pulp and minerals. Sediment content of this compound feed was about 0.9%. This feed was used as such for sample 1.
- A **second compound feed (blank II)** was home made. It consisted of barley, corn, soya, rapeseed, sunflower seeds, tapioca, dicalcium phosphate and other mineral and skimmed milk powder. Its sediment content was about 8.7%. This feed matrix was used for sample 2.
- A **third feed matrix (blank III)** was a pelletized commercial feed for pheasants and guinea fowls. It was mainly composed of wheat, corn, soybean meal, sorghum, sunflower cake, wheat bran, monocalcium phosphate and feed complements (vitamins, salts, zinc…). Its sediment content was about 1.5%. This feed matrix was used for preparing samples 3 and 4.

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

Adulterant material used:

- The **PAP** utilised for preparing samples 4 and 5 was a pure poultry meat and bone meal (category 3 material) from the rendering industry ground at 4mm. Its final bone content was of about 11.2%. Its purity was controlled by microscopy and PCR.

3.2.3. Description of the mixing procedures

The stepwise dilution procedure developed by CRA-W and JRC-IRMM was used to produce sample 4. This procedure has been successfully used in numerous former European interlaboratory studies aiming to evaluate different light microscopy protocols.

Prior to sample preparation, mixing of the materials and filling of vials, the rooms where those activities were performed were cleaned to guarantee absence of any fish containing material.

3.3. Qualitative analysis

Qualitative analysis concerned the presence or absence of terrestrial animal (PAP) and/or fish material. These binary results were analysed by classical statistics: accuracy, sensitivity and specificity. All those statistics were expressed as fractions.

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

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

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

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

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

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

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

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

4. Results

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

4.1. Homogeneity study

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

Table 2: Homogeneity study – Results.

Sample	Material	Light microscopy			% sed	PCR						
		Nr of replicates	Terrestrial	Fish		Nr of replicates	Ruminant	Bovine	Porcine	Chicken	Fish	Sheep
1	blank I	10	-	-	0.87	5	-	-	-	-	-	-
2	blank II	10	-	-	8.71	5	+	+	-	-	-	-
3	blank III	10	-	-	1.52	5	+	+	-	-	-	-
4	blank III + 0.05% poultry PAP	5	+	-	1.51	5	+	+	-	+	-	-
5	poultry PAP	10	+	-	11.25	5	-	-	-	+	-	-

(Legend: sed = sediment, + = present, - = not present)

The homogeneity was studied by light microscopy on 10g of sample material for each replicate. For the homogeneity study both flotate and sediment fractions were analysed. Each time 6 slides were prepared. For PCR analysis of each replicate a double extraction was performed on 100mg of sample material.

Blank I (**sample 1**) was negative for any presence of animal material by light microscopy. By PCR it revealed to be negative for all markers.

Blank II (**sample 2**) was microscopically free from any presence of animal material. By PCR, according to its composition (presence of skimmed milk), it was positive for ruminant and bovine DNA.

Blank III (**sample 3**) revealed to be negative for microscopic animal traces and showed by PCR to be only positive for ruminant and bovine DNA.

Blank III adulterated with 0.05% of poultry PAP (**sample 4**) showed systematically terrestrial bones and feather fragments. By PCR it was positive for the presence of chicken DNA.

The poultry PAP (**sample 5**) always presented traces of terrestrial animal (bones and feathers) by light microscopy. According to its composition only chicken DNA was detected.

Throughout all samples analysed during the homogeneity study there has been no detection of fish derived fragments or of any presence of fish DNA. Results from the homogeneity study indicated the samples as fit for their purpose.

4.2. Qualitative analyses from the NRLs

4.2.1. On the respect of the instructions

Overall instructions have been respected through the study.

4.2.2. Overview of results and performance of the method

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

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

Sample	Material	n	AC	
			Terrestrial	Fish
1	blank I	27	0,704 (8)	0,926 (2)
2	blank II	54	0,963 (2)	0,963 (2)
3	blank III	54	0,870 (7)	0,907 (5)
4	blank III + 0.05% poultry PAP	27	0,926 (2)	0,852 (4)
5	poultry PAP	27	1,000	0,778 (6)

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

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

Problems of sensitivity are only limited to 2 cases on 27 (or 7%) for the detection of poultry PAP at concentration of adulteration of 0.05%. It has to be noted that all samples of pure poultry PAP were identified as such.

Specificity problems are much more numerous and represent about 10% of total responses.

A lot of positive deviations are observed for fish and this independently of the sample type (either blank or poultry adulterated materials). Although the percentage of false positive results for fish is low for the blank II sample (4%) and blank I (7%), this percentage increases for blank III (9%), blank III + 0.05% poultry PAP (15%) and the poultry PAP (22%).

False positive results are furthermore observed for terrestrial animal presence. The percentage of lack of specificity for terrestrial material is the lowest for blank II (4%) while for the two other blanks it is abnormally high (13% for blank III and 30% for blank I).

4.2.3. Individual performances of NRLs in qualitative analysis

Individual performances were assessed for each participant by calculating the accuracy, sensitivity and specificity over the blind samples. This was performed separately for both the detection of terrestrial material and fish material. A ranking of the labs was prepared based on the consolidated accuracy (in the present study as no fish was present in the samples, ranking was only based on specificity scores).

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

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

Terrestrial			
lab code	AC	SE	SP
3	1,000	1,000	1,000
4	1,000	1,000	1,000
5	1,000	1,000	1,000
7	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
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
21	1,000	1,000	1,000
24	1,000	1,000	1,000
27	1,000	1,000	1,000
1	0,857	1,000	0,800
13	0,857	1,000	0,800
22	0,857	1,000	0,800
25	0,857	1,000	0,800
26	0,857	1,000	0,800
16	0,857	0,500	1,000
2	0,714	1,000	0,600
6	0,714	1,000	0,600
20	0,714	1,000	0,600
28	0,714	1,000	0,600
9	0,714	0,500	0,800
8	0,571	1,000	0,400

Fish	
lab code	SP
2	1,000
3	1,000
4	1,000
5	1,000
7	1,000
8	1,000
10	1,000
11	1,000
12	1,000
14	1,000
15	1,000
17	1,000
19	1,000
21	1,000
24	1,000
27	1,000
28	1,000
9	0,857
13	0,857
18	0,857
22	0,857
26	0,857
1	0,714
16	0,714
20	0,714
25	0,571
6	0,286

Concerning the ability to detect terrestrial animal constituents (table 4), 12 labs provided incorrect results according to the following details:

- PD for PAP in blank I : labs 1, 2, 6, 8, 13, 20, 26 and 28
- PD for PAP in blank II : labs 9 and 22
- PD for PAP in blank III : labs 2, 6, 8 (for both replicates), 20, 25 and 28
- ND for PAP in blank III + 0.05% poultry PAP : labs 9 and 16

Concerning the ability to detect fish material (table 5), 10 labs delivered incorrect results according to the following details:

- PD for fish in blank I : labs 6 and 22
- PD for fish in blank II : labs 6 and 20

- PD for fish in blank III : labs 1, 6 (for both replicates) and 25 (for both replicates)
- PD for fish in blank III + 0.05% poultry PAP : labs 6, 9, 16 and 25
- PD for fish in poultry PAP : labs 1, 13, 16, 18, 20 and 26

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)

Table 6: General NRL proficiency regarding the detection of terrestrial and fish material. Ranking follows AC values as primary key and SE as second key.

Consolidated			
lab code	**AC**	**SE**	**SP**
3	1,000	1,000	1,000
4	1,000	1,000	1,000
5	1,000	1,000	1,000
7	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
14	1,000	1,000	1,000
15	1,000	1,000	1,000
17	1,000	1,000	1,000
19	1,000	1,000	1,000
21	1,000	1,000	1,000
24	1,000	1,000	1,000
27	1,000	1,000	1,000
18	0,929	1,000	0,917
2	0,857	1,000	0,833
13	0,857	1,000	0,833
22	0,857	1,000	0,833
26	0,857	1,000	0,833
28	0,857	1,000	0,833
1	0,786	1,000	0,750
8	0,786	1,000	0,750
9	0,786	0,500	0,833
16	0,786	0,500	0,833
20	0,714	1,000	0,667
25	0,714	1,000	0,667
6	0,500	1,000	0,417

Table 6 illustrates the very good level of global performance (= consolidated AC superior or equal to 0.90, i.e. having no more than one false result) for 15 labs out of 27 NRLs or in other words for 56% of the NRLs. A second category of NRLs (cells in blue in table 6) having a satisfying global performance is defined (= consolidated AC below 0.90 and having no more than three false results without any ND for terrestrial material). Seven NRLs fall into this category. NRLs included in this category are nevertheless

asked to report to the EURL-AP on the possible source of these deviations. A third category (cells in red in table 6) includes five NRLs that are underperforming for the present test (= consolidated AC below 0.90 and having either at least three false results including one ND for terrestrial or more than three false results). Those labs require improvement of their proficiency. In agreement with the EURL-AP SOP for managing underperformances (available on the EURL-AP intranet since 18 January 2012), these five participants are asked to report on the origin of those multiple errors as well as on the actions they will undertake in order to solve this key issue.

4.3. Qualitative analyses from the non-EU participants

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

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

Individual performances from the 6 participants outside the EU were assessed exactly as in previous section (4.2.3.). 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.

Terrestrial			
lab code	AC	SE	SP
29	1,000	1,000	1,000
30	1,000	1,000	1,000
31	1,000	1,000	1,000
35	1,000	1,000	1,000
33	0,857	1,000	0,800
34	0,857	1,000	0,800

Fish	
lab code	SP
29	1,000
30	1,000
33	0,857
35	0,857
34	0,286
31	*n.a.*

Legend: *n.a.* = not applicable

Lab 31 only performed the detection for terrestrial animal remains and did not deliver results for fish detection.

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

- PD for PAP in blank II : lab 34
- PD for PAP in blank III : lab 33

Concerning the ability to detect fish material, 3 laboratories delivered erroneous results with the following details:

- PD for fish in blank I : lab 34
- PD for fish in blank II : lab 34
- PD for fish in blank III : lab 34 (for both replicates)

- PD for fish in blank III + 0.05% poultry PAP : lab 34
- PD for fish in poultry PAP : lab 33 and 35

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**
29	1,000	1,000	1,000
30	1,000	1,000	1,000
35	0,929	1,000	0,917
33	0,857	1,000	0,833
34	0,571	1,000	0,500
31	*n.a.*	*n.a.*	*n.a.*

Legend: *n.a.* = not applicable

Three participants obtained a very good level of global performance.

Lab 33 obtained a satisfying result (cells in blue in table 9).

Lab 34 was classified as underperforming (cells in red in table 9) according to the applied criteria.

Lab 31 was not classified.

5. Conclusions

This study aimed strictly at evaluating the proficiency levels of the participants to detect the presence of animal by-products in feed. The EU participants, the NRLs, were required to apply the official method as described in Annex VI of regulation EC/152/2009 [3].

The global performance was excellent for 56% of the NRLs. Compared to previous studies involving the NRL network [4, 5], this percentage is low. A satisfying global performance was achieved by 26% of the NRLs. The remaining 18% of the NRLs were underperforming.

The percentage of incorrect results for the NRL network is synthetized as follows:

- Total errors: 10% (or 38 errors on a total of 378 results)
- False positive errors rate over the total errors: 95% (36 on 38) distributed as such:
  - 53% for fish
  - 47% for terrestrial animals
- False negative errors rate over the total errors: 5% (2 on 38) all for terrestrial – as no fish material were incorporated into the sample set.

As reported, the major problems were linked to erroneous detection of animal remains (both terrestrial and fish) in blank feed matrices – or specificity issues.

Results showed a lack of specificity of 30% for terrestrial particles detection in an external reference material which was a compound feed for rabbits. As this feed was the only pelleted samples and since the homogeneity study showed it as to be blank, a possible carry-over of animal particles due to the milling/grinding process during sample preparation could account as an explanation. Data collected after the EURL-AP workshop from the participants shed nevertheless a new light on this case. First of all in each case of false positive result for terrestrial, the number of particles identified as such was barely 1 or 2. In case of more than one particle, the second one was found after having observed almost the entire sediment, i.e. having prepared an unusual high number of slides. This situation is known to lead to false positive results as confirmed in the validation study of the revised microscopic method [4]. Furthermore none of the pictures of the particles identified as terrestrial were presenting *all* classical morphological features permitting a reliable identification: for instance a "bone" (Annex 4 - A) was coloured by Alizarin red but failed to present lacunae or, quite the opposite, was presenting "lacunae" but was not coloured by the latter staining (Annex 4 – B). The same conclusion was taken for the observation of "feathers" (Annex 4 - C) : the type of structure corresponds more likely to plant structure rather than real feathers although the exact nature could not be determined.

The second major lack of specificity of 22% was noted for the presence of fish particles in the pure poultry PAP. The homogeneity for this sample, as well as through all samples, failed at detecting any fish particles. It cannot be ruled out that some poultry fragments, i.e. feathers, could have been erroneously detected as fish remains. The overall problem of specificity for fish is a well-known and frequently reported case [6, 7]. Although several potential sources of errors were discussed by Veys et al. [7], the accurate explanation for it nevertheless remains obscure.

Through this proficiency test, some specificity issues also originate in the home made blank II. In the sediment some particles were identified as "fish scales" (Annex 4 - D) although they remained unstained by Alizarin red. Some rectangular structures were also confused with muscle fibres although no characterized striated banding could be detected (Annex 4 - E), such structures could correspond to minerals.

As reported in previous studies, false positive results are thus to be pointed out due to decisions taken on the observations of a too small number of suspicious particles or in other terms a number of particles being below the limit of detection. The interpretation of observations in this study was the sole responsibility of the participants without consideration of such limit. The changes on results interpretation in the new regulation EU/51/2013 [8] amending annex VI or regulation EC/152/2009 will certainly improve this situation. From the discussions with the participants having faced specificity issues it appeared also that hesitation is the most influencing factor for generating taking a wrong decision; generally the first impression is the correct one. Experience is therefore essential.

Finally, these problems of specificity are to be put into perspective by the underperformance of some NRLs: labs 6, 20 and 25 account for respectively 19%, 11% and 11% of all specificity issues. Follow up of these underperformance cases will help to shed light on this problem.

Concerning the non-EU participants, and on the exception of one participant that did not deliver results for fish detection, four out of five performed satisfyingly or excellently. Comparatively to the NRL network, false positive results for fish were also numerous (7 on a total of 9 erroneous results) but majorly limited to one lab.

Acknowledgment

We are grateful to the EURL-AP staff and the participants for their fruitful collaboration. We also thank the members of the IAG Section Feedingstuff Microscopy for their help in the declaration and the research on suspicious particles, especially L. van Raamsdonk and P. Czajkowski.

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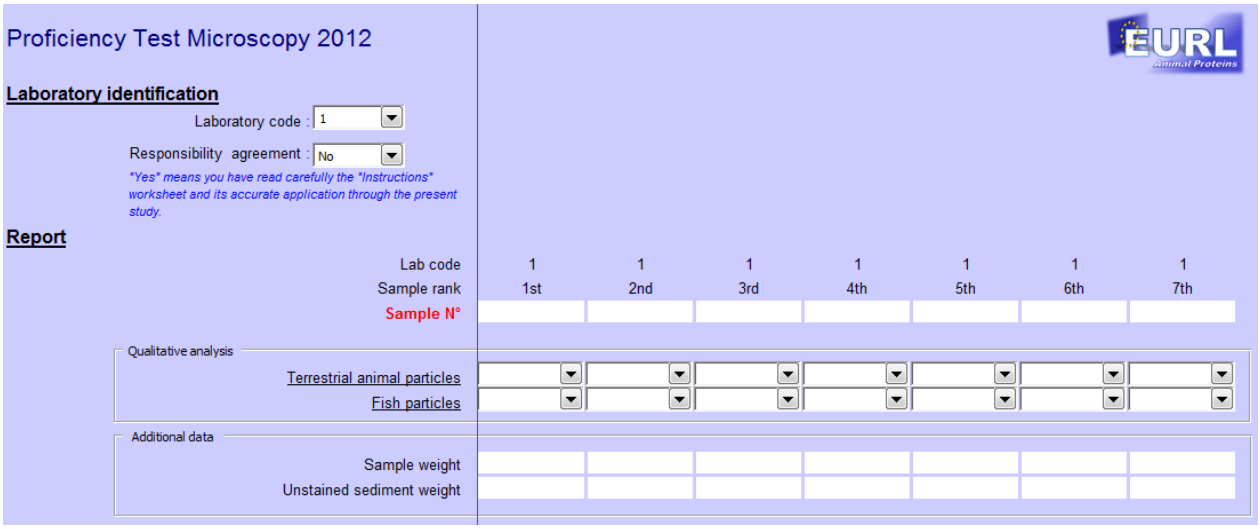
Annex 1

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
Argentina	*Servicio Nacional de Sanidad y Calidad Agroalimentaria*
Belgium	Federal Agency for the Safety of the Food Chain
Bulgaria	National Diagnostic Research Veterinary Medical Institute
China	*China Agricultural University Beijing*
Croatia	Croatian Veterinary Institute
Cyprus	Cyprus Veterinary Services
Czech republic	Central Institute of sampling and testing in Agriculture
Denmark	The Danish Plant Directorate
Estonia	Veterinary and Food Laboratory
Finland	Finnish Food Safety Authority
France	DG for Fair Trading, Consumer Affairs and Fraud Control-Laboratory Directorate Rennes
Germany	Federal Institute for Risk Assessment
Greece	Feedstuffs Control Laboratory
Hungary	Central Agricultural Office-Directorate Food and Feed Safety-Central Feed Investigation Lab.
Ireland	Department of Agriculture and Food Microscopy Laboratory - Seed Testing Station
Italy	National Reference Centre for the Surveillance and Monitoring of Animal Feed
Japan	*Food and Agricultural Materials Inspection Center*
Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
Lithuania	National Veterinary Laboratory
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Norway	*LabNett AS*
Poland	National Veterinary Research Institute
Portugal	Laboratorio Nacional de Investigaçao Veterinaria
Romania	Hygiene Institute of Veterinary Health
Serbia	*Institute of Veterinary Medicine*
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 2012

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
Sample rank	1st	2nd	3rd	4th	5th	6th	7th
Sample N°							
Qualitative analysis							
Terrestrial animal particles							
Fish particles							
Additional data							
Sample weight							
Unstained sediment weight							

Annex 3

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

Laboratory identification code : 1

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	60	Absent	Present	10,00	0,199
2	184	Absent	Absent	10,00	0,793
3	293	Absent	Absent	10,00	0,216
4	322	Present	Absent	10,00	0,239
1	425	Present	Absent	10,00	0,091
2	597	Absent	Absent	10,00	0,888
5	782	Present	Present	10,00	1,245

Laboratory identification code : 2

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	203	Present	Absent	10,00	0,148
5	431	Present	Absent	5,00	0,670
4	439	Present	Absent	10,00	0,162
2	624	Absent	Absent	10,00	0,730
1	785	Present	Absent	10,01	0,062
3	843	Absent	Absent	10,01	0,110
2	850	Absent	Absent	10,01	0,750

Laboratory identification code : 3

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
4	61	Present	Absent	10,38	0,180
2	121	Absent	Absent	3,02	0,230
2	372	Absent	Absent	3,05	0,230
5	395	Present	Absent	3,06	0,350
1	398	Absent	Absent	10,10	0,060
3	401	Absent	Absent	10,18	0,140
3	492	Absent	Absent	10,17	0,220

Laboratory identification code : 4

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
5	143	Present	Absent	10,00	1,093
2	489	Absent	Absent	10,00	0,437
3	617	Absent	Absent	10,00	0,289
2	625	Absent	Absent	10,00	0,760
3	717	Absent	Absent	10,00	0,275
4	727	Present	Absent	10,00	0,146
1	758	Absent	Absent	10,00	0,044

Laboratory identification code : 5

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
5	224	Present	Absent	10,01	1,205
4	277	Present	Absent	10,04	0,140
2	291	Absent	Absent	10,01	0,862
3	312	Absent	Absent	10,01	0,140
2	391	Absent	Absent	10,01	0,876
3	563	Absent	Absent	10,01	0,163
1	578	Absent	Absent	10,01	0,093

Laboratory identification code : 6

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
5	314	Present	Absent	10,00	0,848
2	552	Absent	Present	10,01	0,374
4	637	Present	Present	10,00	0,091
2	769	Absent	Absent	10,01	0,235
3	779	Present	Present	10,01	0,072
3	834	Absent	Present	10,01	0,144
1	875	Present	Present	10,01	0,018

Laboratory identification code : 7

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
5	323	Present	Absent	10,01	1,260
2	336	Absent	Absent	10,01	0,800
4	340	Present	Absent	10,01	0,160
3	573	Absent	Absent	10,01	0,210
1	614	Absent	Absent	10,00	0,070
2	724	Absent	Absent	10,01	0,770
3	734	Absent	Absent	10,02	0,170

Laboratory identification code : 8

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	330	Present	Absent	10,00	0,159
2	562	Absent	Absent	10,00	0,939
2	570	Absent	Absent	10,00	0,863
4	628	Present	Absent	10,00	0,218
5	629	Present	Absent	10,00	1,260
1	830	Present	Absent	10,00	0,089
3	833	Present	Absent	10,00	0,179

Laboratory identification code :	9

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
5	152	Present	Absent	10,03	1,287
2	192	Absent	Absent	10,01	0,780
4	304	Absent	Present	10,03	0,196
3	509	Absent	Absent	10,05	0,228
2	517	Present	Absent	10,02	0,850
1	587	Absent	Absent	10,09	0,041
3	870	Absent	Absent	10,05	0,345

Laboratory identification code :	10

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	222	Absent	Absent	10,10	0,240
1	434	Absent	Absent	10,00	0,110
5	530	Present	Absent	10,10	1,290
2	606	Absent	Absent	10,00	0,960
3	698	Absent	Absent	10,00	0,170
4	736	Present	Absent	10,30	0,180
2	760	Absent	Absent	10,10	1,040

Laboratory identification code :	11

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
4	232	Present	Absent	5,22	0,078
1	515	Absent	Absent	5,49	0,040
5	593	Present	Absent	5,53	0,682
2	859	Absent	Absent	5,31	0,322
3	861	Absent	Absent	5,27	0,086
2	867	Absent	Absent	5,66	0,365
3	878	Absent	Absent	5,24	0,067

Laboratory identification code :	12

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
2	156	Absent	Absent	10,00	0,740
5	449	Present	Absent	10,00	1,247
4	583	Present	Absent	10,00	0,245
3	654	Absent	Absent	10,00	0,182
3	806	Absent	Absent	10,00	0,203
1	857	Absent	Absent	10,00	0,087
2	877	Absent	Absent	10,00	0,745

Laboratory identification code :	13

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
4	70	Present	Absent	10,00	0,198
5	107	Present	Present	5,00	0,643
3	339	Absent	Absent	10,00	0,207
3	383	Absent	Absent	10,00	0,212
2	526	Absent	Absent	10,00	0,870
2	543	Absent	Absent	10,00	0,820
1	866	Present	Absent	10,00	0,144

Laboratory identification code :	14

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
2	175	Absent	Absent	10,37	0,995
4	178	Present	Absent	10,20	0,246
5	233	Present	Absent	10,13	1,160
3	437	Absent	Absent	10,26	0,227
1	542	Absent	Absent	10,18	0,155
2	660	Absent	Absent	10,07	0,915
3	663	Absent	Absent	10,33	0,308

Laboratory identification code :	15

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	123	Absent	Absent	10,00	0,170
5	215	Present	Absent	3,00	0,350
3	635	Absent	Absent	10,00	0,144
2	715	Absent	Absent	10,01	0,592
2	723	Absent	Absent	10,01	0,567
1	731	Absent	Absent	10,00	0,088
4	862	Present	Absent	10,00	0,167

Laboratory identification code :	16

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	303	Absent	Absent	10,01	0,160
3	338	Absent	Absent	10,01	0,156
5	440	Present	Present	10,01	0,950
1	596	Absent	Absent	10,01	0,014
2	733	Absent	Absent	10,01	0,654
4	808	Absent	Present	10,00	0,089
2	849	Absent	Absent	10,01	0,599

Laboratory identification code :	17

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
2	138	Absent	Absent	10,02	0,860
1	389	Absent	Absent	9,98	0,077
2	544	Absent	Absent	10,01	0,806
3	581	Absent	Absent	10,00	0,153
4	592	Present	Absent	10,00	0,144
3	753	Absent	Absent	10,07	0,133
5	836	Present	Absent	4,98	0,576

Laboratory identification code :	18

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
4	196	Present	Absent	10,00	0,192
1	299	Absent	Absent	10,00	0,047
2	507	Absent	Absent	10,00	0,984
2	661	Absent	Absent	10,00	0,879
5	701	Present	Present	5,00	0,664
3	725	Absent	Absent	10,00	0,159
3	825	Absent	Absent	10,00	0,161

Laboratory identification code :	19

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	185	Absent	Absent	10,58	0,190
4	223	Present	Absent	10,04	0,180
2	229	Absent	Absent	10,06	0,640
2	417	Absent	Absent	10,44	0,880
5	512	Present	Absent	5,13	0,620
3	582	Absent	Absent	10,59	0,190
1	794	Absent	Absent	10,24	0,050

Laboratory identification code :	20

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
2	129	Absent	Absent	5,16	0,420
3	231	Present	Absent	5,96	0,100
2	319	Absent	Present	5,95	0,470
1	380	Present	Absent	5,88	0,010
5	620	Present	Present	5,10	0,640
4	745	Present	Absent	5,89	0,110
3	860	Absent	Absent	5,69	0,130

Laboratory identification code :	21

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	140	Absent	Absent	10,00	0,152
5	368	Present	Absent	3,00	0,354
1	506	Absent	Absent	10,00	0,081
4	709	Present	Absent	10,00	0,160
3	735	Absent	Absent	10,00	0,169
2	741	Absent	Absent	10,00	0,805
2	868	Absent	Absent	10,00	0,826

Laboratory identification code :	22

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
2	58	Absent	Absent	10,18	0,488
3	177	Absent	Absent	10,10	0,179
5	386	Present	Absent	10,16	0,394
3	419	Absent	Absent	10,18	0,239
2	777	Present	Absent	10,13	0,255
1	812	Absent	Present	10,41	0,084
4	844	Present	Absent	10,45	0,210

Laboratory identification code :	24

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
2	183	Absent	Absent	10,00	0,741
1	308	Absent	Absent	10,00	0,077
4	421	Present	Absent	10,00	0,109
3	428	Absent	Absent	10,00	0,276
2	508	Absent	Absent	10,00	0,898
5	521	Present	Absent	10,00	1,324
3	879	Absent	Absent	10,00	0,954

Laboratory identification code :	25

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
5	53	Present	Absent	10,00	0,920
3	104	Present	Present	10,00	0,160
1	353	Absent	Absent	10,00	0,064
2	354	Absent	Absent	10,00	0,355
3	438	Absent	Present	10,00	0,172
4	574	Present	Present	10,00	0,159
2	616	Absent	Absent	10,00	0,461

Laboratory identification code : 26

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
4	124	Present	Absent	10,01	0,120
3	149	Absent	Absent	10,04	0,100
1	155	Present	Absent	10,01	0,040
2	561	Absent	Absent	9,94	0,220
3	708	Absent	Absent	9,66	0,110
2	787	Absent	Absent	10,01	0,260
5	872	Present	Present	10,32	1,050

Laboratory identification code : 27

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
1	164	Absent	Absent	10,00	0,080
3	276	Absent	Absent	10,00	0,150
5	350	Present	Absent	10,00	0,199
2	363	Absent	Absent	10,00	0,769
4	511	Present	Absent	10,00	0,160
2	796	Absent	Absent	10,00	0,744
3	842	Absent	Absent	10,00	0,151

Laboratory identification code : 28

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
2	75	Absent	Absent	10,00	0,725
3	275	Present	Absent	10,00	0,117
2	292	Absent	Absent	10,00	0,615
3	447	Absent	Absent	10,00	0,114
1	749	Present	Absent	10,00	0,080
4	781	Present	Absent	10,00	0,119
5	854	Present	Absent	10,00	0,985

Laboratory identification code : 29

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	167	Absent	Absent	10,00	0,225
4	205	Present	Absent	10,00	0,219
5	359	Present	Absent	10,00	1,369
2	525	Absent	Absent	10,00	0,906
3	618	Absent	Absent	10,00	0,211
2	778	Absent	Absent	10,00	0,784
1	839	Absent	Absent	10,00	0,147

Laboratory identification code :	30

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
1	137	Absent	Absent	5,04	0,031
3	195	Absent	Absent	5,01	0,222
3	230	Absent	Absent	5,04	0,187
2	309	Absent	Absent	5,03	0,326
4	403	Present	Absent	5,03	0,077
5	539	Present	Absent	5,05	0,593
2	706	Absent	Absent	5,02	0,344

Laboratory identification code :	31

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	96	Absent		45,00	0,000
1	191	Absent		45,00	0,000
5	278	Present		45,00	0,000
2	318	Absent		45,00	0,000
3	329	Absent		45,00	0,000
2	400	Absent		45,00	0,000
4	529	Present		45,00	0,000

Laboratory identification code :	33

Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	221	Absent	Absent	10,00	0,142
2	382	Absent	Absent	10,00	0,649
2	516	Absent	Absent	10,00	0,736
4	556	Present	Absent	10,00	0,144
5	557	Present	Present	10,00	1,133
3	645	Present	Absent	10,00	0,172
1	740	Absent	Absent	10,00	0,034

Laboratory identification code :	34

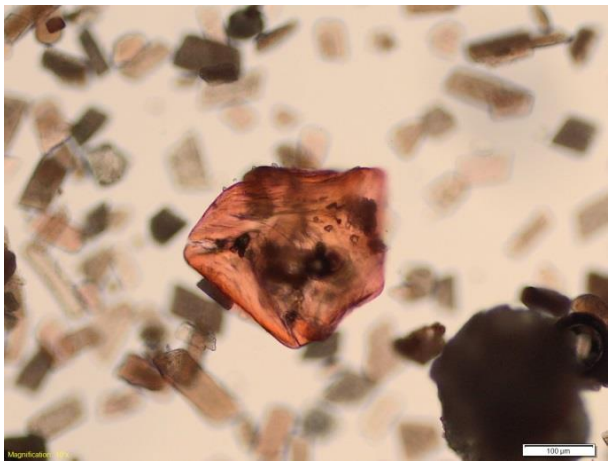
Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
1	110	Absent	Present	1,02	0,240
5	179	Present	Absent	1,00	0,116
2	301	Present	Absent	1,01	0,041
3	482	Absent	Present	1,05	0,094
3	528	Absent	Present	1,00	0,097
4	817	Present	Present	1,01	0,031
2	876	Absent	Present	1,03	0,041

Laboratory identification code :	35

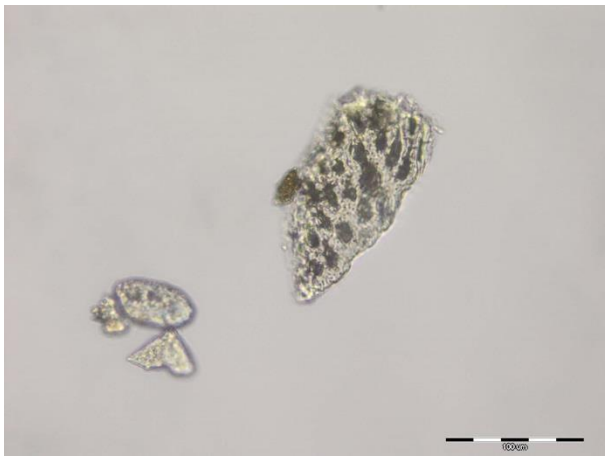
Sample type	Sample N°	Terrestrial animal part.	Fish part.	Sample weight (g)	Sediment weight (g)
3	302	Absent	Absent	10,01	0,140
3	609	Absent	Absent	10,07	0,234
2	211	Absent	Absent	10,01	0,768
1	371	Absent	Absent	10,02	0,112
4	520	Present	Absent	10,01	0,167
5	737	Present	Present	10,01	1,265
2	759	Absent	Absent	10,03	0,813

Annex 4

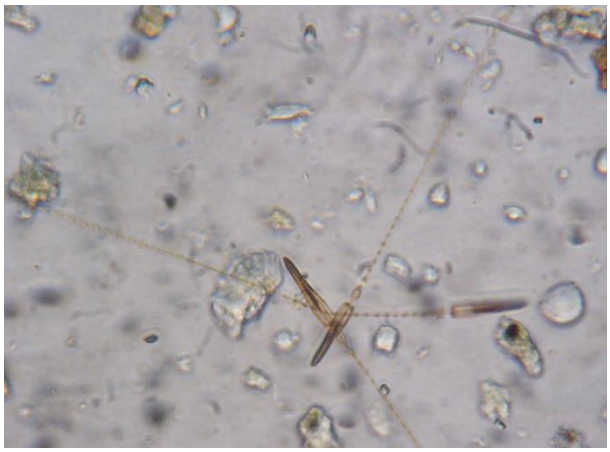
Pictures from false positive results.



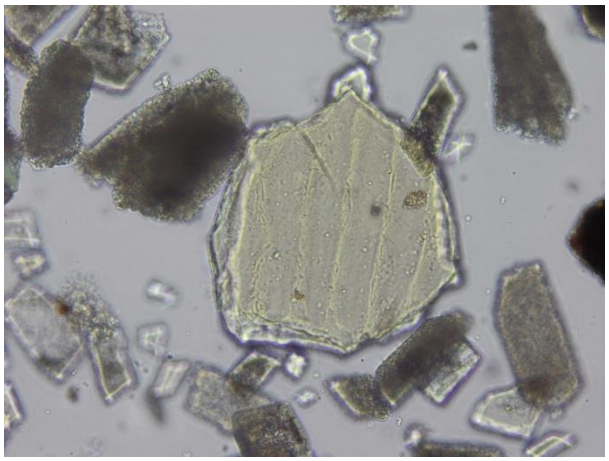
A: particle identified as a terrestrial bone, coloured by Alizarin red. Blank I



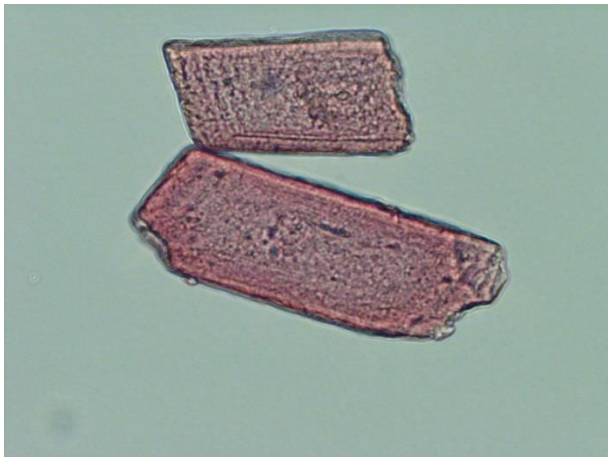
B: particle identified as terrestrial bone, but unstained by Alizarin red. Blank I



C: particles identified as feathers. Blank I



D: particle identified as scale, but unstained by Alizarin red. Blank II



E: Particles identified as muscles. Blank II