Community Reference Laboratory for Animal Proteins in feedingstuffs

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CRL-AP Proficiency Test 2008
Final report

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Summary

On behalf of DG Sanco, the Community Reference Laboratory for animal proteins in feedingstuffs (CRL-AP) organized in 2008 the present proficiency test for all National Reference Laboratories (NRLs) having in charge the analyses of animal proteins in feedingstuffs in each of the Member States of the European Union (EU). The goal of the study was to evaluate the performance of the NRLs to apply the microscopic method for qualitative detection of animal constituents in feedingstuffs as stated by Commission Directive 2003/126/EC*. This study also included some official control labs from countries outside EU.

The number of participants was of 30 (26 NRLs and 4 labs outside EU). A set of 10 blind samples was sent to each participant. Some samples from the set were adulterated with terrestrial meat and bone meal. In a wish to reflect the daily diversity of feed, different matrices were used for the sample preparation. Blank samples were sent in order to check for possible cross-contaminations. The homogeneity of the sample materials was tested by the organiser and considered as sufficient.

The study showed that a majority of participants obtained a very good level of global performance comparable to previous CRL-AP studies. Nevertheless some participants were still underperforming. The organisers asked for action plans in order to remediate to those underperformances.

The results indicated that the diversity of sample material did not appear to be a problem for the majority of the participants as revealed by the global method performance. Nevertheless some issues on the detection of animal constituents were highlighted during the study: a lack of specificity for the detection of fish, more precisely when feather meal is present in a feed, and a lack of sensitivity for animal particle detection when a feed is adulterated with a pure muscle meal with only scarce presence of bone fragments. Furthermore the study demonstrated the difficulty of decision for the participants in absence of particles with well-characterised microscopic features allowing them to be classified as from terrestrial or fish origin.

Keywords :

Meat and bone meals – Processed animal proteins – Microscopy – Qualitative analysis

* Referred through the document as EC 126/2003 directive

1. Foreword and aim of the study

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

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

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

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

(iii) To develop new analytical methods for the detection of animal proteins in feedingstuffs (classical 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.

It is in this framework that the CRL-AP has organized in 2008 a proficiency test aiming to evaluate the performance of the NRLs to detect the presence of processed animal proteins by the microscopic method as stated in the EC 126/2003 directive [1]. On proposal of the Commission, invitations to participate the proficiency test were also sent to some official control labs outside the EU.

The present final report is based on a preliminary version submitted as a working document on 20 February 2009 to the NRL network for approval. Results were presented and discussed during the 3rd CRL-AP Annual Workshop held in Gembloux on 11-12 March 2009. This final version was modified taking into account the NRL's comments received on 30 March 2009.

2. Introduction

On the 7th November 2008, a set of 10 blind samples for the CRL-AP Proficiency Test 2008 have been sent by express shipment to the 26 NRLs and to 4 laboratories outside this network. Those four external participants were the Canadian Food Inspection Agency, the Croatian Veterinary Institute, the Institute of Veterinary Medicine of Serbia and the Russian Central Veterinary Lab. The detailed list of the 30 participating labs is included in Annex 1.

The following instructions have been given to each participating lab:

- Analysis of the 10 blind samples by applying the protocol described in EC 126/2003 directive [1].
- Mention has been done that each participating laboratory was itself responsible to reach appropriate homogeneity of the sample sub-portions that had to be taken from the whole sample vial for analysis.
- Qualitative analyses have been requested for each of the 10 samples. Participants were asked to provide additional data such as the number of slides observed, whether or not they observed the whole slides, the sample and sediment weights, the number of particles they had detected to support their conclusions and to further specify the exact nature of the particles when their number were less or equals to 5. Quantifications were not requested.
- The results had to be encoded by way of an Excel report form -downloadable from the CRL-AP intranet (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 without the need for the participant of re-encoding the data. Participants were asked to sign the summarized results sheet and to send it by fax to the CRL-AP. Results were taken into consideration only when both the Excel file and the fax were received.
- The results had to be sent in both forms concomittantly to the CRL-AP by the 1st December 2008. Notification has been done that this date was a deadline and that results arriving later would not be accepted. A shift of the deadline was nevertheless proposed for participants outside EU due to custom related delays in delivery of the samples.

Results from 29 labs were accepted on a total of 30 participating laboratories. One participant of a country outside the EU did not report its results.

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

3. Material and methods

3.1. Material

3.1.1. Description of the samples

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

The composition was established taking into account the following features:

- Target concentration of mammalian meat and bone meal (referred to as MBM through the text) that was selected is 0.1 %, reflecting what is generally considered for the time being as the adulteration level that a method should be able to detect.
- Presence of fishmeal that could interfere with the detection of constituents from terrestrial animals when using classical microscopy [2] (the so-called "masking effect").
- Feed matrix conditioning (milled or pelleted) that requires grinding before analysis as requested by the EC 126/2003 Directive.
- PAPs lacking any classical relevant microscopic features such as bones, fish scales, and cartilages usually found through the sediment observation.

Each participating lab received about 55g of 10 blind samples to which a unique random number was assigned. Details of the samples are indicated in table 1.

Table 1: Composition of blind samples set used in the CRL-AP Proficiency Test 2008.

Sample	Material	Nr of replicates
1	Blank A	1
2	Blank B (Pellets)	1
3	0.1% MBM	1
4	0.5% feather meal	2
5	1% pure muscle MBM	2
6	Pure fish + 0.5% MBM	2
7	Pellets + 0.1% MBM	1
Total		**10**

3.1.2. Materials used in the preparation of the samples

The **first feed matrix** used for Blank A was a classical compound feed produced by a local plant. The matrix is composed of barley, wheat, corn, soya bean meal, milk derivate, beat pulp, potato proteins, salts, minerals and vitamins. Sediment content of this compound feed was about 1.60% (STD 0.12%).

A **second feed matrix** for Blank B was a pelleted feed supplement for bovines from a producer. It is composed of rapeseed and palm cattle cake, wheat and wheat glutenfeed, corn, soya bean, barley, salts, minerals and vitamins. Its sediment content was about 0.61% (STD 0.06%). This feed matrix was also used but after grinding for the preparation of the 0.5% feather meal and the 1% pure muscle MBM materials.

A **third feed matrix** was used for preparing the 0.1% MBM material. This commercial matrix contains corn glutenfeed, soya bean, palm kernel meal, coconut meal, beat and citrus pulp, sunflower seed meal, molasses, salts minerals and vitamins. The matrix sediment was of 1.86% (STD 0.33%).

Finally a **fourth pelleted feed matrix** was used for the elaboration of the Pellets + 0.1% MBM material. Its composition was the following: palm kernel meal, soya meal, wheat, rapeseed, sorghum, sugar beat pulp and molasses. Its sediment was of 2.05% (STD 0.29%).

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

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

- The **MBM** used for sample 3 was a mix of 4 pure porcine and 4 pure bovine meat and bone meals from different temperature treatments (but always at least at 133°C, 3 bars for 20 min). Its final bone content was of about 60%. Purity of each meal was controlled by PCR.
- The **feather meal** used in sample 4 is the flotate from an industrial poultry feather meal obtained after a two successive TCE sedimentation in order to reduce as much as possible the final bone content. Its strict avian origin was controlled by PCR.
- The **pure muscle MBM** used for sample 5 is a flotate of a bovine meat meal treated at 141°C after two successive TCE sedimentations in order to reduce as much as possible its final bone content. The original bovine meat meal purity was investigated by PCR.
- The **fish meal** used in the study was a mix of 5 fish meals used. The fish meals were from different geographical origin (Peru, Panama, France and Iceland) and hence composed of different species. The fishbone content was of about 19%. Purity of the fish meals was investigated by PCR.
- The **MBM** used for sample 6 is a pure bovine meat and bone meal treated at 133°C. Its bone content was of about 80%. Its purity was investigated in DG-Sanco 2004 study [3].
- The **MBM** used for sample 7 was a bovine bone meal with a bone content of about 66%. PCR tests indicated a pure bovine origin.

3.1.3. Description of the mixing procedures

The **stepwise dilution procedure** developed by CRA-W and JRC-IRMM was used to produce the following samples: 3, 5 and 6. This procedure has been successfully used in numerous former European interlaboratory studies aiming to evaluate different classical microscopy protocols.

The **spiking procedure** was used for the production of the other sample materials adulterated by PAPs (samples 4 and 7).

3.2. Qualitative analysis

Qualitative analysis concerned the presence or absence of terrestrial (MBM) and/or fish. For sample 5 containing only muscle fibres which cannot be assigned to a fish or terrestrial origin, the qualitative analysis was restricted to the sole presence of animal particles. These binary results were analysed by classical statistics: accuracy, sensitivity and specificity. All those statistics were expressed as fractions.

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

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

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

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

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

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

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

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

4. Results

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

4.1. Homogeneity study

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

Table 2: Homogeneity – Results of the detection of terrestrial and fish particles by classical microscopy

Material	Nr of replicates analysed	Terrestrial	Fish	Remark
Blank A	10	-	-	
Blank B (Pellets)	10	-	-	grinded samples
0.1% MBM	10	+	-	
0.5% feather meal	10	+	-	use of cystine reagent
1% pure muscle MBM	10	+	-	use of Fehling staining
Pure fish + 0.5% MBM	5	+	+	
Pellets + 0.1% MBM	5	+	-	• grinded samples • use of Fehling staining

Legend: + = present, - = not present

The homogeneity study has been performed on 10g of sample material for each replicate. For the homogeneity study the flotate as well as the sediment fraction were analysed.

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

In the **0.1% MBM** the presence of terrestrial bones was systematically observed. No fish particles were ever noted on a total of more than 30 slides.

For the **0.5% feather meal** sample, the presence of feather fragments was always recorded. A few bone particles could still be detected among slides even though the TCE double sedimentation used in the preparation to reduce their number. On the 15 slides performed from the sample sediments a total of only 25 bone fragments were found, in other terms a mean number of 1.7 bone fragments / slide.

The sample **1% pure muscle MBM** did not contain bone fragments; this results from the analysis of 15 slides from the sediments. The presence of muscle fibres was always confirmed from the flotate.

For the **pure fish + 0.5% MBM** sample, the presence of terrestrial bones was reported for each of the 5 sediments analysed.

Concerning the **pellets + 0.1% MBM** sample, terrestrial bones were systematically reported through the 5 sediments. The presence of muscle fibres was also confirmed by way of Fehling staining on the flotates for all 5 replicates.

4.2. Qualitative analysis

4.2.1. Preliminary remarks

On the respect of the EC 126/2003 directive:

- Labs 3, 10 and 19 did not respect the EC 126/2003 directive instructions stating that at least 5g of the sample are required for analyses (on the sieve fractions or sediment). Amounts ranging from 1g to 2g were taken for sedimentation by those labs, but only for sample 6 (pure fish + 0.5% MBM).
- Labs 3, 6, 8 and 11 did not respect the EC 126/2003 directive instructions indicating that when fish is detected at least 3 slides have to be observed: "… at least two additional slides […] and the total sediment fraction shall be examined."
- Labs 3, 6, 11 and 25 reported some results based on a single slide observation.

On the respect of the instructions given for the present study:

- Lab 26 did not report the number of slides analysed - it actually examined the whole sediment.
- Some labs did not report the detailed data:
    - Labs 19 and 22 forgot to mention the number of fish particles observed for one sample.
    - Lab 14 did not report the number of particles detected for 4 samples.
    - Labs 5, 20, 21 and 23 did not report the number of particles detected for 2 samples.

4.2.2. Overview of results and performance of the method

Table 3 summarizes the results submitted by the 26 NRLs for the 7 types of materials submitted to qualitative analysis.

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

Sample	Material	n	AC	
			Terrestrial	Fish
1	blank A	26	0.885 (3)	0.962 (1)
2	blank B (Pellets)	26	0.923 (2)	0.923 (2)
3	0.1% MBM	26	1.000	0.923 (2)
4	0.5% feather meal	52	0.981 (1)	0.788 (11)
5	1% pure muscle MBM	52	0.865* (7)	
6	pure fish + 0.5% MBM	52	0.923 (4)	1.000
7	pellets + 0.1% MBM	26	0.885 (3)	0.769 (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, * = sensitivity for animal particles detection only).

On the exception of sample 5 which is discussed in a dedicated section, there is no case report of "no results" through the study although it was one of the possible statements in case of inconclusive results on the presence or the absence of relevant material or in case of inability of the laboratory to provide a result. This means that NRL participants were confident enough in their observations.

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

The ratio of false positive results reported for the blank materials A and B is of 6% (or 3/52) and for terrestrial particles of 10% (or 5/52). Both percentages are low.

The correct detection of 0.1% MBM in a grinded matrix is faultless. Nevertheless two NRLs have false positive results for the presence of fish (2/26 or 8%).

The detection of feather meal at a level of 0.5% - with only traces of bone particles - does not present noticeable problems as only one false negative for terrestrial is recorded (2% or 1/52). However 21% (11/52) of false positive records for fish occurs for this sample.

The number of false negative results for the presence of animal particles in the 1% pure muscle MBM is 13% (7/52).

Concerning the detection of 0.5% MBM in a pure fish meal, only 8% (4/52) of false negative results was observed.

Finally the detection of 0.1% MBM added to a pelleted matrix generates only 12% (3/26) of false negative for terrestrial particles but produces a high percentage of false positive results for fish: 23% (6/26).

4.2.3. Detailed review of results for each sample material

Blank A :

Lab 1 reported 11-50 terrestrial particles on a total of 3 slides.

Lab 16 detected 5 particles identified as hairs on a total of 6 slides

Lab 18 detected both terrestrial and fish particles accordingly the following details: 6-10 bone and muscle fragments and 6-10 fish bones, muscle and scales fragments on a total of 4 slides.

Blank B :

Lab 1 reported 11-50 terrestrial particles and 2 fish bones on a total of 3 slides.

Lab 16 detected only 1 fishbone on total of 8 slides.

Lab 26 recorded only 1 terrestrial bone on the whole sediment.

0.1% MBM:

Lab 18 reported the presence of 2 fish particles (fishbone and scale) on a total of 4 slides. It also only detected a few 2 terrestrial particles (bone fragment and a muscle cell) which is the lowest record of terrestrial particles for this sample.

Lab 19 identified 3 fish bones aside the 88 terrestrial bone fragments on a total of 4 slides.

0.5% Feather meal:

Lab 30 did not detect any terrestrial particle although having prepared 4 slides for one of the duplicates of this sample. This lab also presented the lowest value of sediment weight recovered after sedimentation process for both replicates. However the first duplicate was correctly identified. It cannot be ruled out that the sample analysis was only made based on the sole sediment observation.

Some comments are needed on this sample material. Although the detection of terrestrial particles is almost faultless, it seems likely that some labs reported only bones (lab 6, 7, 12, 16, 18, 19, 21, 25, 26, 28). Those

labs do not mention the presence of feather. The question as to whether those participants made the analysis on the sieve fraction (or the flotate) can be raised.

Numerous labs reported false positive results for fish (lab 1, 6, 7, 18, 21, 27 and 31). Presence of fish by those labs is evidenced by the mention of fishbone fragments, some rare fish scales, fish muscle and even krill identification. One possible explanation which could account for this unexpected raise of false positive results for fish would be that some feather meal fragments (e.g. rachis) could settle down and be identified as fish particles. We observed similar particles during the homogeneity study. In such a case the use of the Alizarin Red staining is helpful for a better differentiation. Finally misidentifications of particles from the sediments and considered as from fish origin (see Annex 4) may also explain for some false positive results.

1% pure muscle MBM:

Labs 13, 28 and 30 failed to find any animal particle (terrestrial and fish particles) on both replicates of the sample.

Labs 16 had a false negative result for the presence of animal particles for one on two replicates.

As from the composition of the MBM, which contained only muscle fibres, it was impossible to distinguish their terrestrial or fish origin, some comments on the interpretation of the results is requested.

- Labs 12, 14, 20, 21, 23, 25 and 27 choose the “no results” option for both fish and terrestrial presence. Some of those labs justified this choice in the information cells of the result form.
- Other labs (6, 9, 10 and 24) justified the presence of terrestrial animal fragments as they observed a few particles identified as terrestrial bones.
- All other assertive results on the presence of “terrestrial and fish” particles, or “terrestrial only” and “fish only” are not scientifically justified due to any unambiguous criteria habilitating a 100% certain decision.
- Finally a few labs (1, 6, 7, 8, 16, 18, 19 and 29) reported the presence of fish bones, scales or scale tooth but their numbers are always very limited. Such observations on the presence of fish particles were not noticed during the homogeneity study.

Pure fish + 0.5% MBM :

Lab 21 was unable to detect the presence of terrestrial particles through the two sample replicates.

Labs 9 and 25 failed to detect terrestrial particles in one sample duplicate. For lab 25 this might originate from a poor sediment recovery (1.5% instead of 18.3% as a mean) that would deliver a too scarce number of terrestrial particles.

Pellets + 0.1% MBM :

Lab 7, 14 and 28 did not detect terrestrial particles from this material.

Labs 1, 6, 16, 18, 23 and 31 reported the presence of fish particles according the following details :

- Lab 1 : 4 fish bones
- Lab 16 : 2 scales
- Labs 6, 23 and 18 : 6-10 fish bones and scales
- Lab 31 : 11-50 particles classified as fish

From the homogeneity study, no particles from fish origin were observed. Misidentification of terrestrial bone presenting very elongated lacunae with fish bones as shown in Annex 4 may explain the high rate of PD for fish in this sample.

4.2.4. Individual performances of NRLs in qualitative analysis

Individual performances were assessed for each participant by calculation of the accuracy, sensitivity and specificity over the blind samples. This was performed separately for both the detection of terrestrial material and fish material. A ranking of the labs was prepared based on the accuracy.

Results are to be found in tables 4 and 5.

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

Terrestrial			
lab code	AC	SE	SP
2	1.000	1.000	1.000
3	1.000	1.000	1.000
4	1.000	1.000	1.000
6	1.000	1.000	1.000
8	1.000	1.000	1.000
10	1.000	1.000	1.000
12	1.000	1.000	1.000
19	1.000	1.000	1.000
20	1.000	1.000	1.000
23	1.000	1.000	1.000
24	1.000	1.000	1.000
27	1.000	1.000	1.000
29	1.000	1.000	1.000
31	1.000	1.000	1.000
18	0.900	1.000	0.500
26	0.900	1.000	0.500
7	0.900	0.875	1.000
9	0.900	0.875	1.000
14	0.900	0.875	1.000
25	0.900	0.875	1.000
1	0.800	1.000	0.000
16	0.800	0.875	0.500
13	0.800	0.750	1.000
21	0.800	0.750	1.000
28	0.700	0.625	1.000
30	0.700	0.625	1.000

Fish			
lab code	AC	SE	SP
2	1.000	1.000	1.000
3	1.000	1.000	1.000
4	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
12	1.000	1.000	1.000
13	1.000	1.000	1.000
14	1.000	1.000	1.000
20	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
28	1.000	1.000	1.000
29	1.000	1.000	1.000
30	1.000	1.000	1.000
19	0.875	1.000	0.833
21	0.875	1.000	0.833
23	0.875	1.000	0.833
27	0.875	1.000	0.833
7	0.750	1.000	0.667
16	0.750	1.000	0.667
1	0.625	1.000	0.500
6	0.625	1.000	0.500
31	0.625	1.000	0.500
18	0.375	1.000	0.167

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

- PD for MBM in blank A : labs 1, 16 and 18
- PD for MBM in blank B : labs 1, 26
- ND for MBM in 0.5% feather : lab 30

- ND for animal particles in 1% pure muscle MBM : labs 13, 16, 28 and 30
- ND for MBM in pure fish + 0.5% MBM : labs 9, 21 and 25
- ND for MBM in the pellets + 0.1% MBM : labs 7, 14, 28

Concerning the ability to detect fish material, 10 labs encountered problems :

- PD for fish in blank A : lab 18
- PD for fish in blank B : labs 1, 16
- PD for fish in 0.1% MBM : labs 18 and 19
- PD for fish in 0.5% feather : labs 1, 6, 7, 18, 21, 27 and 31
- PD for fish in the pellets + 0.1% MBM : labs 1, 6, 16, 18, 23 and 31

A general ranking of the participants was performed on a consolidated evaluation; including their proficiency in detecting both terrestrial and fish materials through the 10 blind samples (table 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
2	1.000	1.000	1.000
3	1.000	1.000	1.000
4	1.000	1.000	1.000
8	1.000	1.000	1.000
10	1.000	1.000	1.000
12	1.000	1.000	1.000
20	1.000	1.000	1.000
24	1.000	1.000	1.000
29	1.000	1.000	1.000
19	0.944	1.000	0.875
23	0.944	1.000	0.875
26	0.944	1.000	0.875
27	0.944	1.000	0.875
9	0.944	*0.900*	1.000
14	0.944	*0.900*	1.000
25	0.944	*0.900*	1.000
13	0.889	0.800	1.000
6	0.833	1.000	0.625
31	0.833	1.000	0.625
7	0.833	0.900	0.750
21	0.833	0.800	0.875
28	0.833	0.700	1.000
30	0.833	0.700	1.000
16	0.778	0.900	0.625
1	0.722	1.000	0.375

18	0.667	1.000	0.250

The table illustrates the very good level of global performance (= consolidated AC superior to 0.90, *i.e.* having just one false result) for 16 labs out of 26 NRLs or in other words for 62% of the NRLs. However critical consideration on this apparent good performance rate is needed. First of all, taking into account the present legislation on the feed ban, the type of wrong detection must be considered: one ND for terrestrial particles has to be regarded as more serious than one PD for fish. Secondly this study was performed on a set of 10 blind samples and the general proficiency presented is based on 10 evaluations of sensitivity and 8 evaluation of specificity, thus the impact of a single mistake only accounts for 1/18th. Comparatively to the last CRL-AP Proficiency Test 2007 a single mistake accounted for 1/6th or in other words its impacts was threefold when compared to the present study. Hence any comparison with past studies might be misinterpreted and has not been realised.

Thus among the 16 labs presenting a very good global performance, three labs (9, 14 and 25) failed once to detect the presence of terrestrial material (cells in black italics in table 6). Those labs should be encouraged to hire internally on the possible origin for this accidental mistake.

A second category (cells in blue in table 6) of NRLs having a satisfying global performance is defined (= consolidated AC below 0.90 and having no more than three false results including a maximum of two ND for terrestrial material). NRLs included in this category are nevertheless asked to report to the CRL-AP on the possible source of these deviations. Attention has to be paid by the labs that had missed two times the detection of terrestrial material (cells in blue underlined).

A third category (cells in red in table 6) includes participants that are underperforming (= consolidated AC below 0.90 and having either more than four false results or three ND for terrestrial). Those participants require improvement of proficiency. Those participants are asked to report on the origin of those multiple errors as well as on the actions they will undertake in order to solve this critical issue.

4.2.5. Individual performances of other participants in qualitative analysis

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

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

Tables 7 (left) and 8 (right): Lab proficiencies regarding the detection of terrestrial and fish material. Ranking follows AC values.

Terrestrial			
lab code	**AC**	**SE**	**SP**
5	1.000	1.000	1.000
11	0.900	0.875	1.000
22	0.700	0.750	0.500

Fish			
lab code	**AC**	**SE**	**SP**
5	1.000	1.000	1.000
11	1.000	1.000	1.000
22	0.875	1.000	0.833

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

- PD for MBM in blank B : lab 22
- ND for MBM in 0.1% MBM : lab 22
- ND for MBM in 0.5% feather : lab 11

- ND for MBM in pure fish + 0.5% MBM : lab 22
- PD for fish in 0.1% MBM : lab 22

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

Two participants obtained very good level of global performance: lab 5 had a faultless set of answers and lab 11 failed once to detect the presence of terrestrial material (cells in black italics in table 9).

The other participant is underperforming and therefore needs improvement of proficiency. For this underperforming lab however, contrarily to the NRL network, the major source of error is not related to PD for fish in the 0.5% feather nor to PD for fish in the pellets + 0.1% MBM.

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

Consolidated			
lab code	**AC**	**SE**	**SP**
5	1.000	1.000	1.000
11	0.944	*0.900*	1.000
22	0.778	0.800	0.750

5. Conclusions

The study was based on a diversity of material types (pelleted feeds, different feed matrices, various MBM, use of pure fish meal, some prohibited animal materials without clearly identifiable discriminating criteria) aiming at reflecting the daily diversity of feeds which have to be analysed by control labs. This diversity of materials was successfully overcome by the majority of participants.

Among the NRLs, 62% of them obtained a very good level of global performance. This level of good performance is comparable to previous CRL-AP Proficiency tests [4, 5] and reflects the maturity of the NRL network. Five NRLs are considered as underperforming and are asked to take actions in order to improve their proficiency.

The global results table on the method performance reveals an overall problem of specificity for the detection of fish, more precisely when feather meal is present in a feed. In addition, it also reveals a lack of sensitivity for animal particle detection when a feed is adulterated with a pure muscle meal with a scarce number of bone fragments. On the opposite way this type of pure muscle meal adulteration of feed allowed to experience the prudence of the NRL network: many participants (as well as one foreign participant) admitted to be unable to classify this type of contamination as being either of terrestrial or fish origin, which is effectively impossible to determine. This study thus demonstrates the difficulty of decision in absence of particles with well defined microscopic features for origin identification. The organisers also stress the risk of taking a wrong decision under similar circumstances.

Concerning the participants outside EU, two of them performed well whereas one failed. From external sources the organisers know that those two participants have opportunities to access to training sessions on the detection of PAPs in feed. The organisers do not know if it is the case for the less performing participants. In the negative case, this would once again confirm as previously demonstrated through CRL-AP studies [4, 5] that continuous formation and education is crucial for keeping a high level of proficiency.

Acknowledgment

We are especially grateful to the whole CRL-AP staff and the participants for their fruitful collaboration.

6. References

[1] EU. 2003. Commission Directive 2003/126/EC of 23 December 2003 on the analytical method for the determination of constituents of animal origin for the official controls of feedingstuffs. Official Journal of the European Union L 339/78, 24/12/2003: 78-84.

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

[3] Boix A, von Holst C, Baeten V, Berben G and Vancutsem J. 2004. Determination of Processed Animal Proteins (PAPs) including meat and bone meal (MBM) in feed – Part I : Intercomparison study for the determination of PAPs in feed using microscopy. Joint Research Centre IRMM, Geel, Belgium.

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

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

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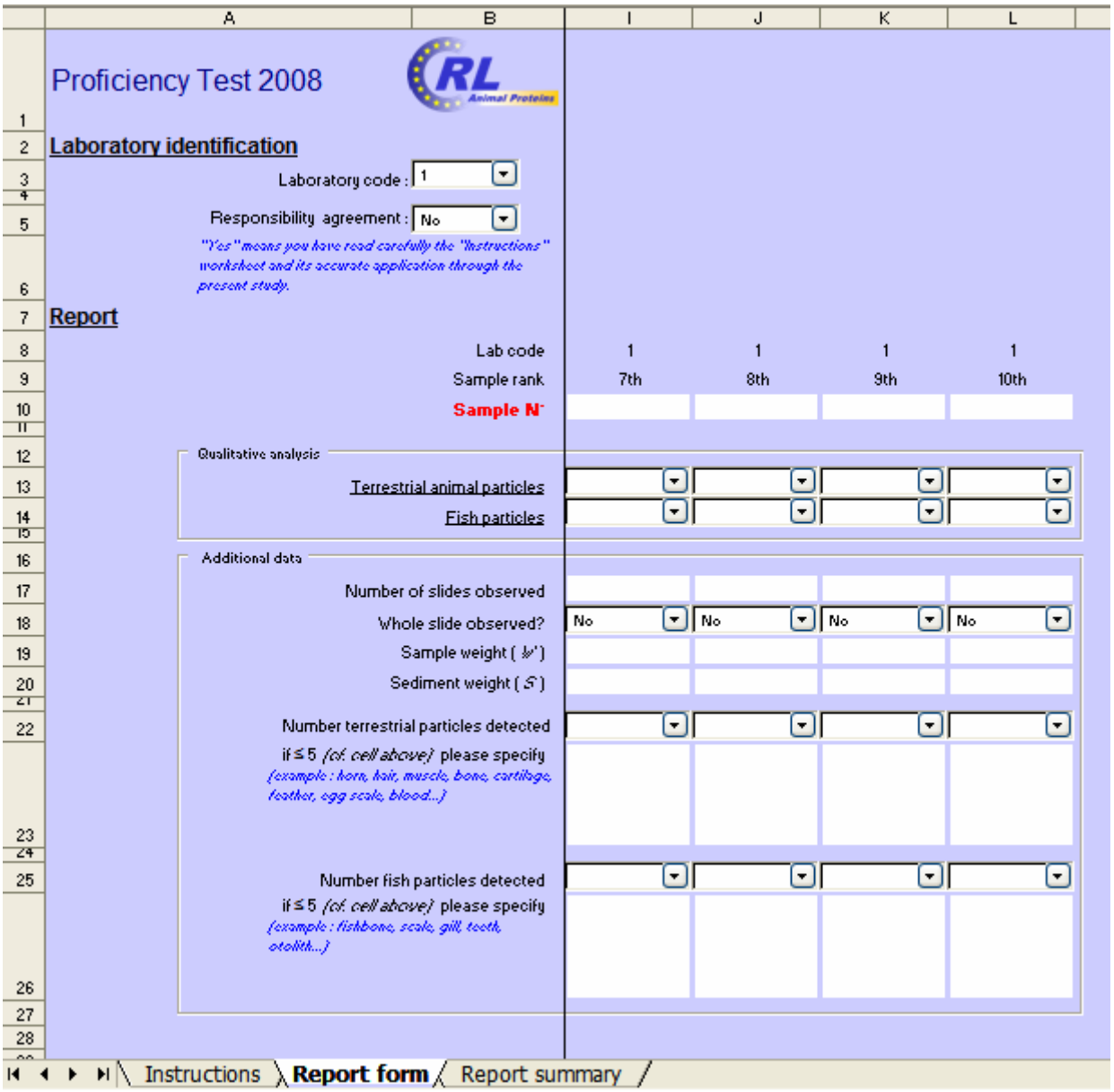
Annex 1

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

Country	Institute Name
Austria	Austrian Agency for Health and Food Safety
Belgium	Federal Agency for the Safety of the Food Chain
Bulgaria	National Diagnostic Research Veterinary Medical Institute
Canada	*Canadian Food Inspection Agency*
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	Service Commun des Laboratoires – Laboratoire de 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
Latvia	National Diagnostic Centre of Food and Veterinary Service
Lithuania	National Veterinary Laboratory
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Poland	National Veterinary Research Institute
Portugal	Laboratorio Nacional de Investigaçao Veterinaria
Romania	Hygiene Institute of Veterinary Health
Russia	*Central Veterinary Laboratory*
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	Veterinary Laboratories Agency

Annex 2

Excel result report form.



Annex 3

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

Laboratory identification code : **1**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
3	Present	Not present	3	Yes	10	0,182	11 to 50			
4	Present	Not present	3	Yes	10	0,065	6 to 10			
6	Present	Present	3	Yes	10	2,018	11 to 50		> 50	
5	Present	Not present	3	Yes	10	0,125	11 to 50			
2	Present	Present	3	Yes	10	0,198	11 to 50		2	2 bones
1	Present	Not present	3	Yes	10	0,287	11 to 50			
7	Present	Present	3	Yes	10	0,255	> 50		4	4 bones
6	Present	Present	3	Yes	10	2,065	11 to 50		> 50	
5	Present	Present	3	Yes	10	0,208	11 to 50		2	2 bones
4	Present	Present	3	Yes	10	0,215	11 to 50		3	3 bones

Laboratory identification code : **2**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
4	Present	Not present	6	Yes	10,03	0,07	11 to 50	bones, feathers		
5	Present	Not present	9	Yes	10,04	0,07	> 50	only muscle fibers		
6	Present	Present	12	Yes	10,02	1,97	11 to 50	bones, muscle fibers?	> 50	fishbones, scales, gills, teeth, otolithe, muscle fibers
3	Present	Not present	6	Yes	10,05	0,11	11 to 50	bones		
1	Not present	Not present	12	Yes	10,03	0,16				
2	Not present	Not present	10	Yes	10,02	0,03				
7	Present	Not present	6	Yes	10,04	0,17	11 to 50	bones, cartilage		
5	Present	Not present	6	Yes	10,03	0,06	> 50	only muscle fibers		
6	Present	Present	9	Yes	10,04	2,07	6 to 10	bones, muscle fibers?	> 50	fishbones, scales, gills, teeth, otolithe, muscle fibers
4	Present	Not present	6	Yes	10,01	0,06	11 to 50	bones, feathers		

Laboratory identification code : **3**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
1	Not present	Not present	3	Yes	10,12	0,0551				
4	Present	Not present	4	Yes	10,16	0,0564	> 50			
6	Present	Present	3	Yes	2,2	0,3629	11 to 50		> 50	
5	Present	Not present	2	Yes	10,18	0,054	> 50			
3	Present	Not present	1	Yes	10,18	0,1088	> 50			
2	Not present	Not present	3	Yes	10,14	0,0547				
7	Present	Not present	3	Yes	10,22	0,1847	11 to 50			
6	Present	Present	1	Yes	2,13	0,353	11 to 50		> 50	
5	Present	Not present	2	Yes	10,18	0,0489	> 50			
4	Present	Not present	2	Yes	10,12	0,0514	> 50			

Laboratory identification code : **4**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
2	Not present	Not present	5	Yes	5,0003	0,0466				
3	Present	Not present	5	Yes	5,0004	0,1076	> 50	bones		
7	Present	Not present	5	Yes	5,0002	0,1141	6 to 10	bones		
5	Present	Present	5	Yes	5,0003	0,0478	> 50	only musclefibres, no diff.between MBM and FM possible	> 50	only musclefibres, no diff.between MBM and FM possible
6	Present	Present	5	Yes	5,0003	1,0813	11 to 50		> 50	
4	Present	Not present	5	Yes	5,0002	0,0526	> 50	bones, feathers		
6	Present	Present	5	Yes	5,0002	1,0783	11 to 50		> 50	
1	Not present	Not present	5	Yes	5,0004	0,0888				
5	Present	Present	5	Yes	5,0003	0,0483	> 50	only musclefibres, no diff.between MBM and FM possible	> 50	only musclefibres, no diff.between MBM and FM possible
4	Present	Not present	5	Yes	5,0002	0,0468	> 50	bones, feathers		

Laboratory identification code : **5**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
2	Not present	Not present	2	Yes	6,35	0,0201				
1	Not present	Not present	3	Yes	6,67	0,0973				
3	Present	Not present	2	Yes	6,85	0,1146	> 50			
4	Present	Not present	2	Yes	6,58	0,0424	> 50			
6	Present	Present	4	Yes	6,31	1,4999	6 to 10		> 50	
7	Present	Not present	2	Yes	6,81	0,1215	> 50			
6	Present	Present	4	Yes	6,7	1,4231	11 to 50		> 50	
4	Present	Not present	2	Yes	7,45	0,0465	> 50			
5	No results	No results	3	Yes	6,6	0,0391				
5	No results	No results	2	Yes	5,68	0,0367				

Laboratory identification code : **6**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
6	Present	Present	3	Yes	10	0,8358	11 to 50		> 50	
5	Present	Not present	2	Yes	10	0,0273	> 50	muscular fibers in sieved fraction (among which numerous with "distorted aspect" as often observed in MBM)	2	2 fishbone fragments not refound in a 2nd extract
3	Present	Not present	2	Yes	10	0,0609	> 50			
2	Not present	Not present	2	Yes	10	0,403				
1	Not present	Not present	1	Yes	10	0,0207				
7	Present	Present	2	Yes	10	0,1452	> 50		6 to 10	1 scale and 6 fishbone fragments in a 1st extract and 3 fishbone fragments in a 2nd extract
4	Present	Present	2	Yes	10	0,0359	6 to 10	6 bone fragments in a 1st extract and 51 bone fragments in a 2nd extract	11 to 50	3 fishbone fragments in a 1st extract and 11 fishbone fragments in a 2nd extraction
6	Present	Present	2	Yes	10	0,8473	11 to 50		> 50	
5	Present	Not present	1	Yes	10	0,276	> 50	muscular fibers in sieved fraction (among which numerous with "distorted aspect" as often observed in MBM) and 1 bone fragment not refound in a second extract)		
4	Present	Present	2	Yes	10	0,039	> 50	12 bone fragments found in a 1st extract and 68 bone fragments found in a 2nd extract	11 to 50	2 fishbone + 1 scale fragments found in a 1st extract and 1 scale + 22 fishbone fragments found in a 2nd extract

Laboratory identification code : **7**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
6	Present	Present	10	Yes	10,01	2,168	5	Bones	> 50	Fishbones, gills, teeth, scales, muscle, etc.
4	Present	Present	10	Yes	10,002	0,148	11 to 50	Bones	6 to 10	Bones
5	Not present	Present	10	Yes	10,018	0,084			6 to 10	Fishbones, muscle
1	Not present	Not present	5	Yes	10,094	0,125				
2	Not present	Not present	7	Yes	7,323	0,023				
7	Not present	Not present	7	Yes	11,485	0,926				
3	Present	Not present	10	Yes	10,004	0,067	6 to 10	Bones		
5	Not present	Present	10	Yes	10,029	0,074			6 to 10	Fishbones, muscle
4	Present	Present	10	Yes	10,644	0,091	6 to 10	Bones	5	Fishbones
6	Present	Present	10	Yes	10,67	4,104	6 to 10	Feathers, muscle, bones	> 50	Fishbones, muscle, scales, gill, etc.

Laboratory identification code : **8**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
3	Present	Not present	2	Yes	10,00g	0,1524	11 to 50			
7	Present	Not present	2	Yes	10	0,2255	11 to 50			
4	Present	Not present	2	Yes	10	0,1079	11 to 50	Feathers; meat fibres; bones		
6	Present	Present	2	Yes	10	2,1424	11 to 50	bones	> 50	All kinds
1	Not present	Not present	2	Yes	10	0,1768				
2	Not present	Not present	2	Yes	10	0,0928				
5	Not present	Present	2	Yes	10	0,0792			> 50	one scale tooth; many meat fibres (fish?)
4	Present	Not present	2	Yes	10	0,0854	> 50	bones (small);feathers		
5	Not present	Present	2	Yes	10	0,0789			> 50	Many meat fibres (fish?)
6	Present	Present	2	Yes	10	2,1644	11 to 50	bones	> 50	all kinds

Laboratory identification code : **9**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
6	Not present	Present	10	Yes	5,1708	0,988			> 50	
5	Present	Not present	4	Yes	5,1938	0,029	2	2 bones, muscle fibres = meat meal		
7	Present	Not present	5	Yes	5,1942	0,094	2	bone		
2	Not present	Not present	3	Yes	5,1738	0,027				
1	Not present	Not present	6	Yes	5,0024	0,051				
4	Present	Not present	3	Yes	5,0549	0,028	5	5 bones, feather meal hydrolysed		
3	Present	Not present	5	Yes	5,0555	0,066	11 to 50			
4	Present	Not present	5	Yes	5,0502	0,028	2	2 bones, feather meal hydrolysed		
6	Present	Present	11	Yes	5,0871	1,015	6 to 10		> 50	
5	Present	Not present	5	Yes	5,0198	0,027	1	one bone, muscle fibres = meat meal		

Laboratory identification code : **10**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
4	Present	Not present	4	Yes	10	0,0697	6 to 10			
7	Present	Not present	3	Yes	10	0,2155	11 to 50			
5	Present	Not present	4	Yes	10	0,0495	2	Bone		
5	Present	Not present	5	Yes	10	0,0062	1	Bone		
1	Not present	Not present	4	Yes	10	0,1396				
6	Present	Present	3	Yes	2	0,334	3	Bone	> 50	
6	Present	Present	4	Yes	2	0,4078	4	Bone	> 50	
3	Present	Not present	3	Yes	10	0,2068	6 to 10			
2	Not present	Not present	4	Yes	10	0,1068				
4	Present	Not present	4	Yes	10	0,0955	6 to 10			

Laboratory identification code : **11**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
6	Present	Present	1	Yes	5	1,07	5	bones, cartilage	> 50	
5	Present	Not present	2	Yes	10	0,103 g	11 to 50			
2	Not present	Not present	2	Yes	10	0,087 g				
4	Not present	Not present	2	Yes	10	0,163 g				
3	Present	Not present	2	Yes	10	0,219 g	11 to 50			
7	Present	Not present	1	Yes	10	0,272 g	> 50			
1	Not present	Not present	2	Yes	10	0,222 g				
5	Present	Not present	2	Yes	10	0,145 g	> 50			
6	Present	Present	1	Yes	5	1,092 g	6 to 10		> 50	
4	Present	Not present	3	Yes	10	0,133	2	bones		

Laboratory identification code : **12**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
3	Present	Not present	5	Yes	5	0,064	11 to 50	bones, cartilage		
1	Not present	Not present	10	Yes	5	0,131				
4	Present	Not present	6	Yes	5	0,07	11 to 50	bones		
6	Present	Present	4	Yes	5	1,06	11 to 50	bones	> 50	bones, scale, cartilage
2	Not present	Not present	2	Yes	5	0,011				
7	Present	Not present	4	Yes	5	0,075	4	bones		
5	No results	No results	4	Yes	5	0,043				
4	Present	Not present	4	Yes	5	0,029	3	bones		
5	No results	No results	2	Yes	5	0,027				
6	Present	Present	4	Yes	5	1,083	11 to 50	bones	> 50	bones, scale, cartilage

Laboratory identification code : **13**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
4	Present	Not present	4	Yes	10,0001	0,0761	11 to 50			
5	Not present	Not present	4	Yes	10,0001	0,046				
6	Present	Present	4	Yes	10,0005	1,8816	6 to 10		> 50	
2	Not present	Not present	4	Yes	10,0004	0,1708				
1	Not present	Not present	4	Yes	10,0002	0,4147		.		
3	Present	Not present	4	Yes	10,0002	0,1558	11 to 50			
7	Present	Not present	4	Yes	10,0004	0,2635	> 50			
4	Present	Not present	4	Yes	10	0,1667	11 to 50			
5	Not present	Not present	4	Yes	10,0006	0,1216				
6	Present	Present	4	Yes	10,001	1,9554	11 to 50		> 50	

Laboratory identification code : **14**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
3	Present	Not present	10	Yes	10	0,0959	5	muscle, poultry bones		
7	Not present	Not present	10	Yes	10	0,2254				
5	No results	No results	10	Yes	10	0,0728		just musclefiber		
6	Present	Present	10	Yes	10	1,8914	3	feather, poultry bone		
4	Present	Not present	10	Yes	10	0,0499	5	feather, poultry bones		
5	No results	No results	10	Yes	10	0,0665		just musclefiber		
1	Not present	Not present	10	Yes	10	0,2021				
2	Not present	Not present	10	Yes	10	0,0418				
6	Present	Present	10	Yes	10	2,1369	2	feather, poultry bone		
4	Present	Not present	10	Yes	10	0,0461	5	feather, poultry bones		

Laboratory identification code : **16**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
5	Not present	Not present	8	Yes	10,2	0,05				
6	Present	Present	6	Yes	9,98	1,85	11 to 50		> 50	
2	Not present	Present	8	Yes	10,19	0,4			1	bone
4	Present	Not present	6	Yes	10,16	0,41	4	bones		
7	Present	Present	6	Yes	10,28	0,2	6 to 10		2	scales
3	Present	Not present	6	Yes	10,25	0,15	5	4 bones; 1 muscle		
1	Present	Not present	6	Yes	10	0,57	5	hairs		
5	Present	Present	6	Yes	10,18	0,6	6 to 10		3	2 bones; 1 scale
6	Present	Present	6	Yes	10,21	2,24	11 to 50		> 50	
4	Present	Not present	8	Yes	10	0,61	3	bones		

Laboratory identification code : **18**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
5	Present	Present	4	Yes	9,21	0,05	2	bone, muscle,blood	2	fishbone, scale,muscle
6	Present	Present	4	Yes	11,19	1,3	6 to 10	bone,muscle,	> 50	fishbone,scale,muscle
2	Not present	Not present	4	Yes	11,14	0,01				
3	Present	Present	4	Yes	9,64	0,07	2	bone, muscle	2	fishbone,scale
4	Present	Present	4	Yes	10,03	0,04	2	bone, muscle	2	fishbone,scale
5	Present	Present	4	Yes	10,64	0,03	1	muscle, bone	2	fishbone,scale
6	Present	Present	4	Yes	10,37	0,44	5	bone, muscle	11 to 50	fishbone,scale
7	Present	Present	4	Yes	10,62	0,16	6 to 10	bone,muscle	6 to 10	fishbone, muscle,scale
1	Present	Present	4	Yes	9,9	0,15	6 to 10	bone,muscle	6 to 10	fishbone, muscle, ccale
4	Present	Present	4	Yes	10,36	0,03	1	bone, muscle	2	fisbone, muscle,scale

Laboratory identification code : **19**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
7	Present	Not present	6	Yes	10	0,191	> 50	116 bones		
4	Present	Not present	4	Yes	10	0,05	11 to 50	13 bones		
1	Not present	Not present	5	Yes	10	0,148				
6	Present	Present	6	Yes	2	0,401	11 to 50	12 bones	> 50	muscle
5	Not present	Present	3	Yes	10	0,05			2	many muscles and 2 fishbones
6	Present	Present	5	Yes	2	0,379	11 to 50	37 bones		
3	Present	Present	4	Yes	10	0,148	> 50	88 bones	3	fishbones
2	Not present	Not present	3	Yes	10	0,059				
4	Present	Not present	3	Yes	10	0,058	6 to 10	bones and a few musclefibers		
5	Not present	Present	3	Yes	10	0,049			> 50	fish muscle

Laboratory identification code : **20**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
2	Not present	Not present	10	Yes	10,01	0,073	2	bone		
4	Present	Not present	10	Yes	10,01	0,046	11 to 50			
5	No results	No results	15	Yes	10,01	0,042	11 to 50	muscle		
6	Present	Present	15	Yes	10	1,914	6 to 10			
7	Present	Not present	10	Yes	10	0,188	11 to 50			
1	Not present	Not present	10	Yes	10	0,13				
3	Present	Not present	10	Yes	10	0,134	6 to 10			
6	Present	Present	15	Yes	10	1,88	6 to 10			
4	Present	Not present	10	Yes	10	0,048	11 to 50			
5	No results	No results	15	Yes	10	0,052	11 to 50	2 bone, muscle		

Laboratory identification code : **21**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
4	Present	Not present	5	Yes	10,008	0,181	6 to 10			
5	No results	No results	5	Yes	10,021	0,187		Muscle fibres only		
7	Present	Not present	5	Yes	10,001	0,229	6 to 10			
4	Present	Present	5	Yes	10	0,172	2	Bone	2	Bone
3	Present	Not present	5	Yes	10,084	0,284	6 to 10			
2	Not present	Not present	5	Yes	10,572	0,146				
1	Not present	Not present	5	Yes	10,008	0,213				
5	No results	No results	5	Yes	10,008	0,153		Muscle fibres only		
6	Not present	Present	5	Yes	10,031	2,774			> 50	
6	Not present	Present	5	Yes	10,001	2,511			> 50	

Laboratory identification code : **22**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
4	Present	Not present	3	Yes	10	0,0514	11 to 50	feather		
2	Present	Not present	3	Yes	10,04	0,053	6 to 10			
6	Present	Present	5	Yes	10,04	1,98	5	bone		
3	Not present	Present	5	Yes	10,04	0,181			11 to 50	
1	Not present	Not present	3	Yes	9,95	0,13				
5	Present	Not present	5	Yes	10,04	0,039	6 to 10			
7	Present	Not present	5	Yes	10,02	0,226	6 to 10			
5	Present	Not present	3	Yes	10,03	0,63	6 to 10			
4	Present	Not present	3	Yes	9,96	0,048	6 to 10			
6	Not present	Present	5	No	9,96	1,51			> 50	

Laboratory identification code : **23**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
2	Not present	Not present	6	Yes	5	0,169				
4	Present	Not present	6	Yes	5	0,144	11 to 50			
3	Present	Not present	6	Yes	5	0,098	11 to 50			
7	Present	Present	6	Yes	5	0,173	11 to 50		6 to 10	
5	No results	No results	6	Yes	5	0,14		muscles of fish or chicken (unspecified) , no bone particles detected		muscles of fish or chicken (unspecified) , no bone particles detected
6	Present	Present	6	Yes	5	0,878	4	bone	> 50	
1	Not present	Not present	6	Yes	5	0,197				
5	No results	No results	6	Yes	5	0,127		muscles of fish or chicken (unspecified) , no bone particles detected		muscles of fish or chicken (unspecified) , no bone particles detected
4	Present	Not present	6	Yes	5	0,148	11 to 50			
6	Present	Present	6	Yes	5	0,937	6 to 10		> 50	

Laboratory identification code : **24**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
5	Present	Not present	10	Yes	5,371	0,05	2	We detected ~20 muscle fibers per slide - in the sive fraction, but no more than 1-2 bone particle in the whole sediment		
2	Not present	Not present	10	Yes	5,699	0,028				
6	Present	Present	4	Yes	5,605	1,103	11 to 50		> 50	
4	Present	Not present	4	Yes	5,633	0,051	6 to 10	Beside the bone fragments in the sediment, we found also feather fragments in the sive fraction.		
3	Present	Not present	4	Yes	5,772	0,084	11 to 50			
1	Not present	Not present	10	Yes	5,681	0,09				
5	Present	Not present	10	Yes	5,42	0,049	2	We detected ~20 muscle fibers per slide - in the sive fraction, but no more than 1-2 bone particle in the whole sediment		
7	Present	Not present	4	Yes	5,633	0,12	> 50			
6	Present	Present	4	Yes	5,405	1,063	5	bone fragments in the sediment	> 50	
4	Present	Not present	4	Yes	5,688	0,044	6 to 10	Beside the bone fragments in the sediment, we found also feather fragments in the sive fraction.		

Laboratory identification code : **25**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
3	Present	Not present	1	Yes	10	0,0662	11 to 50			
5	No results	No results	3	Yes	10	0,0916	11 to 50	Muscle		
2	Not present	Not present	3	Yes	10	0,084				
4	Present	Not present	3	Yes	10	0,3291	5	bone		
6	Not present	Present	3	Yes	10	0,1462			> 50	
1	Not present	Not present	3	Yes	10	0,1699				
7	Present	Not present	2	Yes	10	0,1538	6 to 10			
5	No results	No results	3	Yes	10	0,1262	> 50	Muscle		
4	Present	Not present	3	Yes	10	0,1423	4	Bone		
6	Present	Present	3	Yes	10	0,5397	5	Bone	> 50	

Laboratory identification code : **26**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
7	Present	Not present		Yes	5,07	0,147	> 50			
2	Present	Not present		Yes	5,61	0,056	1	bone		
1	Not present	Not present		Yes	5,23	0,096				
5	Not present	Present		Yes	5,42	0,066			> 50	only muscles
5	Not present	Present		Yes	5,81	0,074			> 50	only muscles
3	Present	Not present		Yes	5,75	0,087	11 to 50			
6	Present	Present		Yes	5,24	1,132	11 to 50		> 50	
4	Present	Not present		Yes	5,08	0,08	1	bones		
4	Present	Not present		Yes	5,5	0,081	6 to 10	bones		
6	Present	Present		Yes	5,17	1,056	> 50		> 50	

Laboratory identification code : **27**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
5	No results	No results	3	Yes	10	0,062	11 to 50	muscles only		
2	Not present	Not present	4	Yes	10	0,056				
3	Present	Not present	3	Yes	10	0,192	11 to 50			
5	No results	No results	4	Yes	10	0,072	11 to 50	muscles only		
6	Present	Present	3	Yes	8	1,568	6 to 10		> 50	
1	Not present	Not present	5	Yes	10	0,142				
4	Present	Present	4	Yes	10	0,062	11 to 50		5	1krill, 4 bones
7	Present	Not present	4	Yes	10,01	0,218	> 50			
4	Present	Not present	5	Yes	10	0,063	11 to 50			
6	Present	Present	3	Yes	8	1,498	4	bones	> 50	

Laboratory identification code : **28**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
1	Not present	Not present	3	Yes	6,288	0,053				
4	Present	Not present	3	Yes	6,384	0,049	5	Bones		
2	Not present	Not present	2	Yes	6,322	0,022				
6	Present	Present	3	Yes	6,176	1,065	5	Bones	> 50	
7	Not present	Not present	3	Yes	6,476	0,094				
5	Not present	Not present	3	Yes	6,393	0,034				
4	Present	Not present	3	Yes	6,472	0,036	5	Bones		
3	Present	Not present	3	Yes	6,496	0,087	6 to 10			
5	Not present	Not present	3	Yes	6,375	0,043				
6	Present	Present	3	Yes	6,302	1,061	6 to 10		> 50	

Laboratory identification code : **29**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
3	Present	Not present	10	Yes	10	0,1378	6 to 10			
5	Not present	Present	10	Yes	10	0,055			5	Muscle, bones
6	Present	Present	5	Yes	10	2,157	6 to 10		> 50	
7	Present	Not present	10	Yes	10	0,161	5	bones, muscle		
1	Not present	Not present	10	Yes	10	0,156				
2	Not present	Not present	10	Yes	10	0,056				
4	Present	Not present	10	Yes	10	0,11	5	feather, bones		
4	Present	Not present	10	Yes	10	0,168	5	feather, bones		
6	Present	Present	5	Yes	10	2,035	4	bones	> 50	
5	Not present	Present	10	Yes	10	0,147			3	muscle, bones

Laboratory identification code : **30**

Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
2	Not present	Not present	4	Yes	10,01	0,09				
3	Present	Not present	4	Yes	10	0,062	11 to 50			
4	Present	Not present	4	Yes	10,06	0,035	5	bone		
5	Not present	Not present	4	Yes	10	0,024				
7	Present	Not present	6	Yes	10,02	0,052	2	bone		
6	Present	Present	5	Yes	10,01	0,317	3	bone	> 50	
1	Not present	Not present	4	Yes	10	0,092				
5	Not present	Not present	4	Yes	10	0,027				
6	Present	Present	4	Yes	10,02	0,289	5	bone	> 50	
4	Not present	Not present	4	Yes	10,01	0,016				

Laboratory identification code : **31**

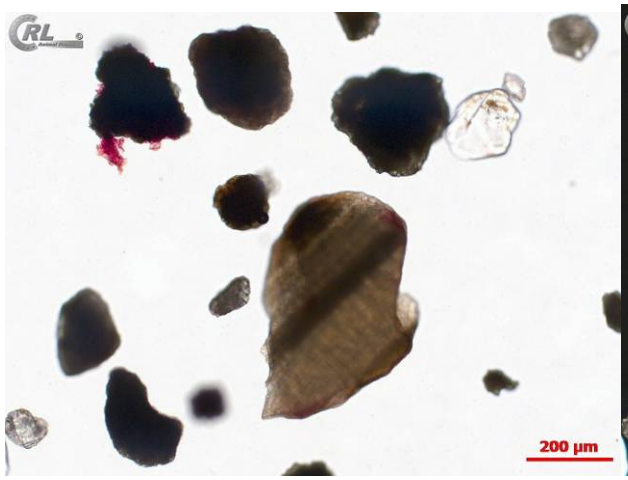
Sample N°	Terrestrial animal part.	Fish part.	Number of slides	Whole slide ?	W (g)	S (g)	Number of terrestrial part. detected	Comment if number of terr. part. ?5	Number of fish part. detected	Comment if number of fish part. ?5
2	Not present	Not present	8	Yes	10	0,137				
6	Present	Present	8	Yes	10	2,231	11 to 50		> 50	
4	Present	Present	8	Yes	10	0,183	> 50		6 to 10	
7	Present	Present	8	Yes	10	0,318	> 50		11 to 50	
3	Present	Not present	8	Yes	10	0,213	> 50			
5	Not present	Present	8	Yes	10	0,117			11 to 50	
5	Not present	Present	8	Yes	10	0,089			11 to 50	
1	Not present	Not present	8	Yes	10	0,207				
4	Present	Present	8	Yes	10	0,146	> 50		11 to 50	
6	Present	Present	8	Yes	10	2,135	11 to 50		> 50	

Annex 4

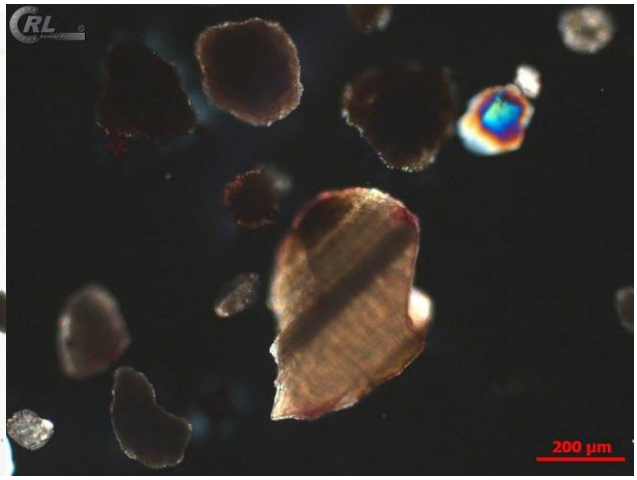
Example of possible misidentifications for fish.

1. Sample 4 : 0.5 % Feather meal.

Confusion with otolith :



Plant particle. Bright Field. 100x.



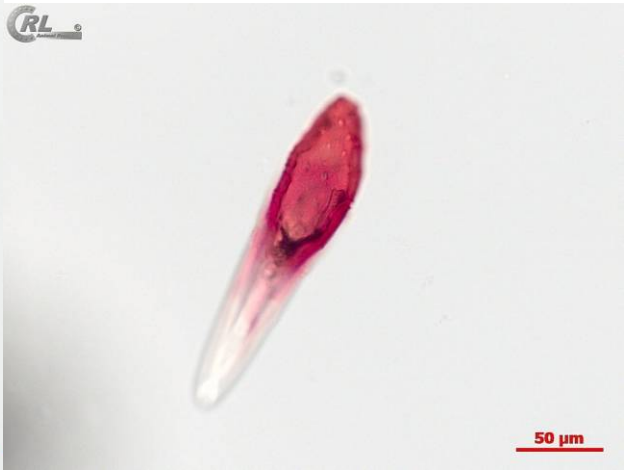
Plant particle.Polarization.100x.

Confusion with fish scale:

Mineral particle. Bright Field. Alizarine Red. 400x

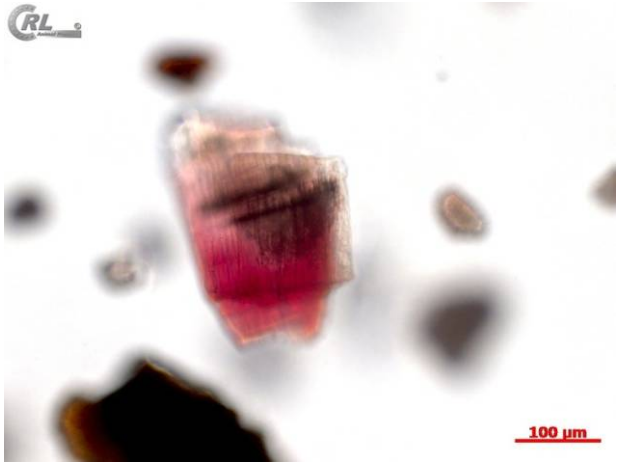
Confusion with fish teeth:

Trichome. Alizarine Red. BF. 400x



Trichome. Alizarine Red. Bright Field. 400x

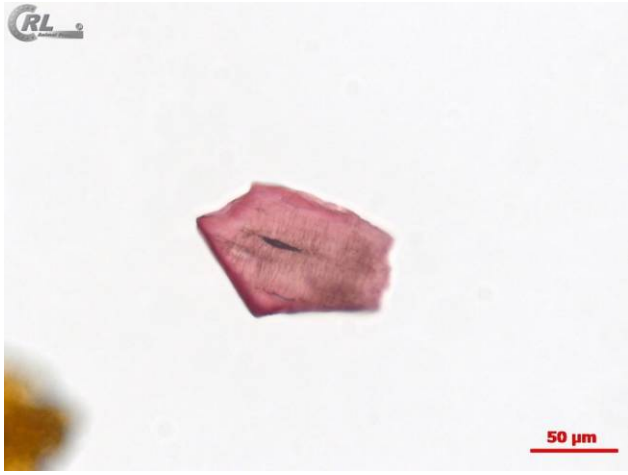
Confusion with fishbone:



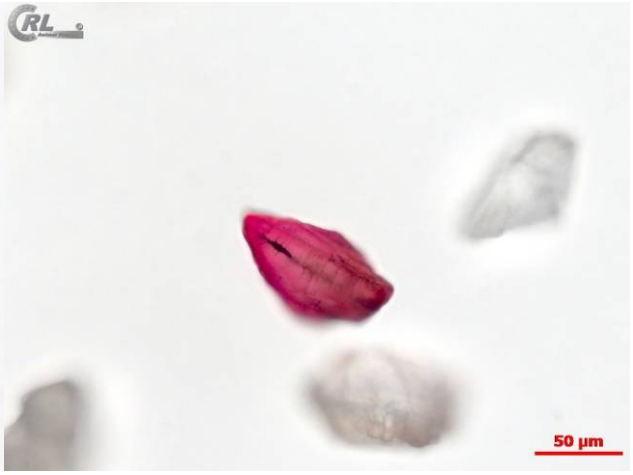
Plant particle. Alizarine Red. Bright Field. 200x

2. Sample 7: Pellets + 0.1% MBM.

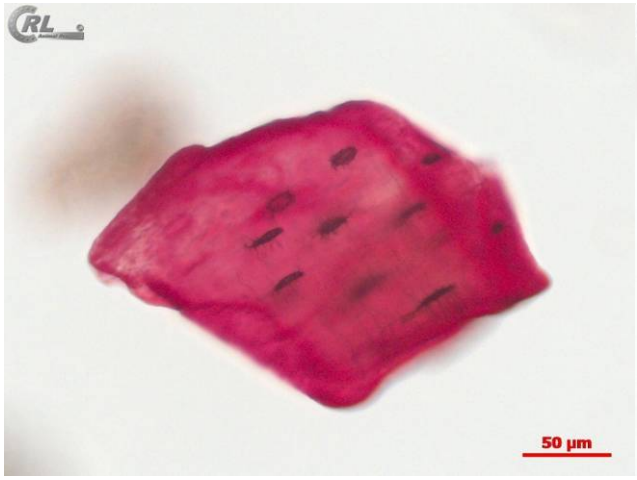
Confusion with fishbones:



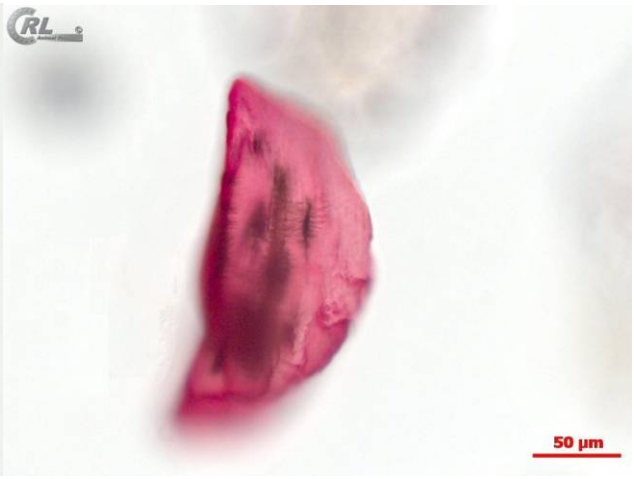
MBM. Alizarine Red. Bright Field. 400x.



MBM. Alizarine Red. Bright Field. 400x



MBM. Alizarine Red. Bright Field. 400x.



MBM. Alizarine Red. Bright Field. 400x