Community Reference Laboratory for Animal Proteins in feedingstuffs

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CRL-AP Interlaboratory Study 2006
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 2006 the present interlaboratory study for all National Reference Laboratories (NRLs) of the European Union nominated at that time. The goals of the study were (i) to evaluate the performance of the NRLs to apply the microscopic method for qualitative purpose indicated in Commission Directive 2003/126/EC[§] and (ii) to investigate the robustness of the EC 126/2003 directive method regarding the quantitative evaluation of animal constituents in feedingstuffs.

The number of participants was 23 and each participant received 19 blind samples of materials adulterated by mammalian meat and bone meal and/or fish meal at different levels of concentration. Blank materials were also sent in order to check for possible cross-contamination. Instructions were the following ones: applying strictly the EC 126/2003 directive for both qualitative detection of animal particles and quantification. Participants had to quantify only when fish (and not terrestrial) particles were detected by the qualitative analysis. In this respect each participant had 10 quantifications to realise.

The homogeneity of the materials was tested by the CRL-AP and considered as sufficient.

From the qualitative results, it appeared that 77% of participants performed above 95% consolidated accuracy. This result is positive taking into consideration the high number of participating NRLs. It is the best performance obtained since interlaboratory studies on this issue are organized within the European Union using the method described in EC 126/2003 directive. Nevertheless future improvement of the proficiency of the remaining 23% of NRLs performing worse is expected and possible because there are only a few ones underperforming. We can rely on the EC 126/2003 directive as it stands for further improvement of the qualitative detection of animal proteins in feedingstuffs by NRLs.

Concerning the robustness of the EC 126/2003 directive for quantification of animal constituents in feedingstuffs, the present study revealed that only two NRLs on three are able to realise quantitative analysis. Quantifications from those NRLs indicated that the variation of the results *between* laboratories (reproducibility standard deviation) is that low that we cannot rely on the present protocol. Therefore CRL-AP recommends major improvements of the EC 126/2003 directive. Improvements have to focus on more detailed instructions to be implemented in the present procedure. This study also demonstrated that, for the labs that were able to carry out this type of calculation, the variation of the results *within* laboratories for the batch of samples analysed (repeatability standard deviation) is nonetheless acceptable.

Keywords :

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

[§] 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 an uniformity of the results provided by European control laboratories. On 29 April 2004, the European Parliament and the Council have 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, has 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. The new 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, polymerase chain reaction, 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 where Member States contest the results of analysis.

It is in this framework that the CRL-AP has organized in 2006 its first interlaboratory study aiming to:

- firstly evaluate the performance of the NRL to apply the microscopic method for qualitative purpose, as stated in the EC 126/2003 directive [1] which is the sole reference method.
- secondly investigate the robustness of the EC 126/2003 directive method regarding the quantitative determination of animal constituents in feedingstuffs.

Results of this study were presented and discussed with the participants during the CRL-AP Workshop organised at Gembloux, Belgium, from 16 April to 18 April 2007. The present final report, based on a working document diffused to all NRL on 11 April 2007, includes some enhancements and additional comments that were made during the discussions of the workshop.

2. Introduction

Beginning of December 2006, 19 blind samples for the CRL-AP Interlaboratory Study 2006 have been sent by express shipment to the nominated NRLs at that time (23 laboratories in total). The detailed list of the participating labs is included in Annex 1.

The following instructions have been given to each participating lab:

- Analysis of the blind samples by applying the protocol described in EC 126/2003 directive [1].
- Mention has been done that each participating laboratory was sole responsible to reach appropriate homogeneity of the sample sub-portions that had to be taken from the whole sample vial for analysis. Therefore the CRL-AP strongly recommended them to take appropriate mixing and/or grinding procedure for achieving this sub-sample homogeneity.
- Qualitative and quantitative results were requested. Qualitative analyses have been requested for each of the 19 samples whereas quantitative analyses were only required when a qualitative result demonstrated that only fish constituents had been detected.
- For quantitative analysis, it was asked to provide also the detailed way of calculations as well as to explain how they determined precisely the correction factor *d*. An example of calculation on one of the treated sample has also been 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 at once to the CRL-AP by 22 January 2007. Notification has been done that this date was a deadline and that results arriving later would not be accepted.

On the 23 participating laboratories, only results from 22 labs were accepted after exclusion of one participant, lab number 34, because the later did not report its results.

According to the purpose of the Interlaboratory study, two types of analysis - qualitative and quantitative - were thus carried out on the results.

3. Material and methods

3.1. Material

3.1.1. Description of the samples

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

The composition was established taking into account the following features:

- For the qualitative part of the study
  - Target concentration of mammalian meat and bone meal (referred 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 at 5 % that could interfere with the detection of constituents from terrestrial animals when using classical microscopy [2] (often so-called “masking effect”).
- For the quantitative part of the study
  - Target concentrations of fish meal were selected at 4 different levels: 0.25%, 0.5%, 1% and 1.5 % in order to evaluate the potential of the method assuming that a tolerance level of 1% could be introduced.
  - Variation in the composition and the microscopic structures observed in fish meal samples.

Each participating lab received 19 blind samples to which a unique random number was assigned. Details of the samples are indicated in table 1.

Sample	Material	Nr of replicates
A	Blank	3
B	0.1% MBM	3
C	0.5% Fish III	2
D	0.25% Fish III	2
E	1.5% Fish III	2
F	1% Fish III	2
G	0.1% MBM + 5% Fish I	3
H	1%Fish I	2
Total		**19**

Table 1: Composition of blind samples set used in the CRL-AP Interlaboratory Study 2006.

3.1.2. Materials used in the preparation of the samples

The **base of the test material** (Sample A or blank) was a mixture of 50/50 in weight of two compound feeds produced in the framework of the STRATFEED EC project (www.stratfeed.cra.wallonie.be) for bovine containing corn, soya beans and hulls, rapeseed, beet, straw, molasses, sunflower, palmkernel, coconut, citruspulp, wheat, vegetable fat and various feed additives such as limestone, salts, minerals and vitamins. The sediment content of the compound feed was about 0.69% (STD = 0.07%). Prior to use the material it was tested by classical microscopy, Polymerase Chain Reaction (PCR), immunoassay kits, near-infrared microscopy (NIRM) and near-infrared imaging 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 in the study was a mix of bovine and porcine processed material with a bone content of about 10.07% (STD = 0.79%). The MBM was treated at temperature 133°C and 3 bars for 20 minutes. This MBM has been analyzed by classical microscopy in order to check if the particles issued from this sample had the microscopic features normally observed for that kind of product. PCR was also applied in order to check the species present in the sample, confirming that these samples mainly contained bovine and porcine materials with low amounts of poultry material.
- Two different **fish meals (Fish I and Fish III)** have been used in this study. Fish meals were obtained directly from fish meal producers and the proportion of bones for Fish I and Fish III were respectively about 11.75% (STD = 0.15%) and 10.81% (STD = 0.08%). Fish I is made of one single pure fish meal material, while Fish III is made of a mixture of three pure fish meal materials. All fish samples were analyzed by classical microscopy in order to check if the particles issued from this sample had the microscopic features normally observed for that kind of product. The microscopic observations confirmed that the materials had effectively the expected specific features such as fish bones and scales. PCR was performed in order to check the species present in the sample; its results confirmed that these samples only included material from fish origin.

3.1.3. Description of the mixing procedures

Prior to using the base of the material and the processed animal proteins (MBM, Fish I and Fish III) in the preparation of the different mixtures, those have been sieved in order to keep only particles from the 0 – 1000 µm fraction.

The blind sample materials have been prepared according to two procedures:

- Two materials, 0.1% MBM and 0.1% MBM + 5% Fish I, have been produced following the **stepwise dilution procedure** developed by CRA-W and JRC-IRMM. This procedure has been successfully used in three former European interlaboratory studies (DG-Sanco 2003 [2], DG-Sanco 2004 [3], STRATFEED [4] studies) aiming to evaluate different classical microscopy protocols. This procedure is described in detail in the final report of DG-Sanco 2003 study [2]. The use of the stepwise dilution procedure to produce these samples allows comparing the results of the first CRL-AP interlaboratory study with those former European studies. All the samples spiked at 0.1% MBM (without and with 5 % fish meal) used in the four studies have thus a same degree of difficulty. About 55g of the samples prepared according to the stepwise dilution procedure have been put in the vial used.
- The five other adulterated materials, i.e. either by Fish I or Fish III, have been produced following the **spiking procedure**. In this procedure, consisting of four steps, 25g of the base material has been weighted and has been put in the vial. Afterwards the required quantity of fish meal has been weighted and added in the vial in three successive steps. Between each step, the pre-mixture was mixed with a spoon. During the third step, the remaining required quantity of base material necessary to reach the targeted concentration was finally added. During the last step each sample prepared has been thoroughly agitated during 2 minutes using a whisk. The spiking procedure chosen to prepare the samples used to evaluate the quantitative performance of the method described in the EC 126/2003 directive was preferred to the stepwise dilution because it ensures an exact percentage of PAPs for each produced sample.

3.2. Qualitative analysis

Qualitative analysis concerned the presence or absence of terrestrial (MBM) and/or fish material for the 19 blind samples. 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, 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, was calculated as follows:

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

Specificity is the ability of classifying negative results as negative, 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 (terrestrial material and 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.

The 95% confidence intervals were calculated from the Binomial distribution (with lower limit p = 0.025 and upper limit p = 0.975).

3.3. Quantitative analysis

The main objective for this part of the Interlaboratory study was that of delivering a portrait of the current performance of the method as stated in the EC 126/2003 directive from a quantitative perspective. Therefore labs were explicitly asked to strictly follow the protocol described in the directive.

Quantification was asked if strictly only fish particles where detected in the samples. So the participants had normally 10 quantifications to realise, i.e. for each of the 2 replicates of the following materials:

- 0.25% Fish III
- 0.50% Fish III
- 1% Fish III
- 1% Fish I
- 1.50% Fish III

Results were expressed in terms of estimated value (in %) of constituents of fish origin, in compliance to the EC 126/2003 directive.

The CRL-AP wanted to consider all results from the analysis. No randomization tests for quantitative results, such as Grubb's or Cochran's test, were performed for the detection of possible outliers. Therefore robust statistics [5] for data analysis was chosen. The use of robust statistics shows some striking advantages in comparison to the traditional approach: the detection and rejection of outliers is not required, thus the impact of extreme values on the average and the standard deviation is downweighted. Moreover, robust statistic works well for distributions that deviate from normal distribution due to extreme values which is typical for data received in a collaborative study.

The estimates of the mean values and the precision of the data expressed in terms of within-laboratory variation (*repeatability* standard deviation) and between-laboratory variation (*reproducibility* standard deviation) are obtained by analysis of variance (ANOVA) as specified in the IUPAC guideline for method validation [6]. Robust statistics have been applied to the estimation of the standard deviation and the average.

An indicative proficiency for each participant was assessed by *z*-score analyses. Assessment of the performance on each material was realised by *z*-score analysis. The global performance was estimated by pooling of *z*-scores and obtaining the *z*-score global mean or rescaled sum of scores (RSZ) [7] for each participant. A ranking of the participants was realised by sorting them by their absolute value of RSZ, in order to establish whether they reported consistently to high or to low concentrations of the fish meal content.

4. Results

Gross results from all participants are to be found in Annex 6.

4.1. Homogeneity study

4.1.1. Homogeneity of samples 0.1% MBM and 0.1% MBM + 5% Fish I

It has been demonstrated that the stepwise dilution procedure used to produce sample 0.1% MBM and sample 0.1% MBM + 5% Fish I gives a sufficient homogeneity of the prepared material for the qualitative evaluation of the EC 126/2003 directive [2].

Five samples of material 0.1% MBM and four samples of material 0.1% MBM + 5% Fish I have been analysed. Results from these analyses are shown in table 2. Terrestrial bones have been found in the five samples 0.1% MBM and the four samples 0.1% MBM + 5% Fish I (table 2).

Material	Sediment	Terrestrial	Fish
0.1% MBM	0.48%	+	-
	0.76%	+	-
	0.66%	+	-
	0.67%	+	-
	0.68%	+	-
0.1% MBM + 5% Fish I	1.13%	+	+
	1.07%	+	+
	1.13%	+	+
	0.87%	+	+

Table 2: Homogeneity – Detection of terrestrial or fish particles. (Legend: + = present, - = not present)

4.1.2. Homogeneity of samples adulterated only by fish

4.1.2.1. Analysis on the raw fraction

As mentioned in point 3.1.3., in order to obtain samples with the highest achievable homogeneity, the five following materials (0.25%, 0.50%, 1%, 1.50% Fish III and 1% Fish I) have been prepared by the spiking procedure. Table 3 gives for each prepared material the standard deviation on the weight percentage.

Material	n	Mean %	STD %
0.25% Fish III	10	0.253	0.00086
0.5%Fish III	10	0.503	0.00111
1% Fish III	10	1.011	0.00150
1% Fish I	10	1.010	0.00109
1.5% Fish III	10	1.525	0.00058

Table 3: Homogeneity – Weight percentage of fish spiking. (Legend: n = number of samples).

In addition, PCR and NIR microscopy analyses have been done on the raw fraction of the materials (cf. tables in Annex 3).

4.1.2.2. Analysis on the sediment fraction

From each material spiked with fish meal, the entire 55g of five samples have been sedimented and quantification has been performed according to EC 126/2003 directive. Table 4 gives the summary of the quantification by way of classical microscopy. Details of these results can be found in Annex 4.

Material	n	Fish content %	Mean %	STD %
0.25% Fish III	1	0.28	0.38	0.11
	2	0.28		
	3	0.48		
	4	0.51		
	5	0.32		
0.5% Fish III	1	0.68	0.64	0.05
	2	0.69		
	3	0.66		
	4	0.57		
	5	0.63		
1% Fish III	1	1.03	1.12	0.22
	2	1.44		
	3	0.97		
	4	1.23		
	5	0.91		
1.5% Fish III	1	1.63	1.56	0.21
	2	1.52		
	3	1.67		
	4	1.23		
	5	1.78		

Table 4: Homogeneity – Quantification results.

4.2. Qualitative analysis

4.2.1. Overview of results and performance of the method

Table 5 summarizes the results submitted by the 22 laboratories for the eight types of materials submitted to qualitative analysis.

Sample	Material	n	AC	
			Terrestrial	Fish
A	Blank	66	1.000	0.879 (8)
B	0.1% MBM	66	0.985 (1)	0.909 (6)
C	0.5% Fish III	44	1.000	1.000
D	0.25% Fish III	44	1.000	1.000
E	1.5% Fish III	44	0.955 (2)	1.000
F	1% Fish III	44	1.000	1.000
G	0.1% MBM + 5% Fish I	66	0.879 (8)	1.000
H	1%Fish I	44	0.955 (2)	1.000

Table 5: Global results expressed as accuracy (AC) for the 8 samples (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).

It must be highlighted that there is no case report of "no results" although it was one of the possible choice of value in case a participant could not either determine the presence or the absence of relevant particles, or the result of the analysis was not conclusive. This means that participants were confident enough in their observations.

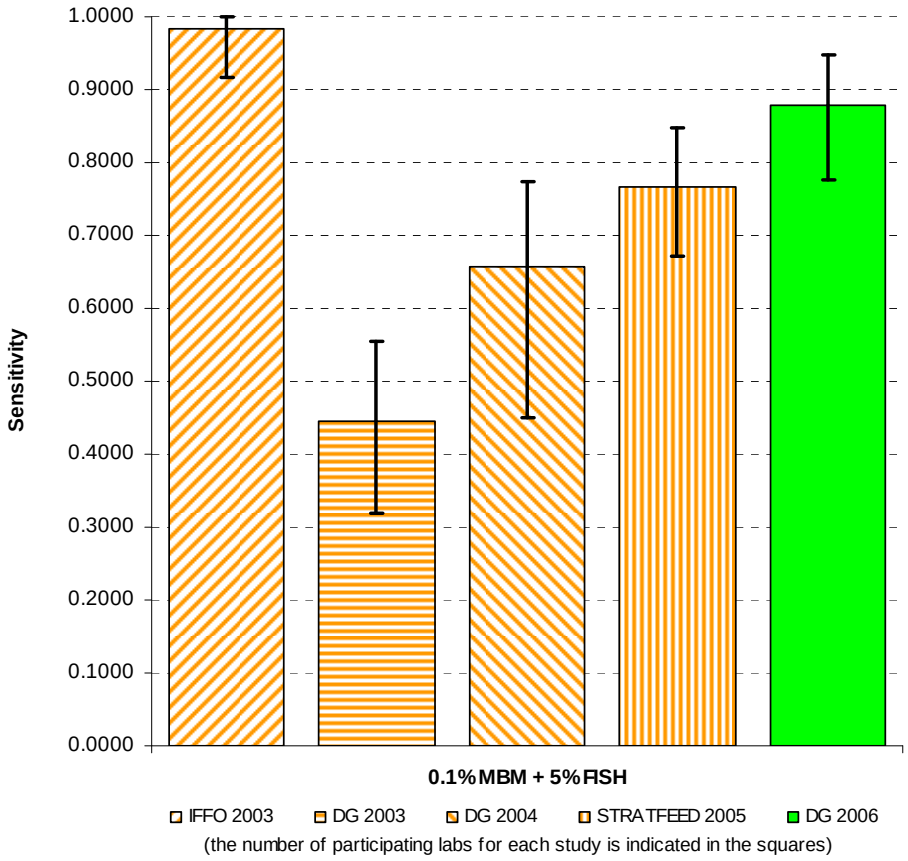
The overall results, expressed in terms of accuracy, indicate a satisfying global performance for the method.

Nevertheless some laboratories obtained false positive results for fish in the blank (12% or 8/66) and in the 0.1% MBM (9% or 6/66). Furthermore, for the 0.1% MBM, one case of false negative result was observed.

The material containing 0.1% MBM + 5% Fish I presents also some false negatives (12% or 8/66). This material type of material, with a percentage of MBM close to the limit of detection together with a high percentage of fish meal, has been used for other interlaboratory studies [2, 3, 4, and 8]. The relative high content of fish classically engenders a masking effect on the MBM particles detection [2, 8]. It is the case in the present study which again indicates a relative lower sensitivity, although being very satisfying as that obtained from previous studies (cf. next section).

4.2.2. Comparison with other studies

For an estimation of the evolution of the performance through the different European studies over the past years, using that same type of adulterated material, 0.1% MBM in presence of 5% Fish, we compared the sensitivity of the present results with that from past studies in graph 1.



Graph 1: Comparison of sensitivity for a 0.1% MBM + 5% Fish material through different European studies. Error bars indiquate the 95% confidence interval.

The sensitivity obtained for 0.1% MBM + 5% Fish I in the present study is the second highest observed since the IFFO study in 2003 [8]. Moreover it must be emphasized that the calculations through this study take into consideration all results meaning no outliers rejections as it was the case for all other studies referred on the graph. A continuous progress is thus observed.

Noteworthy the present sensitivity of 0.879 shows rather large 95% confidence limits: the explanation for this is the presence of some few underperforming participants (labs 2, 5 and 32) on the total number of labs. The detailed information on this is illustrated in the next section.

Finally, when considering the number of participants in comparison to that of IFFO 2003, the sensitivity score obtained in the present study reflects an overall better proficiency from the participants.

4.2.3. Performances of individual labs in qualitative analysis

Individual performances were assessed for each participant by calculation of the accuracy, sensitivity and specificity over the 19 blind samples. This was realized 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 find in tables 6 and 7.

Terrestrial			
lab code	AC	SE	SP
1	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
7	1.000	1.000	1.000
9	1.000	1.000	1.000
10	1.000	1.000	1.000
14	1.000	1.000	1.000
15	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
22	1.000	1.000	1.000
29	1.000	1.000	1.000
33	1.000	1.000	1.000
23	0.947	1.000	0.923
30	0.947	1.000	0.923
35	0.947	1.000	0.923
20	0.947	0.833	1.000
5	0.895	0.667	1.000
2	0.842	0.667	0.923
32	0.789	0.333	1.000

Fish			
Lab code	AC	SE	SP
1	1.000	1.000	1.000
4	1.000	1.000	1.000
6	1.000	1.000	1.000
7	1.000	1.000	1.000
9	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
18	1.000	1.000	1.000
20	1.000	1.000	1.000
21	1.000	1.000	1.000
22	1.000	1.000	1.000
23	1.000	1.000	1.000
29	1.000	1.000	1.000
30	1.000	1.000	1.000
32	1.000	1.000	1.000
33	1.000	1.000	1.000
35	1.000	1.000	1.000
5	0.947	1.000	0.833
10	0.947	1.000	0.833
19	0.842	1.000	0.500
2	0.789	1.000	0.333
3	0.737	1.000	0.167

Tables 6 (left) and 7 (right): Lab proficiencies regarding the detection of terrestrial and fish material. Ranking follows AC values.

Concerning the ability to detect terrestrial material, four labs (20, 5, 2 and 32) are underperforming (cells in red in table 6). For those labs the underperformance is due to either a high number of false negative results, i.e. they did not detected MBM when it was present, or to a high number of false positive, i.e. they detected MBM when it was actually not present, or to both type of false responses. Details for those incorrect answers for the detection of terrestrial material are hereunder.

- ND for MBM in the 0.1% MBM
  - **Lab 32: 1/3 samples**
- ND for MBM in the 0.1% MBM + 5% Fish I (masking effect)
  - Lab 20: 1/3 samples **Labs 2, 5: 2/3 samples** **Lab 32: 3/3 samples**
- PD for MBM in the 1% Fish I
  - Lab 2: 1/2 samples

Concerning the ability to detect fish material five labs (5, 10, 19, 2 and 3) encountered problems (cells in red in table 7). For those labs the underperformance is only due to false positive results, i.e. they detected fish when it was actually not present. Details for those incorrect answers for the detection of fish material are hereunder.

- PD for fish in the blank
  - Lab 5: 1/3 samples **Labs 3, 19: 2/3 samples** **Lab 2: 3/3 samples**
- PD for fish in the 0.1% MBM
  - Labs 2, 10, 19: 1/3 samples **Lab 3: 3/3 samples**

We suppose a cross contamination for labs 5 and 19 because their false positives were found in the blank. Data reporting the very low number of fish particles detected by those labs support this assumption. For the other labs reporting false positive in the blank, situation is unexplained.

A general ranking of the participants was realized on a consolidated evaluation, including their proficiency in detecting both terrestrial and fish materials through the 19 blind samples (table 8 next page):

The individual global performance is excellent (= full set of correct answers) for twelve labs on twenty-two or in other words for 55% of the participants. Five participants (cells in blue italics in table 8) had good performances, with a single false positive for labs 10, 23, 30, 35 and one false negative for lab 20 (cell in red in table 8) in 0.1% MBM + 5% Fish I. This occurrence of a single error on a set of 19 samples has to be considered as an inherent feature for such study.

Five participants were underperforming (cells in red through the whole line in table 8) and require improvement of proficiency.

As a conclusion and expressed in percentage this means that 77% of participants performed above 95% consolidated accuracy and implemented the method correctly whereas only 23% of participants performed less well.

Consolidated			
lab code	AC	SE	SP
1	1.000	1.000	1.000
4	1.000	1.000	1.000
6	1.000	1.000	1.000
7	1.000	1.000	1.000
9	1.000	1.000	1.000
14	1.000	1.000	1.000
15	1.000	1.000	1.000
18	1.000	1.000	1.000
21	1.000	1.000	1.000
22	1.000	1.000	1.000
29	1.000	1.000	1.000
33	1.000	1.000	1.000
10	*0.974*	*1.000*	*0.947*
20	*0.974*	0.947	*1.000*
23	*0.974*	*1.000*	*0.947*
30	*0.974*	*1.000*	*0.947*
35	*0.974*	*1.000*	*0.947*
5	0.921	0.895	0.947
19	0.921	1.000	0.842
32	0.895	0.789	1.000
3	0.868	1.000	0.737
2	0.816	0.895	0.737

Table 8: General lab proficiency regarding the detection of terrestrial and fish material. Ranking follows AC values.

4.3. Quantitative analysis

4.3.1. Preliminary remark

This interlaboratory study intended to sketch a global inventory view on the appliance of the quantitative method described in the EC 126/2003 directive. For reminder the purpose was thus that of assessing the robustness of the method and not that of evaluating the participants. Nevertheless a ranking of the labs was realized for the purpose of the study (in order to check the variability between labs for instance). This ranking is logically purely informative.

From the 22 participants, five did not perform the quantitative part of the study. Some of them recognized to be unable to address this type of analysis on the sole basis of the EC 126/2003 directive. That means that data treatment was made on the results of 17 laboratories.

As a reminder, the formula of the EC 126/2003 directive [1] to use for the calculation of the estimated value (in %) of constituents of fish origin is the following:

$$\% = \frac{S \times d}{W \times f} \times 100$$

Where *S* is the weight of the sediment (in mg), *W* the weight of the sample material for the sedimentation (in mg), *d* is the correction factor for the estimated portion of fish bones and scale fragments in the sediment (in

%), *f* is the correction factor for the proportion of bones in the constituents of animal origin in the sample examined.

The participants had to indicate on the result form the four terms of the formula (cf. Annex 2) for each sample for which quantification was required.

Additionally, as stated in the instructions, the participants had to explain how they calculated the *d* factor and to provide an example on a dedicated worksheet. Concerning this issue, and on the exception of the 5 labs that did not perform the quantitative analysis, only 8 participants on 17 (or 47%) respected the instructions (see table 9 hereunder), whereas all other did not and provided incomplete answers.

Worksheet "d factor"

	#	Lab ID	
Did not explain how to calculate d factor and did not realise quantification at all)	5	30, 20, 15, 9, 2	**23%**
Did not explain how to calculate d factor, but realised quantification without this factor	1	1	**5%**
Explained how to calculate d factor without the asked example	6	23, 22, 21, 18, 6, 5	**27%**
Did not explain how to calcultate d factor but gave an example of final estimation	2	10, 4	**9%**
Explained how to calculate d factor and provided the asked example	8	35, 33, 32, 29, 19, 14, 7, 3	**36%**
Total	**22**		**100%**

Table 9: Overview of the respect of instructions for worksheet "d factor".

4.3.2. Results on quantitative analysis

The estimated values of constituents of fish origin as reported by the 17 labs that realized the quantitative analysis are shown in table 10 (next page).

Two figures from the 1.5% Fish III are missing: one from lab 23 and one from lab 35 because they originate from false positive results for MBM and were therefore effectively not calculated.

Basic statistics (lower part table 10) illustrates heterogeneity of results: whereas the means of estimated values of constituents of fish origin of each material increase according to the target values, the standard deviations which are almost equal to the means indicate a very high variability between the results. This can also be deduced from the observed minima and maxima for each material. Noteworthy two labs, 3 and 10, have respectively the majority of maxima (cells in red) and minima (cells in blue).

The means are always higher than the target values, but this does not seems to reflect any global overestimation as the medians are closed to the expected values of percentage. The medians thus indicate a same number of overestimations as underestimations. The higher observed means might therefore be more likely related to some few aberrant overestimated percentages. Robust statistics were thus applied in order to take the suspected aberrant values into consideration.

	0.25% Fish III		0.5% Fish III		1% Fish III		1% Fish I		1.5% Fish III	
Lab ID	D1	D2	C1	C2	F1	F2	H1	H2	E1	E2
1	0.09%	0.07%	0.16%	0.25%	0.40%	0.84%	1.18%	0.52%	1.03%	1.03%
3	**2.73%**	2.69%	2.33%	**3.22%**	3.23%	3.08%	3.79%	5.18%	4.75%	**5.64%**
4	0.04%	0.03%	0.10%	0.32%	1.04%	0.15%	**0.03%**	0.47%	0.33%	0.96%
5	1.00%	0.90%	1.25%	1.23%	1.34%	0.98%	1.29%	1.07%	2.01%	1.81%
6	0.50%	0.50%	0.50%	0.50%	1.00%	1.00%	2.00%	2.00%	2.00%	2.00%
7	0.54%	0.34%	0.65%	1.82%	1.14%	1.69%	3.37%	2.70%	2.60%	2.88%
10	**0.01%**	0.03%	0.06%	**0.05%**	**0.02%**	0.09%	0.09%	0.05%	**0.04%**	0.06%
14	0.80%	1.10%	1.80%	1.70%	3.30%	3.60%	4.20%	**5.20%**	4.50%	4.70%
18	0.21%	0.17%	0.19%	0.77%	1.05%	1.03%	0.74%	0.88%	1.03%	1.25%
19	0.14%	0.13%	0.36%	0.11%	0.65%	0.39%	0.59%	0.52%	0.80%	0.61%
21	0.23%	0.23%	0.48%	0.13%	0.75%	0.95%	0.98%	0.79%	1.65%	1.90%
22	1.60%	1.70%	1.70%	1.40%	**4.60%**	2.60%	3.60%	3.60%	5.30%	5.20%
23	0.40%	0.70%	1.80%	0.70%	3.20%	2.60%	3.10%	2.80%	*PD*	5.10%
29	0.20%	0.20%	0.50%	0.50%	1.50%	1.50%	1.00%	1.50%	1.50%	1.00%
32	0.10%	0.13%	0.32%	0.25%	0.44%	0.36%	0.53%	0.56%	0.44%	0.43%
33	0.09%	0.14%	0.25%	0.29%	0.27%	0.45%	0.24%	0.85%	0.19%	0.76%
35	0.20%	0.10%	0.50%	0.50%	1.00%	1.00%	1.00%	0.50%	*PD*	1.50%

n	34	34	34	34	32
mean	0.53%	0.79%	1.39%	1.67%	2.03%
STD	0.70%	0.77%	1.17%	1.50%	1.76%
min	0.01%	0.05%	0.02%	0.03%	0.04%
max	2.73%	3.22%	4.60%	5.20%	5.64%
median	0.21%	0.50%	1.00%	1.00%	1.50%

Table 10: Quantitative results. The upper part table gives the results expressed in %. The lower part table provides some basic statistics (Legend: n = nr of quantifications, STD = standard deviation, min = minimum value, max = maximum value)

	Average	STD	s_r	RSD_r	s_R	RSD_R
0.25% Fish III	0.32 (± 0.18)	0.09	0.05	17	0.37	113
0.5% Fish III	0.66 (± 0.31)	0.16	0.19	30	0.65	98
1% Fish III	1.14 (± 0.47)	0.24	0.26	23	0.97	85
1% Fish I	1.18 (± 0.66)	0.33	0.35	30	1.37	116
1.5% Fish III	1.79 (± 0.82)	0.41	0.22	12	1.70	95

All data are expressed in percentage (%)

Average	*Robust mean of all submitted results (± two times the standard error. or range with $\alpha = 0.05$)*
STD	*Standard deviation of the average. calculated from the reproducibility standard deviation divided by the square root of the number of laboratories*
s_r	*Repeatability standard deviation (within-laboratory variability)*
RSD_r	*Relative repeatability standard deviation*
s_R	*Reproducibility standard deviation (between-laboratory variability)*
RSD_R	*Relative reproducibility standard deviation*

Table 11: Robust statistics results.

From the robust analysis (table 11 previous page), all averages -or robust means- are relative close to the target values. This reflects indirectly the good sample preparation as revealed by the homogeneity study.

The repeatability or within-laboratory variability, expressed by RSD_r, ranges from 12% to 30% which is low. Considering the fact that all samples duplicates where blind and that consequently all measurements of the estimated values of fish content are thus independent, we can consider that the measurement capability within a lab is not that bad. The repeatability actually reflects a combined variability from the measurement uncertainty and the homogeneity. As the homogeneity is satisfying, the variability might be more linked to the measurement uncertainty. Although the repeatability looks good from this study we cannot ascertain that it actually reflects the real capability of a laboratory rather than the repeatability of a single operator.

Contrary to the repeatability the reproducibility or between-laboratory variability, expressed by RSD_R, presents an extremely high variation: about 100% (ranging from 85% to 116%). This obviously indicates very strong lab biases and supports the high standard deviations observed from the reported results (table 10). This very poor reproducibility was investigated by the z-score analysis (in order to normalise the data and not for any lab proficiency ranking) and its related distribution.

Differences between estimated values and target values (i.e. the robust averages) over the standard deviation, or z-scores, allows not only comparison of each lab measurements but also a ranking of each lab. Furthermore it permits to detect outlying results possibly influencing the reproducibility. Results of z-scores are in able 12.

z-scores											
Lab ID	**0.25% Fish III**		**0.5% Fish III**		**1% Fish III**		**1% Fish I**		**1.5% Fish III**		**|mean|**
29	-0.341	-0.341	-0.247	-0.247	0.372	0.372	-0.137	0.243	-0.171	-0.465	0.096
6	0.511	0.511	-0.247	-0.247	-0.144	-0.144	0.623	0.623	0.123	0.123	0.173
21	-0.269	-0.270	-0.278	-0.814	-0.405	-0.194	-0.152	-0.296	-0.084	0.062	0.270
18	-0.313	-0.426	-0.727	0.170	-0.093	-0.114	-0.334	-0.228	-0.447	-0.318	0.283
35	-0.341	-0.625	-0.247	-0.247	-0.144	-0.144	-0.137	-0.516		-0.171	0.286
1	-0.668	-0.699	-0.768	-0.629	-0.766	-0.311	-0.002	-0.504	-0.448	-0.450	0.524
5	1.932	1.648	0.912	0.881	0.206	-0.165	0.084	-0.084	0.129	0.012	0.556
19	-0.511	-0.540	-0.464	-0.850	-0.506	-0.774	-0.448	-0.501	-0.582	-0.694	0.587
32	-0.625	-0.540	-0.526	-0.634	-0.722	-0.805	-0.494	-0.471	-0.794	-0.800	0.641
33	-0.653	-0.511	-0.634	-0.572	-0.898	-0.712	-0.714	-0.251	-0.941	-0.606	0.649
4	-0.795	-0.824	-0.866	-0.526	-0.103	-1.022	-0.873	-0.539	-0.859	-0.488	0.689
7	0.625	0.057	-0.015	1.793	0.000	0.568	1.663	1.154	0.476	0.641	0.696
10	-0.881	-0.824	-0.928	-0.943	-1.156	-1.084	-0.828	-0.858	-1.029	-1.017	0.955
23	0.227	1.080	1.763	0.062	**2.126**	1.507	1.458	1.230		1.946	1.266
14	1.364	**2.216**	1.763	1.608	**2.229**	**2.539**	**2.293**	**3.053**	1.594	1.711	**2.037**
22	**3.636**	**3.920**	1.608	1.144	**3.571**	1.507	1.838	1.838	**2.064**	**2.005**	**2.313**
3	**6.855**	**6.730**	**2.580**	**3.961**	**2.156**	1.997	1.980	**3.034**	1.738	**2.261**	**3.329**

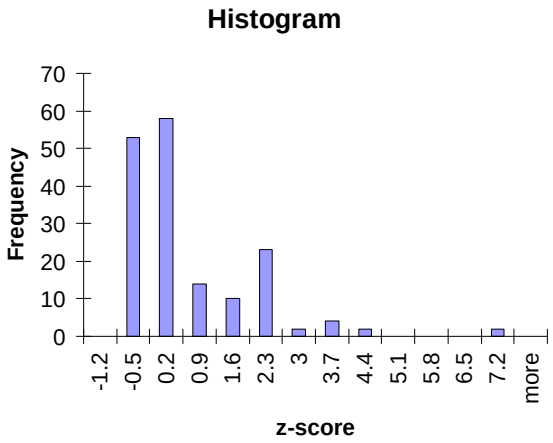
Table 12: z-Scores results. Ranking follows absolute mean of pooled individual z-scores (greyish column).

From table 12 it appears that, although the method presents a very poor reproducibility, successfully only few labs presents global out-of-range z-scores:

- Labs 14 and 22 have figures equivalent to a global warning value (cell in blue bold, |mean| column)
- Lab 3 has a figure equivalent to an action value (cell in red bold, |mean| column)

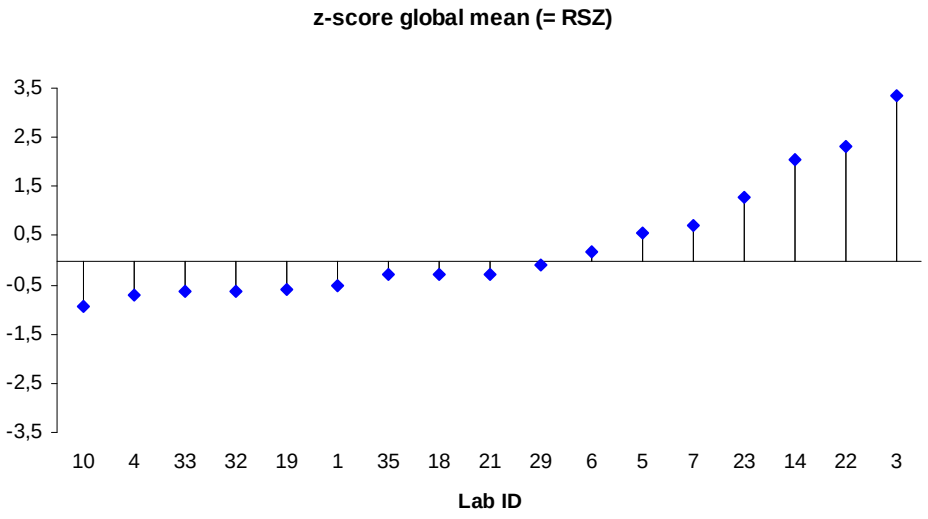
Of note also the fact that the out-of-range z-scores are condensed on the 0.25% Fish III, i.e. the sample with the smallest concentration of adulteration. Results from labs 3, 14 and 22 correspond to the first observation of potential outliers when considering that all maximum values from the reported estimations originate from those labs (table 10). We searched at determining if there could be a possible correlation between z-scores and the number of analysis a year which the participants reported to realised – this information was to find through the inquiry form that was send concomitantly with the invitation letter to the present study. It appears that no correlation at all could be found.

In order to get further insights on the very high values of the between laboratory variation, the distribution of the 168 obtained z-scores was studied.



Graph 2: Distribution of z-scores.

From the histogram of z-scores (graph 2) we see that the distribution is clearly shifted to the left and is not typical of a Gaussian one. It actually looks more like a Poisson distribution which is classical for counting results [9]. This skewing of the distribution partly clarifies the very poor reproducibility: it could be related to some few strong lab biases responsible for the high RSR_R. The z-score global mean or rescaled sum of z-score (RSZ) provides evidence for this (graph 3).



Graph 3: z-Score global mean.

The RSZ for all labs indicates a few numbers of high positive z-scores compared to a general trend of numerous low negative z-scores (graph 3). This partly explains for the skewing of the distribution (strong positive bias for labs 3, 22, 14, 23). But the skewing is also linked to the fact that a symmetrical normal distribution cannot be expected from this type of study. Actually if the distribution was normal, then two times the standard deviation which is typical for a 95% range would include negative results for the estimated values of constituents of fish origin which is not possible.

Whereas the shape of distribution can be explained, the exact cause of biases, or possible factors impacting the measurement uncertainty, still has to be investigated. Those factors can only be directed by the formula terms: *S*, *W*, *d* and *f*.

4.3.3. Respective influence of formula terms on quantification

4.3.3.1. Sedimentation

The first possible cause of uncertainty to consider is the sedimentation process itself as counting of particles can only be realized on the sediment containing the bones and fish scales particles. We calculated from the data of the report form the percentage of sediment obtained for each sample by following formula:

$$\% = \frac{S}{W} \times 100 .$$

The results are expressed in table 13.

		0.25% Fish III		0.5% Fish III		1% Fish III		1% Fish I		1.5% Fish III		
weight of sample (g)	Lab ID	D1	D2	C1	C2	F1	F2	H1	H2	E1	E2	mean/lab
10	**1**	1.10%	1.24%	1.28%	1.31%	0.91%	1.50%	1.14%	1.18%	1.53%	1.59%	1.28%
5	**3**	1.64%	1.86%	1.32%	1.65%	1.33%	1.21%	1.17%	1.68%	1.34%	1.60%	1.48%
5	**4**	**0.40%**	0.60%	**0.20%**	0.40%	**0.60%**	0.80%	**0.40%**	0.60%	**0.60%**	0.60%	0.52%
5	**5**	0.42%	0.64%	0.72%	1.12%	0.64%	0.60%	0.56%	0.64%	0.92%	0.76%	0.70%
5	**6**	1.54%	1.18%	1.74%	1.46%	1.48%	1.48%	1.48%	1.44%	1.66%	1.64%	1.51%
5	**7**	1.74%	1.58%	1.26%	1.79%	1.78%	1.35%	1.76%	1.21%	2.05%	1.21%	1.57%
5	**10**	2.46%	**3.56%**	**3.64%**	1.94%	1.24%	3.56%	**5.48%**	3.16%	**4.34%**	4.00%	3.34%
5	**14**	1.15%	1.53%	1.29%	1.20%	1.14%	1.26%	1.47%	1.45%	1.26%	1.30%	1.30%
5	**18**	1.54%	1.28%	0.94%	1.30%	1.96%	1.28%	1.22%	1.20%	1.40%	1.88%	1.40%
10	**19**	1.41%	1.75%	1.25%	1.08%	1.17%	1.01%	1.32%	1.40%	1.51%	1.15%	1.30%
10	**21**	1.30%	1.50%	1.10%	1.00%	1.20%	1.20%	1.20%	1.00%	1.10%	1.50%	1.21%
10	**22**	0.81%	0.84%	0.85%	0.70%	0.77%	0.66%	0.91%	0.60%	0.88%	0.86%	0.79%
5	**23**	1.66%	1.42%	1.26%	1.56%	1.62%	1.52%	1.24%	1.64%	1.70%	1.70%	1.53%
10	**29**	1.20%	1.20%	1.10%	1.10%	1.00%	1.30%	1.10%	1.20%	1.30%	1.20%	1.17%
10	**32**	1.09%	0.89%	0.88%	0.70%	0.86%	**10.03%**	1.06%	1.08%	1.00%	0.95%	1.85%
5	**33**	1.20%	1.22%	1.34%	1.16%	1.28%	1.42%	1.18%	1.00%	1.22%	1.34%	1.24%
5	**35**	1.11%	1.31%	1.10%	1.18%	1.08%	0.94%	1.00%	0.97%	0.89%	1.74%	1.13%

mean	1.33%	1.23%	1.50%	1.33%	1.46%
STD	0,58%	0,56%	1,60%	0,87%	0,78%
min	0.40%	0.20%	0.60%	0.40%	0.60%
max	3.56%	3.64%	10.03%	5.48%	4.34%

Table 13: Percentage of sedimentation. The upper part table gives the results expressed in %. The lower part table provides some basic statistics (Legend: STD = standard deviation, min = minimum value, max = maximum value). The column on the right gives the global mean % of sediment obtained per lab through all samples.

The standard deviations observed on the percentage of sediment obtained for each material are high (see lower part of table 13). The between-laboratory variation is thus relatively high. This does not seem to hold true for the repeatability. It appears also that *W*, the weight of the sample material taken for the sedimentation (often 10 or 5 g according to EC 126/2003 directive), does not impact on the percentage of sediment obtained. Nevertheless some labs present some unexpected results:

- Lab 10 (blue rectangle in table 13) is obtaining repeatedly abnormal high % of sediment (2 to 5 times the mean percentages for each sample material). This lab, although presenting the highest percentage of sedimentation has the majority of minima (table 10).

- Labs 4 and 5 (red rectangle in table 13) obtained repeatedly low % of sediment. Investigations on the follow up of the protocol of sedimentation are recommended for those labs. Nevertheless those performed correct.
- Lab 32 reported a sediment of 10.03% for only one duplicate of the 1% Fish III. An error of transcription in the report form could account for an explanation.

Thus, on a very few exception, the sedimentation process seemingly does not influence so much the quantification.

4.3.3.2. *d* Factor

The correction factor for the estimated portion of fish bones and scale fragments in the sediment might influence the results has it must be calculated by each microscopist on each sample. It depends itself on the lab procedure for animal particles counting in the sediment.

The EC 126/2003 directive does not mention how to evaluate this factor. For that reason, the CRL-AP asked each participant to define precisely this factor and to provide a concrete example of its calculation based on a sample replicate from the study. Results for this part of the study have been presented in the preliminary remarks from the quantitative analysis (cf. point 4.3.1 and table 9): they are alarming, only 36% of the total participants were able to answer.

Thus the scarce data collected by this study did not allow investigating further on the possible influence of this factor which is critical as it originates straight from the microscopic observations and is a counting result reflecting the proficiency of each microscopist to discriminate – in the present case- fish particles from other particles in the sediment. Considering this, we have to highlight the fact that lab 1 realized all its quantifications with correct results but admitted not using the *d* factor as it is however required in the directive.

It is critical in the future to know how precisely particles are being counted and how many particles are counted for each lab and samples of similar interlaboratory studies.

4.3.3.3. *f* Factor

For the present study this correction factor for the proportion of bones/scales in the constituents of fish origin ranges normally in the order of 10 to 20 % as indicated by the EC 126/2003 directive. This factor is depending from the category and origin of the fishmeal. It is thus impossible for a lab receiving a blind sample material for analysis to know the precise value of this *f* factor. It was the case for the present study. Setting the *f* factor to a value is consequently an empirical choice of the analyst. This has been observed to through this test.

In general participating labs usually selected one single value of *f* factor (table 14 next page) and applied it to all samples for quantification, however (table 14).

- Lab 4 assigned for each blind sample replicate a single unique *f* factor. Moreover it appears from its result sheet that the values attributed varied in between replicates of a same material (e.g. for 0.5% Fish III first replicate: 7.69% - second replicate: 28.57%).
- Lab 32 used two values of *f* factor: 10% (for 5 samples) and 15% (for 5 samples)

lab ID	*f* factor
1	7%
3	11%
4	from 5.88% to 50%
5	15%
6	15%
7	10%
10	7%
14	7%
18	15%
19	15%
21	20%
22	5%
23	20%
29	not used
32	10% and 15 %
33	10%
35	15%

Table 14 : *f* Factors.

From all quantifications that were realised we calculated the global average *f* factor used through all analysis: 11.9%. This value or *f* is very close to the actual *f* factors which were 10.8% for Fish III and 11.8% for Fish I meals (cf. Annex 5).

Some labs (cells in red) also used a lower *f* factor inconsistent with values indicated by the EC 126/2003 directive: labs 14 and 22 have a few performing z-score global mean, while labs 1 and 10 have correct ones.

Finally the specific case of lab 29 (cell in blue in table 14) is worth mentioning. This lab indicated explicitly that it did not use any *f* factor, or in other terms chooses a default value of 100% for *f.* As the *f* factor is a dividing term in the formula it would logically imply that this lab would have the lowest estimated values of constituents of fish origin. It was not the case. On the contrary it is the lab performing the best z-scores (table 12 and graph 3).

In order to check the impact of the *f* factor, we performed the following test: we converted all results from the quantification to that obtained with a *f* factor set to 100% (table 15). The following results were obtained:

	Average	STD	s_r	RSD_r	s_R	RSD_R
0.25% Fish III	0.04 (± 0.02)	0.01	0.01	15	0.04	101
0.5% Fish III	0.08 (± 0.03)	0.02	0.01	17	0.06	81
1% Fish III	0.14 (± 0.05)	0.02	0.03	20	0.10	68
1% Fish I	0.17 (± 0.07)	0.03	0.04	23	0.14	80
1.5% Fish III	0.21 (± 0.08)	0.04	0.02	11	0.16	74

All data are expressed in percentage (%)

Table 15: Robust statistics results (with f set to 100%). (Legend: see table 11).

As expected the averages are logically smaller. Interesting is that using a same *f* factor for all measurements impacts slightly positively on the within-laboratory variability, RSD_r, which is now between 11% and 23% (instead of 12% - 30%). This reflects probably a smoothing effect from the few labs that were

using different *f* factors through their quantifications. Nonetheless using a unique value of *f* factor the reproducibility, RSD_R, remains almost unchanged i.e. not satisfactory.

As a conclusion it comes out that this factor, which cannot be calculated from a sample of unknown composition and origin, does not seems to influence that much the results that can be obtained. As the reproducibility is still very poor when using a same *f* factor, it becomes evident that the *d* factor remains the main likely candidate term from which the high variability between laboratories originates.

4.3.4. Ability of the method for discriminating different contamination levels

Evaluation of the official method as stated in EC 126/2003 directive includes also, independently of the over or underestimation analysis, the ability to discriminate between different levels or percentages of adulteration. This is especially important in order to investigate if the method is reliable for any threshold handling, knowing that the repeatability inside a lab has been demonstrated to be satisfying.

Thus we assessed this ability of the quantification method by comparing the value of the step ranges for each lab between and over the different percentages of fish meal adulteration of the samples (table 15).

	0.25% Fish III	**x 2**	**0.5% Fish III**	**x 2**	**1% Fish III**	**x 1.5**	**1.5% Fish III**
Lab ID	mean		mean		mean		mean
1	0.08%	*2.6*	0.21%	*3.0*	0.62%	*1.7*	1.03%
3	2.71%	*1.0* =	2.78%	*1.1* =	3.15%	*1.6*	5.19%
4	0.04%	***6.0*** ↑↑	0.21%	*2.8*	0.60%	*1.1* =	0.65%
5	0.95%	*1.3*	1.24%	*0.9* ↓	1.16%	*1.6*	1.91%
6	0.50%	*1.0* =	0.50%	*2.0*	1.00%	*2.0*	2.00%
7	0.44%	*2.8*	1.24%	*1.1* =	1.42%	*1.9*	2.74%
10	0.02%	*2.8*	0.06%	*1.0* =	0.06%	*0.9* ↓	0.05%
14	0.95%	*1.8*	1.75%	*2.0*	3.45%	*1.3*	4.60%
18	0.19%	*2.5*	0.48%	*2.2*	1.04%	*1.1* =	1.14%
19	0.14%	*1.7*	0.24%	*2.2*	0.52%	*1.4*	0.71%
21	0.23%	*1.4*	0.31%	*2.8*	0.85%	*2.1*	1.77%
22	1.65%	*0.9* ↓	1.55%	*2.3*	3.60%	*1.5*	5.25%
23	0.55%	*2.3*	1.25%	*2.3*	2.90%	*1.8*	5.10%
29	0.20%	*2.5*	0.50%	*3.0*	1.50%	*0.8* ↓	1.25%
32	0.12%	*2.5*	0.29%	*1.4*	0.40%	*1.1* =	0.44%
33	0.12%	*2.3*	0.27%	*1.3*	0.36%	*1.3*	0.48%
35	0.15%	***3.3*** ↑	0.50%	*2.0*	1.00%	*1.5*	1.50%
mean step range		***x 2.3***		***x 2.0***		***x 1.5***	

All decreases are indicated by red arrows pointing down. Increases higher than 1/3 of the theoretical values are indicated by red arrows pointing up. Status quo are indicated by =

Table 15: Step range analysis.

The calculated mean step ranges globally fit the theoretical ones. Some few labs nevertheless presented some less satisfying results (blue rectangle):

- Lab 4 had a step range of 6 between 0.25% and 0.5% Fish III.
- Lab 3 reported systematically overestimations of percentages and was unable to achieve satisfying progression in step ranges.

The variability around the step ranges, expressed by the standard deviation (results not shown) increased contrariwise the increase in percentage of adulteration. In order terms the reliability of the method diminished as we approach the limit of detection – or better expressed the limit of quantification. This limit for the quantitative analyse, or reliability, might therefore be stated around 0.25% of contamination by fish meal.

5. Conclusions

Two separated conclusions have to be drawn with respect to the two goals of the CRL-AP Interlaboratory Study 2006.

Concerning the qualitative part of the study, results are good and encouraging: 77% of participants performed above 95% consolidated accuracy. This result is positive taking into consideration the high number of participating NRLs. The individual global performance is excellent (= full set of correct answers) for twelve labs on twenty-two or in other words for 55% of the participants. Five participants had good performances, with one single false positive for labs or one false negative in 0.1% MBM + 5% Fish I. It is the best performance obtained since interlaboratory studies using the method described in Directive 126/2003 are organized within the European Union. From the detailed analysis of the lab performances we can be confident on a future improvement of the proficiency of other NRLs, this as the present study demonstrates that only a very few are underperforming severely. The EC 126/2003 directive as it stands now is believed to be suitable for further improvement of the detection of animal proteins in feedingstuffs by way of the microscopic method.

Conclusions on investigations on the robustness of the EC 126/2003 directive regarding the quantitative determination of animal constituents in feedingstuffs are somewhat more temperate. The present study revealed that one NRL on three is unable to realise quantitative analysis by following of the current directive instructions. Among the NRLs that were able to perform quantitative analysis, the study gives evidence of such a high variability of results between NRLs that one should conclude that the method as such is not reliable for the purpose of quantification. Therefore we suggest improvements of the quantitative protocol of the EC 126/2003 directive. The enhancements needed concern mainly further detailed information and precise instructions to be implemented on the actual proposed formula. This held true because the present study demonstrated that the variability within labs, at least for the batch of samples analysed, is nonetheless acceptable.

Acknowledgment

We are especially grateful to the whole technical staff of the CRL-AP and CRA-W for their help in the sample preparation and the homogeneity study : T. Buhigiro, I. Fissiaux, A. Marien, F. Rwagasore, C. Teller, R. Van Belleghem and M. Wiedig.

6. References

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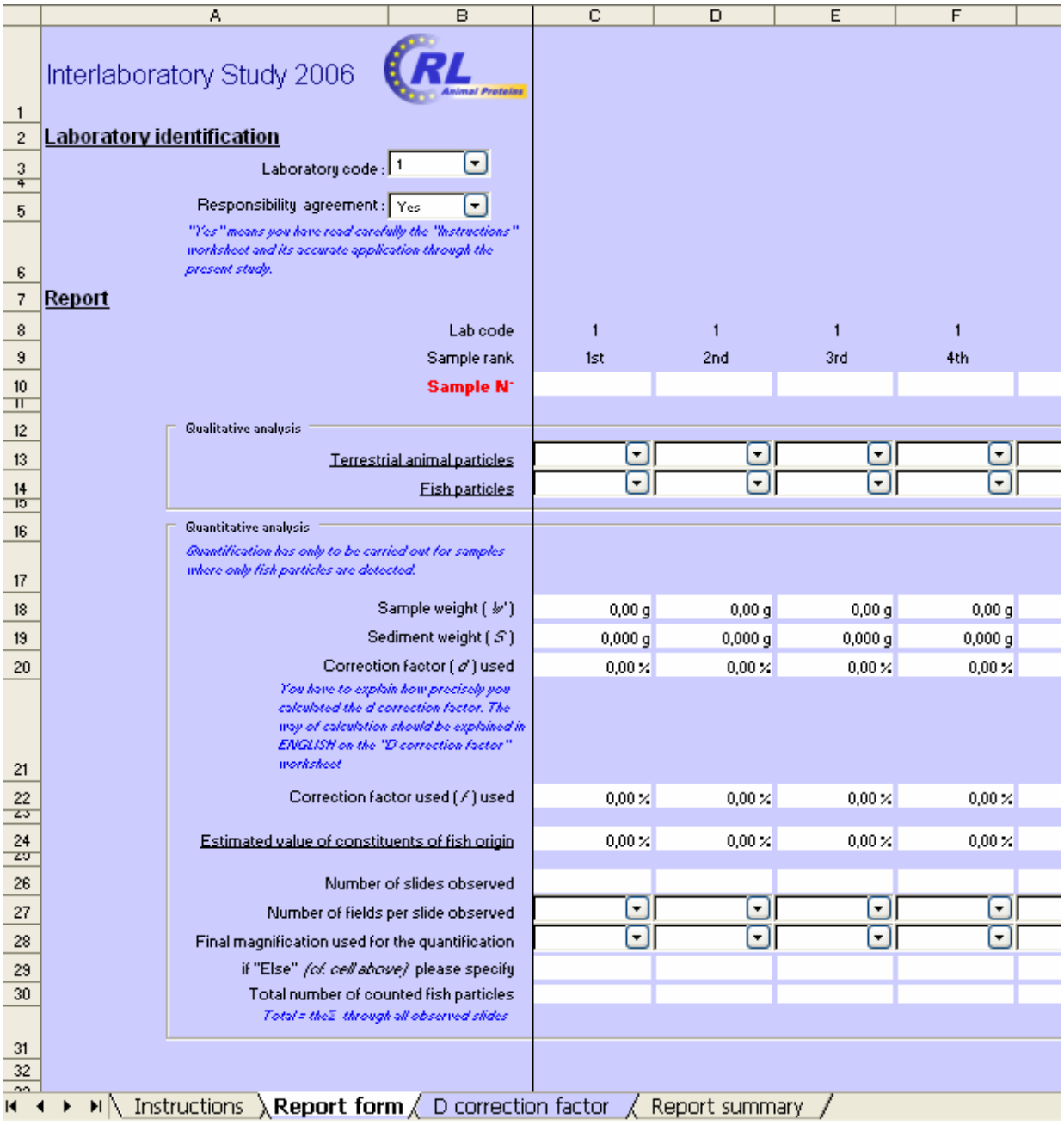
Annex 1

List of participating NRLs

Country	Institute Name
Austria	Austrian Agency for Health and Food Safety
Belgium	Federal Agency for the Safety of the Food Chain
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 Center for the Surveillance and Monitoring of Animal Feed
Latvia	National Diagnostic Centre of Food and veterinary Service
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
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, Dept of Animal Feed
United Kingdom	Veterinary Laboratories Agency

Annex 2

Excel result report form.



Interlaboratory Study 2006

Laboratory identification

Laboratory code : 1

Responsibility agreement : Yes

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

Report

	1	1	1	1
Lab code				
Sample rank	1st	2nd	3rd	4th
Sample N°				

Qualitative analysis

Terrestrial animal particles				
Fish particles				

Quantitative analysis

Quantification has only to be carried out for samples where only fish particles are detected.

Sample weight (*w*)	0,00 g	0,00 g	0,00 g	0,00 g
Sediment weight (*S*)	0,000 g	0,000 g	0,000 g	0,000 g
Correction factor (*d*) used	0,00 %	0,00 %	0,00 %	0,00 %
You have to explain how precisely you calculated the d correction factor. The way of calculation should be explained in ENGLISH on the "D correction factor" worksheet				
Correction factor used (*f*) used	0,00 %	0,00 %	0,00 %	0,00 %
Estimated value of constituents of fish origin	0,00 %	0,00 %	0,00 %	0,00 %
Number of slides observed				
Number of fields per slide observed				
Final magnification used for the quantification				
if "Else" *(cf. cell above)* please specify				
Total number of counted fish particles *Total = the Σ through all observed slides*				

Instructions | **Report form** | D correction factor | Report summary

Annex 3

PCR table from the homogeneity study.

(Realised on the raw fraction)

Sample number		Content	Fish		
			Mean Ct	Min Ct	Max Ct
CRL 151	DQ-06-0815 -1179	0.5% Fish III	32.78	32.19	33.36
CRL 152	DQ-06-0815 -1403		29.42	26.8	32.03
			31.1	**26.8**	**33.36**
CRL 153	DQ-06-0816 -942	0.25% Fish III	35.24	34.47	36
CRL 154	DQ-06-0816 -1124		34.6	34.5	34.69
			34.92	**34.47**	**36**
CRL 155	DQ-06-0817 -943	1.5% Fish III	29.21	28.51	29.91
CRL 156	DQ-06-0817 -1013		31.08	30.59	31.57
			30.15	**28.51**	**31.57**
CRL 157	DQ-06-0818 -426	1% Fish III	29.95	29.54	30.36
CRL 158	DQ-06-0818 -1392		27.94	27.38	28.5
			28.95	**27.38**	**30.36**
CRL 159	DQ-06-0968 -92	1% Fish I	32.64	32.61	32.67
CRL 160	DQ-06-0968 -218		32.9	31.72	34.08
			32.77	**31.72**	**34.08**

Mean CT = mean cycle threshold

NIR microscopy table from the homogeneity study.

(Realised on the raw fraction)

Material	# Analysed particles	# Fish particles	Mean # fish particles
0.25% Fish III	947	0	0
	1222	0	
	1318	0	
0.5% Fish III	926	0	0.67
	1202	1	
	1184	1	
1% Fish III	1049	3	3
	1127	3	
	1265	3	
1% Fish I	1133	3	5
	1087	7	
	1060	5	
1.5% Fish III	1100	6	7
	792	5	
	1227	10	

Annex 4

Details of homogeneity study for materials spiked with Fish III.

(*f* factor used = 11%)

0.25 % Fish III					0.5 % Fish III				
n	**Sed. %**	**Fish content %**	**# Fish part.**	**# Total part.**	**n**	**Sed. %**	**Fish content %**	**# Fish part.**	**# Total part**
1	0.81	0.28	33	862	1	0.75	0.68	56	565
2	0.82	0.28	32	866	2	0.85	0.69	61	684
3	0.83	0.48	66	1042	3	0.82	0.66	40	454
4	0.84	0.51	61	906	4	0.81	0.57	47	602
5	0.76	0.32	42	904	5	0.91	0.63	53	699
Mean %	0.82	0.38			0.83	0.64			
STD %	0.03	0.11			0.06	0.05			

1% Fish III					1.5 % Fish III				
n	**Sed. %**	**Fish content %**	**# Fish part.**	**# Total part**	**n**	**Sed. %**	**Fish content %**	**# Fish part.**	**# Total part**
1	0.83	1.03	93	684	1	0.84	1.63	177	828
2	0.81	1.44	93	482	2	0.89	1.52	204	1090
3	0.82	0.97	121	932	3	0.84	1.67	156	709
4	0.68	1.23	109	555	4	0.87	1.23	150	965
5	0.79	0.91	103	801	5	0.86	1.78	210	938
Mean %	0.79	1.12			0.86	1.56			
STD %	0.06	0.22			0.02	0.21			

Annex 5

Calculated *f* factors for the 2 fish meals used in the study.

Fish III (DQ/06/0980)		
Sediment Nr.	Sample weight (at 0.1g)	Sediment weight (at 0.001g)
1502	3.0	0.327
1503	3.0	0.324
1504	3.0	0.326
1505	3.0	0.324
1506	3.0	0.321
Total	15.0	1.622

***f* factor [%] = (total weight sediment / total weight sample)*100**	**10.8%**

Fish I (DQ/06/1000)		
Sediment Nr.	Sample weight (at 0.1g)	Sediment weight (at 0.001g)
1508	3.0	0.346
1509	3.0	0.353
1510	3.0	0.355
1511	3.0	0.351
1512	3.0	0.358
Total	15.0	1.763

***f* factor [%] = (total weight sediment / total weight sample)*100**	**11.8%**

Annex 6

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

Laboratory identification code : 1

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	F	Not present	Present	10	0,091	0,00%	7,00%	0,40%	2	Whole slide	50 x	0
2	E	Not present	Present	10	0,153	0,00%	7,00%	1,03%	2	Whole slide	50 x	0
3	C	Not present	Present	10	0,128	0,00%	7,00%	0,16%	2	Whole slide	50 x	0
4	A	Not present	Not present	10	0,135	0,00%	0,00%	0,00%	2	Whole slide	50 x	0
5	E	Not present	Present	10	0,159	0,00%	7,00%	1,03%	2	Whole slide	50 x	0
6	G	Present	Present	10	0,163	0,00%	0,00%	0,00%	3	Whole slide	50 x	0
7	H	Not present	Present	10	0,114	0,00%	7,00%	1,18%	2	Whole slide	50 x	0
8	D	Not present	Present	10	0,11	0,00%	7,00%	0,09%	2	Whole slide	50 x	0
9	A	Not present	Not present	10	0,131	0,00%	0,00%	0,00%	2	Whole slide	50 x	0
10	G	Present	Present	10	0,185	0,00%	0,00%	0,00%	3	Whole slide	50 x	0
11	B	Present	Not present	10	0,134	0,00%	0,00%	0,00%	2	Whole slide	50 x	0
12	F	Not present	Present	10	0,15	0,00%	7,00%	0,84%	3	Whole slide	50 x	0
13	B	Present	Not present	10	0,126	0,00%	0,00%	0,00%	3	Whole slide	50 x	0
14	G	Present	Present	10	0,19	0,00%	0,00%	0,00%	3	Whole slide	50 x	0
15	A	Not present	Not present	10	0,134	0,00%	0,00%	0,00%	3	Whole slide	50 x	0
16	C	Not present	Present	10	0,131	0,00%	7,00%	0,25%	3	Whole slide	50 x	0
17	D	Not present	Present	10	0,124	0,00%	7,00%	0,07%	3	Whole slide	50 x	0
18	H	Not present	Present	10	0,118	0,00%	7,00%	0,52%	2	Whole slide	50 x	0
19	B	Present	Not present	10	0,09	0,00%	0,00%	0,00%	2	Whole slide	50 x	0

Laboratory identification code : 2

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	E	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
2	D	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
3	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
4	A	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
5	C	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
6	C	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
7	F	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
8	G	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
9	H	Not present	Present	10	0,07	14,80%	15,00%	0,69%	4	10	100 x	0
10	E	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
11	F	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
12	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
13	A	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
14	G	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
15	D	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
16	B	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
17	H	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
18	A	Not present	Present	0	0	0,00%	0,00%	0,00%	0			0
19	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0

Laboratory identification code : 3

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	F	Not present	Present	5,118	0,068	26,73%	11,00%	3,23%	3	5	100 x	0
2	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
3	A	Not present	Present	5,01	0,049	0,57%	11,00%	0,05%	3	5	100 x	0
4	C	Not present	Present	5,005	0,066	19,43%	11,00%	2,33%	3	5	100 x	0
5	F	Not present	Present	5,053	0,061	28,02%	11,00%	3,08%	3	5	100 x	0
6	E	Not present	Present	5,003	0,067	38,98%	11,00%	4,75%	3	5	100 x	0
7	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
8	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
9	B	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
10	E	Not present	Present	5,006	0,0803	38,64%	11,00%	5,64%	3	5	100 x	0
11	H	Not present	Present	5,0204	0,0589	35,51%	11,00%	3,79%	3	5	100 x	0
12	H	Not present	Present	5,0415	0,0845	33,97%	11,00%	5,18%	3	5	100 x	0
13	A	Not present	Present	5,0826	0,0829	0,52%	11,00%	0,08%	3	5	100 x	0
14	C	Not present	Present	5,0581	0,0837	21,42%	11,00%	3,22%	3	5	100 x	0
15	D	Not present	Present	5,0495	0,0826	18,38%	11,00%	2,73%	3	5	100 x	0
16	B	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
17	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
18	B	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
19	D	Not present	Present	5,2351	0,0972	15,93%	11,00%	2,69%	3	5	100 x	0

Laboratory identification code : **4**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	E	Not present	Present	5,02	0,03	9,20%	16,67%	0,33%	3	5	100 x	26
2	F	Not present	Present	5,01	0,03	14,94%	8,57%	1,04%	3	5	100 x	42
3	E	Not present	Present	5,04	0,03	9,46%	5,88%	0,96%	3	5	100 x	17
4	D	Not present	Present	5,01	0,02	1,80%	16,67%	0,04%	3	5	100 x	3
5	B	Present	Not present	0	0	0,00%	0,00%	0,00%	3	5	100 x	0
6	H	Not present	Present	5,03	0,02	3,76%	50,00%	0,03%	3	5	100 x	12
7	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	3	5	100 x	0
8	G	Present	Present	0	0	0,00%	0,00%	0,00%	3	5	100 x	80
9	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	3	5	100 x	0
10	D	Not present	Present	5,02	0,03	1,99%	45,46%	0,03%	3	5	100 x	13
11	C	Not present	Present	5,02	0,01	14,29%	28,57%	0,10%	3	5	100 x	14
12	C	Not present	Present	5,01	0,02	6,20%	7,69%	0,32%	3	5	100 x	8
13	F	Not present	Present	5,01	0,04	5,25%	27,27%	0,15%	3	5	100 x	19
14	H	Not present	Present	5,02	0,03	13,64%	17,39%	0,47%	3	5	100 x	37
15	G	Present	Present	0	0	0,00%	0,00%	0,00%	3	5	100 x	113
16	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	3	5	100 x	0
17	B	Present	Not present	0	0	0,00%	0,00%	0,00%	3	5	100 x	0
18	G	Present	Present	0	0	0,00%	0,00%	0,00%	3	5	100 x	108
19	B	Present	Not present	0	0	0,00%	0,00%	0,00%	3	5	100 x	0

Laboratory identification code : **5**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	E	Not present	Present	5.0001 g	0.046 g	32,65%	15,00%	2,01%	3	6	50 x	77
2	F	Not present	Present	5.4866 g	0.035 g	31,42%	15,00%	1,34%	3	6	50 x	90
3	H	Not present	Present	5.0065 g	0.028 g	34,98%	15,00%	1,29%	3	6	50 x	104
4	D	Not present	Present	5.0035 g	0.021 g	35,55%	15,00%	1,00%	3	6	50 x	54
5	B	Present	Not present	0	0	0,00%	0,00%	0,00%	3	6	50 x	0
6	G	Present	Present	5.0927 g	0.041 g	61,64%	15,00%	3,30%	3	6	50 x	276
7	H	Not present	Present	5.0034 g	0.032 g	25,26%	15,00%	1,07%	3	6	50 x	83
8	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	3	6	50 x	0
9	B	Present	Not present	0	0	0,00%	0,00%	0,00%	3	6	50 x	0
10	E	Not present	Present	5.0279 g	0.038 g	35,60%	15,00%	1,81%	3	6	50 x	136
11	D	Not present	Present	5.0025 g	0.032 g	21,06%	15,00%	0,90%	3	6	50 x	34
12	A	Not present	Not present	0.00 g	0	0,00%	0,00%	0,00%	3	6	50 x	0
13	C	Not present	Present	5.0024 g	0.036 g	26,14%	15,00%	1,25%	3	6	50 x	50
14	F	Not present	Present	5.0002 g	0.030 g	20,57%	15,00%	0,98%	3	6	50 x	46
15	G	Not present	Present	5.0063 g	0,053	34,82%	15,00%	2,46%	3	6	50 x	48
16	C	Not present	Present	5.0005 g	0.056 g	16,57%	15,00%	1,23%	3	6	50 x	21
17	G	Not present	Present	5.0040 g	0.049 g	37,34%	15,00%	2,43%	3	6	50 x	99
18	B	Present	Not present	0	0	0,00%	0,00%	0,00%	3	6	50 x	0
19	A	Not present	Present	5.0044 g	0,04	NC, 3 partic	NC	NC	3	6	50 x	3

Laboratory identification code : **6**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	E	Not present	Present	5	0,083	20,00%	15,00%	2,00%	1	Whole slide	50 x	400
2	C	Not present	Present	5	0,087	5,00%	15,00%	0,50%	1	Whole slide	50 x	100
3	G	Present	Present	5	0,119	75,00%	15,00%	10,00%	1	Whole slide	50 x	1500
4	A	Not present	Not present	5	0,077	0,00%	0,00%	0,00%	1	Whole slide	50 x	0
5	F	Not present	Present	5	0,074	10,00%	15,00%	1,00%	1	Whole slide	50 x	200
6	B	Present	Not present	5	0,069	0,00%	0,00%	0,00%	1	Whole slide	50 x	0
7	G	Present	Present	5	0,098	75,00%	15,00%	10,00%	1	Whole slide	50 x	1500
8	H	Not present	Present	5	0,074	20,00%	15,00%	2,00%	1	Whole slide	50 x	400
9	E	Not present	Present	5	0,082	20,00%	15,00%	2,00%	1	Whole slide	50 x	400
10	F	Not present	Present	5	0,074	10,00%	15,00%	1,00%	1	Whole slide	50 x	200
11	D	Not present	Present	5	0,077	5,00%	15,00%	0,50%	1	Whole slide	50 x	100
12	B	Present	Not present	5	0,071	0,00%	0,00%	0,00%	1	Whole slide	50 x	0
13	D	Not present	Present	5	0,059	5,00%	15,00%	0,50%	1	Whole slide	50 x	100
14	A	Not present	Not present	5	0,07	0,00%	0,00%	0,00%	1	Whole slide	50 x	0
15	C	Not present	Present	5	0,073	5,00%	15,00%	0,50%	1	Whole slide	50 x	100
16	B	Present	Not present	5	0,084	0,00%	0,00%	0,00%	1	Whole slide	50 x	0
17	H	Not present	Present	5	0,072	20,00%	15,00%	2,00%	1	Whole slide	50 x	400
18	G	Present	Present	5	0,116	75,00%	15,00%	10,00%	1	Whole slide	50 x	1500
19	A	Not present	Not present	5	0,08	0,00%	0,00%	0,00%	1	Whole slide	50 x	0

Laboratory identification code : **7**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	E	Not present	Present	5,0865	0,1041	12,72%	10,00%	2,60%	3	6	100 x	0
2	A	Not present	Not present	5,0226	0,088	0,00%	0,00%	0,00%	0			0
3	C	Not present	Present	5,1117	0,0646	5,11%	10,00%	0,65%	3	6	100 x	0
4	F	Not present	Present	5,0271	0,0897	6,39%	10,00%	1,14%	3	6	100 x	0
5	G	Present	Present	5,0653	0,163	35,28%	10,00%	11,35%	3	6	100 x	0
6	B	Present	Not present	5,0829	0,109	0,00%	0,00%	0,00%	0			0
7	E	Not present	Present	5,0196	0,0607	23,78%	10,00%	2,88%	3	6	100 x	0
8	D	Not present	Present	5,343	0,0928	3,11%	10,00%	0,54%	3	6	100 x	0
9	G	Present	Present	5,546	0,1353	41,40%	10,00%	10,10%	3	6	100 x	0
10	H	Not present	Present	6,022	0,1062	19,11%	10,00%	3,37%	3	6	100 x	0
11	A	Not present	Not present	5,126	0,0741	0,00%	0,00%	0,00%	0			0
12	D	Not present	Present	5,208	0,0825	2,17%	10,00%	0,34%	3	6	100 x	0
13	H	Not present	Present	5,0279	0,0608	22,33%	10,00%	2,70%	3	6	100 x	0
14	B	Present	Not present	5,3316	0,11	0,00%	0,00%	0,00%	0			0
15	F	Not present	Present	5,0211	0,0676	12,56%	10,00%	1,69%	3	6	100 x	0
16	C	Not present	Present	5,1892	0,093	11,16%	10,00%	1,82%	3	6	100 x	0
17	G	Present	Present	5,3541	0,131	35,00%	10,00%	8,55%	3	6	100 x	0
18	A	Not present	Not present	10,0676	0,13	0,00%	0,00%	0,00%	0			0
19	B	Present	Not present	5,3332	0,111	0,00%	0,00%	0,00%	0			0

Laboratory identification code : **9**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	H	Not present	Present	10	0,182	0,00%	0,00%	0,00%	0			0
2	A	Not present	Not present	10	0,168	0,00%	0,00%	0,00%	0			0
3	A	Not present	Not present	10	0,143	0,00%	0,00%	0,00%	0			0
4	C	Not present	Present	10	0,146	0,00%	0,00%	0,00%	0			0
5	A	Not present	Not present	10	0,14	0,00%	0,00%	0,00%	0			0
6	E	Not present	Present	10	0,151	0,00%	0,00%	0,00%	0			0
7	B	Present	Not present	10	0,123	0,00%	0,00%	0,00%	0			0
8	C	Not present	Present	10	0,14	0,00%	0,00%	0,00%	0			0
9	F	Not present	Present	10	0,156	0,00%	0,00%	0,00%	0			0
10	H	Not present	Present	10	0,144	0,00%	0,00%	0,00%	0			0
11	G	Present	Present	10	0,217	0,00%	0,00%	0,00%	0			0
12	B	Present	Not present	10	0,139	0,00%	0,00%	0,00%	0			0
13	D	Not present	Present	10	0,138	0,00%	0,00%	0,00%	0			0
14	F	Not present	Present	10	0,149	0,00%	0,00%	0,00%	0			0
15	G	Present	Present	10	0,192	0,00%	0,00%	0,00%	0			0
16	D	Not present	Present	10	0,195	0,00%	0,00%	0,00%	0			0
17	B	Present	Not present	10	0,153	0,00%	0,00%	0,00%	0			0
18	E	Not present	Present	10	0,183	0,00%	0,00%	0,00%	0			0
19	G	Present	Present	10	0,168	0,00%	0,00%	0,00%	0			0

Laboratory identification code : **10**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
2	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
3	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
4	B	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
5	H	Not present	Present	5	0,274	0,11%	7,00%	0,09%	1	Whole slide	50 x	19
6	D	Not present	Present	5	0,123	0,04%	7,00%	0,01%	1	Whole slide	50 x	7
7	E	Not present	Present	5	0,217	0,06%	7,00%	0,04%	1	Whole slide	50 x	10
8	F	Not present	Present	5	0,062	0,13%	7,00%	0,02%	1	Whole slide	50 x	22
9	H	Not present	Present	5	0,158	0,12%	7,00%	0,05%	1	Whole slide	50 x	20
10	E	Not present	Present	5	0,2	0,10%	7,00%	0,06%	1	Whole slide	50 x	17
11	C	Not present	Present	5	0,182	0,11%	7,00%	0,06%	1	Whole slide	50 x	18
12	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
13	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
14	F	Not present	Present	5	0,178	0,18%	7,00%	0,09%	1	Whole slide	50 x	30
15	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
16	D	Not present	Present	5	0,178	0,05%	7,00%	0,03%	1	Whole slide	50 x	8
17	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
18	C	Not present	Present	5	0,097	0,19%	7,00%	0,05%	1	Whole slide	50 x	33
19	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0

Laboratory identification code : **14**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
2	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
3	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
4	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
5	D	Not present	Present	5	0,0574	5,00%	7,00%	0,80%	2	Whole slide	100 x	0
6	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
7	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
8	D	Not present	Present	5	0,0764	5,00%	7,00%	1,10%	2	Whole slide	100 x	0
9	E	Not present	Present	5	0,0628	25,00%	7,00%	4,50%	2	Whole slide	100 x	0
10	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
11	C	Not present	Present	5	0,0644	10,00%	7,00%	1,80%	2	Whole slide	100 x	0
12	F	Not present	Present	5	0,0572	20,00%	7,00%	3,30%	2	Whole slide	100 x	0
13	E	Not present	Present	5	0,0651	25,00%	7,00%	4,70%	2	Whole slide	100 x	0
14	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
15	C	Not present	Present	5	0,0601	10,00%	7,00%	1,70%	2	Whole slide	100 x	0
16	F	Not present	Present	5	0,0628	20,00%	7,00%	3,60%	2	Whole slide	100 x	0
17	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
18	H	Not present	Present	5	0,0735	20,00%	7,00%	4,20%	2	Whole slide	100 x	0
19	H	Not present	Present	5	0,0724	25,00%	7,00%	5,20%	2	Whole slide	100 x	0

Laboratory identification code : **15**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	B	Present	Not present	5,01	0,045	0,00%	0,00%	0,00%	0	Whole slide		0
2	A	Not present	Not present	9,01	0,103	0,00%	0,00%	0,00%	0	Whole slide		0
3	F	Not present	Present	6,01	0,071	0,00%	0,00%	0,00%	0	Whole slide		0
4	H	Not present	Present	6,009	0,077	0,00%	0,00%	0,00%	0	Whole slide		0
5	B	Present	Not present	6,01	0,066	0,00%	0,00%	0,00%	0	Whole slide		0
6	H	Not present	Present	5,999	0,071	0,00%	0,00%	0,00%	0	Whole slide		0
7	A	Not present	Not present	7,01	0,076	0,00%	0,00%	0,00%	0	Whole slide		0
8	B	Present	Not present	6,01	0,066	0,00%	0,00%	0,00%	0	Whole slide		0
9	F	Not present	Present	6,01	0,06	0,00%	0,00%	0,00%	0	Whole slide		0
10	G	Present	Present	6,007	0,106	0,00%	0,00%	0,00%	0	Whole slide		0
11	C	Not present	Present	8,01	0,092	0,00%	0,00%	0,00%	0	Whole slide		0
12	D	Not present	Present	7,01	0,086	0,00%	0,00%	0,00%	0	Whole slide		0
13	E	Not present	Present	6	0,076	0,00%	0,00%	0,00%	0	Whole slide		0
14	C	Not present	Present	6,01	0,099	0,00%	0,00%	0,00%	0	Whole slide		0
15	D	Not present	Present	6,01	0,073	0,00%	0,00%	0,00%	0	Whole slide		0
16	G	Present	Present	6,01	0,101	0,00%	0,00%	0,00%	0	Whole slide		0
17	E	Not present	Present	6,006	0,101	0,00%	0,00%	0,00%	0	Whole slide		0
18	A	Not present	Not present	6,01	0,072	0,00%	0,00%	0,00%	0	Whole slide		0
19	G	Present	Present	6,005	0,101	0,00%	0,00%	0,00%	0	Whole slide		0

Laboratory identification code : **18**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	A	Not present	Not present	5	0,065	0,00%	0,00%	0,00%	14	Whole slide	100 x	0
2	G	Present	Present	5	0,084	0,00%	0,00%	0,00%	20	Whole slide	100 x	0
3	E	Not present	Present	5	0,07	11,00%	15,00%	1,03%	13	Whole slide	100 x	643
4	H	Not present	Present	5	0,061	9,00%	15,00%	0,74%	9	Whole slide	100 x	275
5	B	Present	Not present	5	0,073	0,00%	0,00%	0,00%	19	Whole slide	100 x	0
6	B	Present	Not present	5	0,085	0,00%	0,00%	0,00%	16	Whole slide	100 x	0
7	C	Not present	Present	5	0,047	3,00%	15,00%	0,19%	12	Whole slide	100 x	117
8	F	Not present	Present	5	0,098	8,00%	15,00%	1,05%	20	Whole slide	100 x	528
9	B	Present	Not present	5	0,092	0,00%	0,00%	0,00%	14	Whole slide	100 x	0
10	D	Not present	Present	5	0,077	2,00%	15,00%	0,21%	10	Whole slide	100 x	54
11	E	Not present	Present	5	0,094	10,00%	15,00%	1,25%	18	Whole slide	100 x	598
12	G	Present	Present	5	0,106	0,00%	0,00%	0,00%	20	Whole slide	100 x	0
13	G	Present	Present	5	0,1	0,00%	0,00%	0,00%	15	Whole slide	100 x	0
14	A	Not present	Not present	5	0,052	0,00%	0,00%	0,00%	12	Whole slide	100 x	0
15	H	Not present	Present	5	0,06	11,00%	15,00%	0,88%	16	Whole slide	100 x	465
16	A	Not present	Not present	5	0,069	0,00%	0,00%	0,00%	13	Whole slide	100 x	0
17	F	Not present	Present	5	0,064	12,00%	15,00%	1,03%	18	Whole slide	100 x	667
18	C	Not present	Present	5	0,065	9,00%	15,00%	0,77%	14	Whole slide	100 x	267
19	D	Not present	Present	5	0,064	2,00%	15,00%	0,17%	15	Whole slide	100 x	94

Laboratory identification code : **19**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	B	Present	Not present	10	0,1337	0,00%	0,00%	0,00%	1	Whole slide	100 x	0
2	B	Present	Not present	10	0,1274	0,00%	0,00%	0,00%	1	Whole slide	100 x	0
3	B	Present	Present	10	0,1107	0,00%	0,00%	0,00%	1	Whole slide	100 x	3
4	E	Not present	Present	10	0,1509	7,98%	15,00%	0,80%	1	Whole slide	100 x	360
5	F	Not present	Present	10	0,1173	8,35%	15,00%	0,65%	1	Whole slide	100 x	325
6	G	Present	Present	10	0,1783	0,00%	0,00%	0,00%	1	Whole slide	100 x	612
7	C	Not present	Present	10	0,1249	4,32%	15,00%	0,36%	1	Whole slide	100 x	161
8	H	Not present	Present	10	0,1322	6,73%	15,00%	0,59%	1	Whole slide	100 x	310
9	E	Not present	Present	10	0,1149	8,01%	15,00%	0,61%	1	Whole slide	100 x	270
10	H	Not present	Present	10	0,1399	5,57%	15,00%	0,52%	1	Whole slide	100 x	275
11	A	Not present	Not present	10	0,1052	0,00%	0,00%	0,00%	1	Whole slide	100 x	0
12	G	Present	Present	10	0,1524	0,00%	0,00%	0,00%	1	Whole slide	100 x	517
13	F	Not present	Present	10	0,1006	5,77%	15,00%	0,39%	1	Whole slide	100 x	231
14	D	Not present	Present	10	0,1407	1,49%	15,00%	0,14%	1	Whole slide	100 x	86
15	C	Not present	Present	10	0,1079	1,48%	15,00%	0,11%	1	Whole slide	100 x	103
16	D	Not present	Present	10	0,1748	1,09%	15,00%	0,13%	1	Whole slide	100 x	59
17	G	Present	Present	10	0,1453	0,00%	0,00%	0,00%	1	Whole slide	100 x	442
18	A	Not present	Present	10	0,1556	0,00%	0,00%	0,00%	1	Whole slide	100 x	8
19	A	Not present	Present	10	0,1071	0,00%	0,00%	0,00%	1	Whole slide	100 x	3

Laboratory identification code : **20**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
2	E	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
3	D	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
4	B	Present	Not present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
5	B	Present	Not present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
6	B	Present	Not present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
7	H	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
8	C	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
9	H	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
10	G	Present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
11	F	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
12	E	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
13	D	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
14	G	Present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
15	F	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
16	C	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
17	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
18	G	Not present	Present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0
19	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	8	Whole slide		0

Laboratory identification code : **21**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	B	Present	Not present	10	0,14	0,00%	20,00%	0,00%	2	Whole slide	200 x	0
2	E	Not present	Present	10	0,11	29,93%	20,00%	1,65%	2	Whole slide	200 x	449
3	H	Not present	Present	10	0,12	16,33%	20,00%	0,98%	2	Whole slide	200 x	245
4	A	Not present	Not present	10	0,13	0,00%	20,00%	0,00%	2	Whole slide	200 x	0
5	E	Not present	Present	10	0,15	25,27%	20,00%	1,90%	2	Whole slide	200 x	379
6	A	Not present	Not present	10	0,13	0,00%	20,00%	0,00%	2	Whole slide	200 x	0
7	D	Not present	Present	10	0,13	3,47%	20,00%	0,23%	2	Whole slide	200 x	52
8	D	Not present	Present	10	0,15	3,00%	20,00%	0,23%	4	Whole slide	200 x	45
9	B	Present	Not present	10	0,14	0,00%	20,00%	0,00%	2	Whole slide	200 x	0
10	B	Present	Not present	10	0,12	0,00%	20,00%	0,00%	2	Whole slide	200 x	0
11	C	Not present	Present	10	0,11	8,73%	20,00%	0,48%	3	Whole slide	200 x	131
12	G	Present	Present	10	0,17	0,00%	20,00%	0,00%	3	Whole slide	200 x	0
13	F	Not present	Present	10	0,12	12,47%	20,00%	0,75%	2	Whole slide	200 x	187
14	A	Not present	Not present	10	0,11	0,00%	20,00%	0,00%	2	Whole slide	200 x	0
15	C	Not present	Present	10	0,1	2,67%	20,00%	0,13%	2	Whole slide	200 x	40
16	F	Not present	Present	10	0,12	15,87%	20,00%	0,95%	2	Whole slide	200 x	238
17	H	Not present	Present	10	0,1	15,80%	20,00%	0,79%	2	Whole slide	200 x	237
18	G	Present	Present	10	0,17	0,00%	20,00%	0,00%	2	Whole slide	200 x	0
19	G	Present	Present	10	0,16	0,00%	20,00%	0,00%	2	Whole slide	200 x	0

Laboratory identification code : **22**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
2	H	Not present	Present	10	0,0905	20,00%	5,00%	3,60%	3	Whole slide	100 x	0
3	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
4	G	Present	Present	10	0,1142	60,00%	5,00%	13,70%	0	Whole slide	100 x	0
5	E	Not present	Present	10	0,0878	30,00%	5,00%	5,30%	3	Whole slide	100 x	0
6	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
7	C	Not present	Present	10	0,0852	10,00%	5,00%	1,70%	3	Whole slide	100 x	0
8	D	Not present	Present	10	0,0809	10,00%	5,00%	1,60%	0			0
9	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
10	H	Not present	Present	10	0,0597	30,00%	5,00%	3,60%	3	Whole slide	100 x	0
11	G	Present	Present	10	0,0954	70,00%	5,00%	13,40%	0	Whole slide	100 x	0
12	F	Not present	Present	10	0,0766	30,00%	5,00%	4,60%	3	Whole slide	100 x	0
13	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
14	C	Not present	Present	10	0,0699	10,00%	5,00%	1,40%	3	Whole slide	100 x	0
15	E	Not present	Present	10	0,0864	30,00%	5,00%	5,20%	3	Whole slide	100 x	0
16	F	Not present	Present	10	0,0656	20,00%	5,00%	2,60%	3	Whole slide	100 x	0
17	D	Not present	Present	10	0,0839	10,00%	5,00%	1,70%	3	Whole slide	100 x	0
18	G	Present	Present	10	0,0905	60,00%	5,00%	10,90%	3	Whole slide	100 x	0
19	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0

Laboratory identification code : **23**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	A	Not present	Not present	5	0,08	0,00%	0,00%	0,00%	5	Whole slide	50 x	0
2	B	Present	Not present	5	0,074	0,00%	0,00%	0,00%	5	Whole slide	50 x	0
3	D	Not present	Present	5	0,083	5,00%	20,00%	0,40%	5	Whole slide	50 x	23
4	B	Present	Not present	5	0,067	0,00%	0,00%	0,00%	4	Whole slide	50 x	0
5	G	Present	Present	5	0,095	0,00%	0,00%	0,00%	6	Whole slide	50 x	0
6	G	Present	Present	5	0,087	0,00%	0,00%	0,00%	5	Whole slide	50 x	0
7	E	Present	Present	5	0,085	0,00%	0,00%	0,00%	5	Whole slide	50 x	0
8	H	Not present	Present	5	0,062	50,00%	20,00%	3,10%	4	Whole slide	50 x	189
9	F	Not present	Present	5	0,081	40,00%	20,00%	3,20%	5	Whole slide	50 x	196
10	C	Not present	Present	5	0,063	30,00%	20,00%	1,80%	4	Whole slide	50 x	87
11	C	Not present	Present	5	0,078	10,00%	20,00%	0,70%	5	Whole slide	50 x	31
12	A	Not present	Not present	5	0,073	0,00%	0,00%	0,00%	4	Whole slide	50 x	0
13	D	Not present	Present	5	0,071	10,00%	20,00%	0,70%	5	Whole slide	50 x	30
14	H	Not present	Present	5	0,082	35,00%	20,00%	2,80%	5	Whole slide	50 x	170
15	E	Not present	Present	5	0,085	60,00%	20,00%	5,10%	5	Whole slide	50 x	270
16	F	Not present	Present	5	0,076	35,00%	20,00%	2,60%	4	Whole slide	50 x	150
17	A	Not present	Not present	5	0,064	0,00%	0,00%	0,00%	4	Whole slide	50 x	0
18	B	Present	Not present	5	0,077	0,00%	0,00%	0,00%	5	Whole slide	50 x	0
19	G	Present	Present	5	0,082	0,00%	0,00%	0,00%	5	Whole slide	50 x	0

Laboratory identification code : **29**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	G	Present	Present	10	0,16	0,00%	0,00%	0,00%	0			0
2	B	Present	Not present	10	0,09	0,00%	0,00%	0,00%	0			0
3	C	Not present	Present	10	0,11	10,00%	0,00%	0,50%	3	Whole slide	100 x	0
4	E	Not present	Present	10	0,13	50,00%	0,00%	1,50%	3	Whole slide	100 x	0
5	G	Present	Present	10	0,17	0,00%	0,00%	0,00%	0			0
6	B	Present	Not present	10	0,09	0,00%	0,00%	0,00%	0			0
7	D	Not present	Present	10	0,12	3,00%	0,00%	0,20%	3	Whole slide	100 x	0
8	C	Not present	Present	10	0,11	6,00%	0,00%	0,50%	3	Whole slide	100 x	0
9	H	Not present	Present	10	0,11	20,00%	0,00%	1,00%	3	Whole slide	100 x	0
10	F	Not present	Present	10	0,1	50,00%	0,00%	1,50%	3	Whole slide	100 x	0
11	A	Not present	Not present	10	0,1	0,00%	0,00%	0,00%	0			0
12	A	Not present	Not present	10	0,09	0,00%	0,00%	0,00%	0			0
13	A	Not present	Not present	10	0,1	0,00%	0,00%	0,00%	0			0
14	B	Present	Not present	10	0,1	0,00%	0,00%	0,00%	0			0
15	G	Present	Present	10	0,14	0,00%	0,00%	0,00%	0			0
16	D	Not present	Present	10	0,12	10,00%	0,00%	0,20%	3	Whole slide	100 x	0
17	E	Not present	Present	10	0,12	40,00%	0,00%	1,00%	3	Whole slide	100 x	0
18	F	Not present	Present	10	0,13	50,00%	0,00%	1,50%	3	Whole slide	100 x	0
19	H	Not present	Present	10	0,12	50,00%	0,00%	1,50%	3	Whole slide	100 x	0

Laboratory identification code : **30**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	A	Not present	Not present	5	0,07	0,00%	0,00%	0,00%	15	Whole slide		0
2	D	Not present	Present	5	0,07	0,00%	0,00%	0,00%	15	Whole slide		0
3	H	Not present	Present	5	0,08	0,00%	0,00%	0,00%	20	Whole slide		0
4	B	Present	Not present	5	0,07	0,00%	0,00%	0,00%	20	Whole slide		0
5	F	Not present	Present	5	0,07	0,00%	0,00%	0,00%	20	Whole slide		0
6	G	Present	Present	5	0,11	0,00%	0,00%	0,00%	20	Whole slide		0
7	D	Not present	Present	5	0,09	0,00%	0,00%	0,00%	20	Whole slide		0
8	A	Not present	Not present	5	0,08	0,00%	0,00%	0,00%	15	Whole slide		0
9	G	Present	Present	5	0,16	0,00%	0,00%	0,00%	20	Whole slide		0
10	C	Not present	Present	5	0,09	0,00%	0,00%	0,00%	20	Whole slide		0
11	E	Not present	Present	5	0,09	0,00%	0,00%	0,00%	20	Whole slide		0
12	G	Present	Present	5	0,11	0,00%	0,00%	0,00%	20	Whole slide		0
13	F	Not present	Present	5	0,09	0,00%	0,00%	0,00%	20	Whole slide		0
14	B	Present	Not present	5	0,07	0,00%	0,00%	0,00%	20	Whole slide		0
15	H	Present	Present	5	0,1	0,00%	0,00%	0,00%	20	Whole slide		0
16	E	Not present	Present	5	0,09	0,00%	0,00%	0,00%	20	Whole slide		0
17	B	Present	Not present	5	0,08	0,00%	0,00%	0,00%	20	Whole slide		0
18	C	Not present	Present	5	0,08	0,00%	0,00%	0,00%	20	Whole slide		0
19	A	Not present	Not present	5	0,08	0,00%	0,00%	0,00%	15	Whole slide		0

Laboratory identification code : **32**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	G	Not present	Present	10,01	0,106	60,65%	20,00%	3,21%	3	Whole slide	100 x	390
2	D	Not present	Present	10,02	0,109	0,88%	10,00%	0,10%	3	Whole slide	100 x	13
3	H	Not present	Present	10	0,106	7,54%	15,00%	0,53%	3	Whole slide	100 x	100
4	A	Not present	Not present	10	0,079	0,00%	0,00%	0,00%	3	Whole slide	100 x	0
5	G	Not present	Present	10	0,1298	25,03%	20,00%	1,62%	3	Whole slide	100 x	192
6	C	Not present	Present	10	0,0877	3,68%	10,00%	0,32%	3	Whole slide	100 x	50
7	B	Not present	Not present	10	0,087	0,00%	0,00%	0,00%	3	Whole slide	100 x	0
8	H	Not present	Present	10,02	0,1085	7,84%	15,00%	0,56%	3	Whole slide	100 x	75
9	D	Not present	Present	10	0,0886	1,46%	10,00%	0,13%	3	Whole slide	100 x	22
10	E	Not present	Present	10	0,1004	6,62%	15,00%	0,44%	3	Whole slide	100 x	72
11	F	Not present	Present	10	0,086	7,77%	15,00%	0,44%	3	Whole slide	100 x	98
12	C	Not present	Present	10	0,0701	3,56%	10,00%	0,25%	3	Whole slide	100 x	34
13	G	Not present	Present	10	0,1351	31,08%	20,00%	2,10%	3	Whole slide	100 x	373
14	A	Not present	Not present	10	0,09	0,00%	0,00%	0,00%	3	Whole slide	100 x	0
15	B	Present	Not present	10	0,083	0,00%	0,00%	0,00%	2	Whole slide	400 x	0
16	A	Not present	Not present	10	0,085	0,00%	0,00%	0,00%	3	Whole slide	100 x	0
17	B	Present	Not present	10,01	0,092	0,00%	0,00%	0,00%	3	Whole slide	400 x	0
18	F	Not present	Present	10	1,003	3,60%	10,00%	0,36%	3	Whole slide	400 x	66
19	E	Not present	Present	10	0,0946	6,80%	15,00%	0,43%	3	Whole slide	100 x	72

Laboratory identification code : **33**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	E	Not present	Present	5	0,061	3,05%	10,00%	0,19%	3	5	100 x	73
2	D	Not present	Present	5	0,06	0,74%	10,00%	0,09%	3	5	100 x	12
3	H	Not present	Present	5	0,059	2,02%	10,00%	0,24%	3	5	100 x	37
4	F	Not present	Present	5	0,064	2,09%	10,00%	0,27%	3	5	100 x	45
5	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
6	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
7	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
8	C	Not present	Present	5	0,067	1,87%	10,00%	0,25%	3	5	100 x	27
9	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
10	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
11	B	Present	Not present	0	0	0,00%	0,00%	0,00%	0			0
12	D	Not present	Present	5	0,061	1,15%	10,00%	0,14%	3	5	100 x	27
13	H	Not present	Present	5	0,05	8,47%	10,00%	0,85%	3	5	100 x	232
14	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0
15	G	Present	Present	0	0	0,00%	0,00%	0,00%	0			0
16	C	Not present	Present	5	0,058	2,47%	10,00%	0,29%	3	5	100 x	53
17	F	Not present	Present	5	0,071	3,18%	10,00%	0,45%	3	5	100 x	70
18	E	Not present	Present	5	0,067	5,79%	10,00%	0,76%	3	5	100 x	113
19	A	Not present	Not present	0	0	0,00%	0,00%	0,00%	0			0

Laboratory identification code : **35**

	Sample	Terrestrial animal part.	Fish part.	*W* (g)	*S* (g)	*d*	*f*	Estimated value of fish const.	Number of slides	Number of fields per slide	Final magnification	Total number of fish part.
1	**G**	Present	Present	5	0,0687	0,00%	0,00%	0,00%	5			0
2	**E**	Present	Present	5	0,0443	0,00%	0,00%	0,00%	5			0
3	**A**	Not present	Not present	5	0,0535	0,00%	0,00%	0,00%	5			0
4	**F**	Not present	Present	5	0,0539	12,50%	15,00%	1,00%	5	Whole slide	100 x	3641
5	**G**	Present	Present	5	0,0722	0,00%	0,00%	0,00%	5			0
6	**C**	Not present	Present	5	0,0551	5,90%	15,00%	0,50%	5	Whole slide	100 x	1188
7	**B**	Present	Not present	5	0,0541	0,00%	0,00%	0,00%	5			0
8	**H**	Not present	Present	5	0,05	15,50%	15,00%	1,00%	5	Whole slide	100 x	3397
9	**F**	Not present	Present	5	0,0468	14,00%	15,00%	1,00%	5	Whole slide	100 x	3290
10	**A**	Not present	Not present	5	0,0458	0,00%	0,00%	0,00%	5			0
11	**D**	Not present	Present	5	0,0556	3,80%	15,00%	0,20%	5	Whole slide	100 x	402
12	**B**	Present	Not present	5	0,0674	0,00%	0,00%	0,00%	5			0
13	**H**	Not present	Present	5	0,0485	9,00%	15,00%	0,50%	5	Whole slide	100 x	2299
14	**B**	Present	Not present	5	0,0494	0,00%	0,00%	0,00%	5			0
15	**C**	Not present	Present	5	0,0591	5,15%	15,00%	0,50%	5	Whole slide	100 x	1049
16	**E**	Not present	Present	5	0,0872	12,75%	15,00%	1,50%	5	Whole slide	100 x	2088
17	**G**	Present	Present	5	0,0825	0,00%	0,00%	0,00%	5			0
18	**A**	Not present	Not present	5	0,0654	0,00%	0,00%	0,00%	5			0
19	**D**	Not present	Present	5	0,0657	2,00%	15,00%	0,10%	5	Whole slide	100 x	510