Combined microscopy-PCR
EURL-AP Proficiency Test 2022

Final version

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Summary

The European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP) organised the present proficiency test for assessing the ability of the NRL network with respect to the detection of processed animal proteins (PAPs) in feed using both light microscopy and PCR according to current legal requirements.

The total number of participating laboratories was 32 (26 NRLs and 6 labs outside the NRL network). Thirty-one laboratories delivered results. The study was based on a set of seven samples (to be analysed both by light microscopy and PCR) consisting of blank feed matrices or feed materials fortified or not with processed animal proteins from terrestrial vertebrates and/or from fish.

Regarding the detection of PAPs by light microscopy the overall results indicated an excellent and satisfactory level of global performance for 81 % of the NRLs. Five NRLs out of the 26 (19 %) were underperforming. The results obtained by light microscopy showed a base-line issue regarding the specificity for fish detection since no fish material was present in the set of samples. Sensitivity issues were observed for the disclosure of milk powder within a feed matrix. The study also stressed the problem of soft tissues processed materials such as liver meal; many participants declared the sample as positive for terrestrial vertebrates, which was a correct answer, but on a very variable number of identifiable particles which was demonstrated to be very low by the homogeneity study.

Concerning the PCR results, it was the first time that the proficiency to perform the three PCR tests for the detection of ruminant, pig and poultry DNA was assessed. Seventy-seven percent of the NRLs (20 out of 26) performed excellently and reported no false result. Five NRLs (19 %) were also considered as excellent as all their results were correct for the detection of ruminant DNA but with a maximum of 2 false results for the detection of pig and poultry DNA. One remaining NRL returned satisfying results corresponding to one false result with the ruminant PCR test. No laboratory was considered as underperforming. The global performance of the network was excellent with three PCR methods.

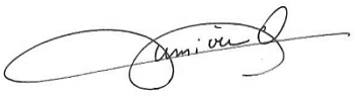
Keywords :

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

This report identified by an ISBN has been prepared from a draft version sent for revision and comments to the participants on the 30th January 2023. After reception of the comments on the 15th February 2023, it was amended accordingly and approved by the signature of the organisers.

ISO 17043 coordinators signature for approval:

Olivier Fumière *Pascal Veys*



1. Foreword

European Union Reference Laboratories (EURL) were created in order to ensure a high level of quality and a uniformity of the results provided by European control laboratories. On 15th March 2017, the European Parliament and the Council adopted Regulation EU/625/2017 [1], improving the effectiveness of the official food and feed controls while redefining the obligations of the relevant authorities and their obligations in the organization of these controls.

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

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

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

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

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

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

In this framework, the EURL-AP has been organising yearly since 2006 proficiency tests for the assessment of the implementation of the reference methods for the detection of animal proteins in feed as described by current Annex VI of Commission Regulation EC/152/2009 [3]. Since 2016, the proficiency tests conducted by the EURL-AP are organised under the ISO17043 standard.

The present study report is part of the activity scope of the EURL-AP annual programme.

2. Introduction

According to modified Annex VI of Commission Regulation EC/152/2009 [3] official controls for the detection of animal proteins in feed inside the EU have to be performed by light microscopy and/or PCR since June 2013 [4]. Standard Operating Procedures (SOP) are supporting the implementation of the two methods.

The objective of the present proficiency test was strictly to evaluate within the network of 26 NRLs the analytical performance to detect processed animal proteins (PAPs) in feed by light microscopy and PCR. Participation of the NRLs is mandatory.

In addition, and on proposal of the Commission, invitations to participate to this test were also sent to a limited number of official control labs outside the EU. Non-EU participants were asked to apply also light microscopy and PCR although strict following of Annex VI of Commission Regulation EC/152/2009 and related SOPs was not imposed to them.

3. Material and methods

3.1. Study organisation

Twenty-six NRLs and six laboratories outside this EU network participated to the study. A detailed list of the 32 participating labs is included in Annex 1.

Official announcement (Annex 2) of the study was made on the 9th September 2022 to all invited participants.

On the 14th October 2022, the sample sets were shipped to the participants. On the same day the Excel report forms containing the instructions (Annex 3) were communicated to all participants – downloadable from the EURL-AP intranet for the NRLs or sent by email to the non-EU participants who have no access to this intranet.

The deadline for the delivery of the results was fixed in the announcement and in the instructions at the 18th November 2022.

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

- Laboratories participating to the proficiency test were themselves responsible to reach appropriate homogeneity of the sample sub-portions that had to be taken from the whole sample vial for analysis. Precautions to avoid laboratory cross-contamination were also highlighted.
- Results had to be encoded by way of an Excel report form (Annex 3). Participants were asked to carefully read the instructions on how to fill in the result form and to testify they did it prior to encoding their results. No other support for communicating the results was accepted.
- Participants were asked to sign the summarized results sheet that is automatically generated when filling the form and to return it by email to the EURL-AP. Results were taken into consideration only when both the Excel file and a copy of the summarized results sheet were received by the EURL-AP.
- Participants were notified that results arriving later would not be accepted.

On the exception of one non-EU participant, all results were delivered on time to the organiser. Thus, the study presents results from 31 participants. The proficiencies of NRLs and other participants were evaluated separately.

3.2. Material

3.2.1. Description of the samples

Seven different blind test materials were prepared for the study. The composition of the sample set was established considering the following factors:

- Use of feed and feed materials intended to different farmed animals ;
- Absence of any ingredient from fish origin ;
- Use of pure ingredients from animal origin commercially available ;
- Use of a mineral premix known to deliver high percentage of sediment ;
- Use of materials prepared from milk known to be detectable by light microscopy ;
- Use of adulterants from animal origins intended to deliver both positive presence for terrestrial vertebrates by light microscopy and positive ruminant, porcine and avian signals by PCR.

Each participating lab received about 40g for each of the seven blind samples to which a unique random number was assigned. Details of the sample set are indicated in Table 1.

Table 1: Composition of the sample set

Sample	Material	Expected results * Microscopy (particles)		PCR (DNA)		
		Terrestrial vert.	Fish	Ruminant	Pig	Poultry
1	Poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP	+	-	+	+	+
2	Pig feed + 2 % milk powder	+	-	+	n.a.	-
3	Feather meal	+	-	-	-	+
4	Poultry feed II (blank)	-	-	-	-	-
5	Mineral premix + 0.5 % TCP	+	-	-	-	-
6	Porcine liver meal	n.a.	-	n.a.	+	+
7	Poultry feed I + 0.2 % pig PAP	+	-	-	+	-
Total		**5**	**0**	**2**	**3**	**3**

(n.a. = not submitted to proficiency assessment,
*** = explanations on expected results are described in section 3.4)**

Expected results were internally determined based on the known composition of the samples (presence or absence of PAP) and the results obtained during the homogeneity study (see 3.5).

3.2.2. Materials used in the preparation of the samples

Six commercially available feed materials or feed were used as matrices:

- The **poultry feed I**, used to prepare sample 1 and 7, was a complete feed made of maize, wheat, dehulled soybean, wheat bran, rapeseed, calcium carbonate, pea, soy oil and natrium chloride. Its sediment was of 2.7 %. No animal DNA was detected by PCR.
- The **poultry feed II** was an organic complete feed for lay hens made of maize, wheat, rapeseed and soy expeller, soy oil, other plant oil, amino acids, calcium carbonate and monocalcium phosphate. Its sediment was of 3.9 %. No animal DNA was detected by PCR.
- The **pig feed** was a complete feed for fatteners made of wheat, maize, rapeseed expeller, soy expeller and oil, other plant oils, amino acids, calcium carbonate, monocalcium phosphate and additives. Its sediment was of 1 %. PCR analyses revealed ruminant DNA.
- The **feather meal** had a sediment content of 0.3 %. PCR analyses revealed chicken and turkey DNA.
- The **mineral premix**, used in the 2019 proficiency test [5], was of unknown composition and had a sediment content of 86 %. PCR analyses revealed it free from ruminant, porcine and poultry DNA.
- The **liver meal** is a feed material intended for fish feeding and labelled as from porcine origin. Its sediment content was negligible. The presence of porcine and poultry DNA was confirmed by PCR.

Adulterant material used:

- A **ruminant PAP** was used for preparing sample 1. Its sediment content reached 60 %. Only ruminant DNA was detected by PCR.
- A **porcine PAP** was used for preparing samples 1 and 7. It had a sediment of 38 %. PCR analyses revealed only the presence of porcine DNA.
- A **poultry PAP** was used for preparing sample 1. It had no sediment. PCR analyses revealed not only poultry DNA presence but ruminant DNA as well.
- A **milk powder** was used for preparing sample 2. It had no sediment.

- **Tricalcium phosphate** (TCP) heat treated in a muffle furnace at 500 °C to destroy any potential remaining presence of DNA was used for preparing sample 5. This material was used in the 2019 proficiency test [5]. PCR analyses proved the absence of any DNA.

3.2.3. Description of the mixing procedures

To avoid presence of interfering material, a cleaning of the rooms where the samples were handled was performed prior to sample preparation, mixing of the materials and filling of the vials.

The poultry feed II (sample 4) was conditioned first in order to avoid contamination.

The feather meal (sample 3) was then prepared.

The poultry feed I based samples (samples 1 and 7) were then conditioned. Adulteration of sample 7 was realised first and a few days later, after cleaning of the rooms, sample 1 was adulterated too.

Then the mineral premix (sample 5) was conditioned and adulterated with TCP.

The pig feed fortified with milk powder (sample 2) was then prepared.

Finally, the most pulverulent material, the liver meal (sample 6) was conditioned.

All adulterations (samples 1, 2, 5 and 7) were proceeded by direct spiking with the adulterant.

3.3. Qualitative analysis

Analyses of qualitative proficiency testing were applied following ISO 13528 [6].

3.3.1. Light microscopy

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

Results are expressed by the participants in three formulations according to regulation EU/56/2013 [4] amending regulation EC/152/2009 [3]:

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

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

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

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

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

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

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

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

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

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

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

3.3.2. PCR

Qualitative analysis concerned the detection of ruminant, pig and poultry (chicken-turkey) DNA as prescribed by Annex VI of Commission Regulation (EC) No 152/2009 in its consolidated most recent version complemented by the corresponding binding SOPs.

The participants delivered Ct values (in cycles) to compare to a cut-off value (in cycles). A cut-of value being specific of a PCR test, one must set for the ruminant, the pig and the poultry DNA detection respectively. For the detection of ruminant DNA and poultry DNA, the respective cut-off are set at 15 copies of the target and validated by a quality criterion (the cut-off Ct value must correspond to a number of copies of the target > 9.00 copies). In the case of the detection of pig DNA, the cut-off is set at 5 copies of the target with a quality criterion > 3.00 copies.

For each sample, DNA is extracted from 2 test portions. The results obtained from the 2 test portions must be consistent, in the sense that both Ct values should be close enough to each other and on the same side compared to the cut-off value. A Ct value < cut-off value corresponds to a positive result. Respectively, a Ct value ≥ cut-off value corresponds to a negative result. Results are expressed by the participants in two formulations:

- Present (= presence of targeted DNA detected)
- Absent (= no targeted DNA detected)

As for light microscopy, these binary results were analysed by classical statistics (accuracy, sensitivity and specificity) with the same formulae as presented in 3.3.1.

3.4. Performance criteria

Evaluation of the performance and scoring were applied as recommended by ISO 13528 [6].

This year again, since all samples had to be analysed by both light microscopy and PCR, the only evaluation done was on the reported analytical results.

3.4.1. Light microscopy

Considering the sample set composition and the announced parameters (Annex 2), the expected results are indicated in Table 1.

Concerning the presence of terrestrial vertebrates :

- Samples 1, 2, 3, 5 and 7 had to be declared as positive.
- Sample 4 had to be declared negative
- Sample 6 was not submitted to any proficiency assessment for this parameter. In the discussion section of the report the explanation for this decision is detailed.

Concerning the presence of fish : all samples had to be declared negative.

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

- **Excellent** level of global performance = consolidated AC superior or equal to 0.90 with no ND for terrestrial vertebrates.
- **Satisfying** level of global performance = consolidated AC superior or equal to 0.90 with one ND for terrestrial vertebrates OR a consolidated AC equal to 0.846 with no ND for terrestrial vertebrates.
- **Underperforming** level of global performance = consolidated AC equal to 0.846 with one ND for terrestrial vertebrates or a consolidated AC inferior to 0.846.

3.4.2. PCR

As for light microscopy, the expected results are indicated in Table 1.

- Samples 1 and 2 were considered to be declared positive for the presence of ruminant DNA.
- Samples 1, 6 and 7 were considered to be positive for the presence of pig DNA.
- Samples 1, 3 and 6 were considered to be positive for the presence of poultry DNA.
- Samples 4 and 5 had to be declared negative.

The detection of ruminant DNA for sample 6 and of pig DNA for sample 2 were out of the performance assessment.

Concerning the PCR, the performance criteria were decided as:

- **Excellent** level of global performance = global AC ≥ 0.90 with no false result (ND or PD) for the detection of ruminant DNA.
- **Satisfying** level of global performance = global AC ≥ 0.85 with maximum 1 false result (ND or PD) for the detection of ruminant DNA.
- **Underperforming** level of global performance = global AC < 0.85 or global AC ≥ 0.85 with 2 false results (ND or PD) for the detection of ruminant DNA.

3.5. Homogeneity study

Homogeneity study has been carried out for all materials used. Table 2 summarizes the results.

Table 2: Homogeneity study – Results

Sample	Material	Light microscopy			PCR			
		Nr of replicates	Terrestrial vert.	Fish	Nr of replicates	Ruminant	Porcine	Poultry
1	Poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP	10	+	-	10	+	+	+
2	Pig feed + 2 % milk powder	10	+	-	10	+	-*	-
3	Feather meal	10	+	-	10	-	-	+
4	Poultry feed II (blank)	10	-	-	10	-	-	-
5	Mineral premix + 0.5 % TCP	10	+	-	10	-	-	-
6	Porcine liver meal	10	< LOD	-	10	-*	+	+
7	Poultry feed I + 0.2 % pig PAP	10	+	-	10	-	+	-

(Legend: ND = not tested, + = systematically detected, - = systematically not detected, < LOD = below the limit of decision, * = results not systematically negative)

The homogeneity was studied by light microscopy on 10 g of sample material (and 3 g for samples 5 and 6) for 10 replicates. Analyses of replicates were performed following strictly EC/152/2009 regulation [3]. For PCR analysis, a DNA extraction was performed on 2 test portions of 100 mg of sample material for each of the 10 replicates.

Through the light microscopic observations performed during the homogeneity study, no single fish particle could be identified through the whole sample set. It demonstrates a total absence of fish material.

Sample 1 (poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP) was systematically positive for the presence terrestrial vertebrates with the observations of bone fragments and muscles. It has to be noted that the presence of muscles was not always observed on the slides prepared from the flotate. PCR analyses detected systematically the presence of ruminant, pig and poultry DNA respectively.

Sample 2 (pig feed + 2 % milk powder) showed systematically the presence of milk globules. The presence of peripheral lactose crystals, as revealed by polarised light on glycerol mounts, was limited to an average of 2 globules per slide of flotate among the observed globules. The presence of ruminant DNA was systematically detected whereas poultry DNA was never detected. For all the replicates analysed, the final conclusion was the absence of pig DNA. Nevertheless, the results were ambiguous for some replicates. For that reason, this parameter was remove from the proficiency assessment.

Sample 3 (feather meal) showed systematically the abundant presence of both feather fragments and terrestrial bones. Muscle fibres were not observed. PCR analyses systematically confirmed the presence of poultry DNA only.

Sample 4 (poultry feed II) revealed to be free from any animal remains. The absence of ruminant, pig and poultry DNA was also confirmed by PCR analyses.

Sample 5 (mineral premix + 0.5 % TCP) was systematically positive for the presence terrestrial vertebrates' bones whereas PCR analyses did not detect the presence of ruminant, pig and poultry DNA.

Sample 6 (porcine liver meal) was delivering only a negligible sediment fraction. Per replicate only one slide from the sediment could be prepared. These slides presented only few bone fragments (9). Slides prepared from the flotate contained only a very limited number of identifiable terrestrial vertebrates' structures: hairs (2) and muscle fibre (1). Other observed structures were lacking histological features allowing reliable characterisation. By PCR, the presence of pig and poultry DNA was confirmed systematically. The presence of ruminant DNA was detected only with one of the two master mixes tested. The parameter "terrestrial vertebrates" was removed from the proficiency assessment.

Sample 7 (poultry feed I + 0.2 % pig PAP) was systematically positive for the presence terrestrial vertebrates' bones. Muscle fibres were not observed. The presence of pig DNA was systematically detected by PCR. The absence of ruminant and poultry DNA was confirmed by the PCR results.

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

3.6. Stability of the samples

Internal stability studies performed on similar samples from past studies have demonstrated that such samples were stable over time (years) for both light microscopic and PCR analyses. There are no reasonable elements which would indicate that present samples should be unstable.

4. Results

Gross results for microscopy and PCR from all participants are to be found in Annexes 4 and 5 respectively.

4.1. Microscopy results

4.1.1. Qualitative analyses from the NRLs

4.1.1.1. Results and performance of the network

Table 3 summarizes the results reported by the 26 NRLs for the sample types submitted to microscopic analysis.

The overall results, expressed in terms of global accuracy (AC) reveal the performance of the NRL network for the detection of PAPs from the present test. The percentage of total error accounted for 16 % of the total responses.

Table 3: Global results expressed as accuracy (AC) – light microscopy

Sample	Material	n	AC	
			Terrestrial Vert.	Fish
1	Poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP	26	1.000	0.923 (2)
2	Pig feed + 2 % milk powder	26	0.423 (15)	0.962 (1)
3	Feather meal	26	1.000	0.808 (5)
4	Poultry feed II (blank)	26	1.000	1.000
5	Mineral premix + 0.5 % TCP	26	0.962 (1)	0.923 (2)
6	Porcine liver meal	26	n.a.	0.923 (2)
7	Poultry feed I + 0.2 % pig PAP	26	1.000	0.923 (2)

Accuracy means sensitivity in case of ND and specificity in case of PD.
In brackets the absolute number of ND or PD. (Legend: n = number of results, n.a. = not submitted to proficiency assessment).

Since the absence of any fish material within the current study, only false positive results could be considered for calculating specificity scores. Such specificity issues arose within each sample, on the exception of sample 4, containing no animal derived product. The percentage of false positive findings of fish material was usually low (7%). However, this percentage was increased up to 19% for sample 3, the feather meal.

Regarding the detection of terrestrial vertebrates' constituents, overall sensitivity and specificity scores did not revealed any major issue since only one false negative case was reported for sample 5. The situation differed totally for sample 2, the pig feed containing 2 % of milk powder. In this sample the rate of false negative results reached 57 %.

The possible origin of the fish specificity issue in sample 3 and the terrestrial vertebrates sensitivity issue in sample 2 are commented into the discussion.

4.1.1.2. Detailed review of results per sample

Sample 1 : Poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP

PD for fish particles :

- Labs 2 and 7 reported fishbones,

Lab 24 reported the sample as < LOD for fish after having reported the presence of bones identified as from fish origin (on two determinations).

Sample 2: Pig feed + 2 % milk powder

ND for terrestrial vertebrates' particles :

- Labs 6, 10, 11, 12, 16, 17, 18, 21, 22 and 26 failed at detecting milk particles
- Labs 7 and 23 declared the sample as negative for terrestrial vertebrates while mentioning in their comments the presence of milk powder.

Labs 5, 20 and 24 reported the sample as < LOD due to the finding of only a few bone fragments.

Looking at the details of the NRLs having declared the sample as positive for terrestrial vertebrates presence, the majority of them reported the observations of : milk, milk globules, milk products, lactose crystals, milk/whey powder.

Two NRLs nevertheless reported the sample as positive without making any reference to dairy products : lab 2 described plasma, while lab 8 described bones and muscle findings.

PD for fish particles :

- Lab 23 reported bones from fish origin.

Sample 3: Feather meal

PD for fish particles :

- Labs 2, 4, 13, 15 and 19 reported erroneously the presence of fish bones.

Among these NRLs, labs 4 and 15 decided to classify the sample as positive for fish although they explicitly commented that either the "fishy" bones were lacking lacunae or that the bones observed could not be identified as from terrestrial origin and therefore decided to be categorised as fish.

Interestingly, among the laboratories that declared the feather meal as effectively positive for terrestrial vertebrates' presence, some participants failed at mentioning in the details of the observations the presence of feathers (labs 8, 12, 15 and 18).

Sample 4: Poultry feed II (blank)

All results were correct.

Sample 5: Mineral premix + 0.5 % TCP

ND for terrestrial vertebrates' particles :

- Lab 7 failed at detecting any terrestrial vertebrates' particles.

PD for fish particles :

- Labs 7 and 24 reported erroneously the presence of fish bones.

Aside the obvious presence of bones and possibly calcified cartilage fragments, some few NRLs also reported the observations of blood particles, muscle fibres and feathers

Sample 6: Porcine liver meal

PD for fish particles :

- Labs 8 and 16 reported fishbones

Lab 7 reported the sample as < LOD for fish after having reported the presence of bones identified as from fish origin (on two determinations).

The parameter terrestrial vertebrate was no submitted to performance assessment but the results delivered by the NRL network are as follows:

- a large majority of labs declared the sample as positive for this parameter (88 %)
- a minority declared it as negative (8 %) or < LOD (4 %).

Sample 7 : Poultry feed I + 0.2 % pig PAP

PD for fish particles :

- Labs 2 and 7 reported fishbones,

Lab 24 reported the sample as < LOD for fish after having reported the presence of bones identified as from fish origin (on two determinations).

4.1.1.3. Individual performances of NRLs in qualitative analysis

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

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

Terrestrial			
lab code	**AC**	**SE**	**SP**
1, 2, 3, 4, 8, 9, 13, 14, 15, 19 and 25	1.000	1.000	1.000
5, 6, 10, 11, 12, 16, 17, 18, 20, 21, 22, 23, 24 and 26	0.833	0.800	1.000
7	0.667	0.600	1.000

Fish		
lab code	**AC**	**SP**
1, 3, 5, 6, 9, 10, 11, 12, 14, 17, 18, 20, 21, 22, 25 and 26	1.000	1.000
4, 8, 13, 15, 16, 19, 23 and 24	0.857	0.857
2 and 7	0.571	0.571

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

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

Consolidated			
lab code	**AC**	**SE**	**SP**
1, 3, 9, 14 and 25	1.000	1.000	1.000
4, 8, 13, 15 and 19	0.923	1.000	0.875
5, 6, 10, 11, 12, 17, 18, 20, 21, 22 and 26	0.923	0.800	1.000
16, 23 and 24	0.846	0.800	0.875
2	0.769	1.000	0.625
7	0.615	0.600	0.625

From the 26 NRLs, 10 performed very well (39 %), 11 performed satisfyingly (42 %) and 5 were underperforming (19 %).

In agreement with the EURL-AP SOP for managing underperformances (available on the EURL-AP intranet since 18 January 2012), the underperforming participants (labs 2, 7, 16, 23 and 24) are asked to report on the origin of their errors as well as on the actions they will undertake in order to solve the problems.

4.1.2. Qualitative analyses and individual performances the non-EU participants

Individual performances from the 9 participants outside the EU were assessed exactly as in the previous section (4.1.1.3). A ranking of those labs was prepared as well based on the consolidated accuracy. Results are to be found in Tables 7 and 8.

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

Terrestrial			
lab code	**AC**	**SE**	**SP**
32	1.000	1.000	1.000
29, 33 and 36	0.833	0.800	1.000
30	0.667	0.600	1.000

Fish		
lab code	**AC**	**SP**
29 and 33	1.000	1.000
30	0.857	0.857
32	0.714	0.714
36	0.571	0.571

The error details are described per sample:

Sample 1 : Poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP

PD for fish particles :

- Labs 30 and 36 reported bones as categorised from fish origin,

Sample 2: Pig feed + 2 % milk powder

ND for terrestrial vertebrates' particles :

- Labs 29, 30 and 33 failed at detecting milk particles

Lab 32 reported the sample as positive without making any reference to dairy products but only describing bone and cartilage fragments

PD for fish particles :

- Lab 32 reported bones from fish origin and possibly gill fragments.

Sample 3: Feather meal

PD for fish particles :

- Lab 32 reported erroneously the presence of fish bones.

Lab 33 declared sample as effectively positive for terrestrial vertebrates' presence, while failing at mentioning in the details of the observations the presence of feathers.

Sample 4: Poultry feed II (blank)

PD for terrestrial vertebrates' particles :

- Lab 30 erroneously reported blood.

Sample 5: Mineral premix + 0.5 % TCP

ND for terrestrial vertebrates' particles :

- Lab 36 failed at detecting any terrestrial vertebrates' particles.

PD for fish particles :

- Lab 36 reported erroneously the presence of fish bones.

This reflects a probable inversion of the results for this participant.

Sample 6: Porcine liver meal

Although the parameter terrestrial vertebrate was no submitted to performance assessment, the results delivered by non-EU participants are as follows:

- a large majority of labs declared the sample as positive for this parameter (80 %)
- a minority declared it as < LOD (20 %).

Sample 7 : Poultry feed I + 0.2 % pig PAP

PD for fish particles :

- Lab 36 reported bones erroneously categorised as fish

A general ranking as for the NRL network was established (Table 9).

Table 9: General non-EU lab proficiency regarding the detection of terrestrial and fish material. Ranking follows AC values as primary key and SE as second key. Lines in black refer to excellent results, lines in blue refer to satisfying results and lines in red refer to underperforming results

Consolidated			
lab code	AC	SE	SP
29 and 33	0.923	0.800	1.000
32	0.846	1.000	0.750
30	0.769	0.800	0.750
36	0.692	0.800	0.625

Three participants performed satisfyingly (lines in blue in Table 9) and two participants were classified as underperforming (lines in red in Table 9) according to the applied criteria.

4.2. PCR results

4.2.1. Qualitative analyses from the NRLs

4.2.1.1. On the respect of the instructions

The NRLs seem to stick generally to the SOPs. Nevertheless, very few labs do not use one of the EURL-AP recommended mastermixes.

4.2.1.2. Overview of results and global performance of the network

Table 10 summarizes the results provided by 26 NRLs for the seven sample types submitted to qualitative PCR analysis.

Table 10: Global results expressed as accuracy (AC) – PCR

Sample	Material	n	AC		
			Ruminant	Pig	Poultry
1	Poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP	26	1.000	1.000	0.923 (2)
2	Pig feed + 2 % milk powder	26	1.000	n.a.	1.000
3	Feather meal	26	1.000	0.962 (1)	0.962 (1)
4	Poultry feed II (blank)	26	1.000	1.000	1.000
5	Mineral premix + 0.5 % TCP	26	1.000	1.000	1.000
6	Porcine liver meal	26	n.a.	1.000	0.923 (2)
7	Poultry feed I + 0.2 % pig PAP	26	0.962 (1)	1.000	1.000

Accuracy means sensitivity in case of ND and specificity in case of PD. The absence of a PCR result is considered as a deviation (ND or PD). In brackets the absolute number of false results. (Legend: n = number of results ; n.a. = not submitted to proficiency assessment)

On the overall results, 7 deviations (1.4 % of the 494 results) were recorded. With the ruminant and the pig targets, the results are almost perfect with only one deviation out of 156 results (0.6 % of false results). The rate of false results obtained with the poultry assay is higher but remains acceptable (2.7 %).

Sample 1 : Poultry feed I + 0.1 % ruminant PAP + 0.1 % pig PAP + 0.1 % poultry PAP

The PCR results expected were the presence of ruminant, pig and poultry DNA. Two negative deviations recorded for the detection of poultry DNA (Labs 20 and 25).

Sample 2 : Pig feed + 2 % milk powder

No deviation recorded with the ruminant and poultry assays. The results for the detection of pig DNA were kept out of the assessment but it must be noticed that all the EU participants reported the sample negative for this parameter.

Sample 3 : Feather meal

Lab 4 reported the presence of pig DNA (PD) and Lab 24 did not detect the presence of poultry DNA (ND).

Sample 4 : Poultry feed II (blank)

No deviation recorded for this sample whatever the target.

Sample 5 : Mineral premix + 0.5 % TCP

No deviation recorded for this sample whatever the target.

Sample 6 : Porcine liver meal

This sample labelled as porcine liver meal was also detected as containing poultry DNA during the homogeneity study. Two negative deviations were recorded for the detection of poultry DNA (Labs 2 and 4). The presence of ruminant DNA was kept out of the proficiency assessment. Only three Labs out of 26 reported a positive result.

Sample 7 : Poultry feed I + 0.2 % pig PAP

Only one positive deviation (PD) reported for the detection of ruminant DNA by Lab 19.

4.2.1.3. Individual performances of NRLs in qualitative analysis

Individual performances were assessed for each participant by calculating the accuracy, sensitivity and specificity over the samples. A ranking of the labs was prepared based on the accuracy. Results are to be found in Table 11 (next page) that summarizes the results obtained by the participants.

Table 11: NRL proficiencies regarding the detection of ruminant, pig and poultry material. Ranking follows AC values. Cells in black refers to excellent NRLs. Cells in blue refers to satisfying NRLs.

Lab code	AC	SE	SP
1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 21, 22, 23 and 26	1.000	1.000	1.000
2, 20, 24 and 25	0.947	0.875	1.000
4	0.900	0.875	0.917
19	0.947	1.000	0.909

Twenty labs out of 26 NRLs (77 % of the NRLs) had faultless scores and were thus classified as excellent. Five other labs were also considered as excellent : all the results obtained with the ruminant PCR test were correct but one negative deviation was recorded for the detection of poultry DNA (Labs 2, 4, 20, 24 and 25) and, for Lab 4, an additional positive deviation was reported with the pig assay.

Lab 19 performed satisfyingly: only one positive deviation was recorded but it was obtained with the ruminant PCR test.

No lab was underperforming.

4.2.1.4. Cut-off quality control

A quality control for the number of copies of the target reached with the Ct value of the cut-off, was developed to minimize the risk of false positive result. A minimum of 9.00 copies at the cut-off is required for the ruminant and the poultry PCR tests whereas it is 3.00 copies at the cut-off for the pig PCR test. Indeed, depending on the variability of the lab (PCR platform + operator), the cut-off value can correspond to a too low number of copies.

All the participants reached the minimum criterion of 9.00 copies for the ruminant cut-off. The range of copies at the cut-off goes from 9.00 copies to 11.89 copies. The cut-off in cycles are comprised between 31.91 cycles and 37.84 cycles. The percentage of the labs with a cut-off corresponding to a number of copies > 10 for this proficiency test was 69.2 %.

For the pig cut-off too, all the participants reached the minimum criterion set at 3.00 copies. The range of copies at the cut-off goes from 3.10 copies to 4.73 copies. The cut-off in cycles are comprised between 35.14 cycles and 41.66 cycles. The percentage of the labs with a cut-off corresponding to a number of copies > 3.50 for this proficiency test was 61.5 %.

Considering the poultry cut-off, two NRLs did not reach the minimum criterion of 9.00 copies. Nevertheless, it did not impact their results as no deviation was recorded. The range of copies at the cut-off goes from 7.92 copies to 13.40 copies. The cut-off in cycles are comprised between 35.12 cycles and 43.20 cycles. No clear link between the cut-off (expressed in cycles or in number of copies) and the false results obtained by the participants can be made.

4.2.2. Qualitative analyses from the non-EU participants

4.2.2.1. Individual performances

Individual performances were assessed for three non-EU participants who reported PCR results by calculating the accuracy, sensitivity and specificity over the samples. Their results are to be found in Table 12.

Table 12 : Non-EU participant proficiencies regarding the detection of ruminant, pig and poultry material. Ranking follows AC values. Cells in black refers to excellent labs. Cells in blue refers to satisfying labs.

Lab code	AC	SE	SP
32 and 33	1.000	1.000	1.000
29	0.895	0.750	1.000

Labs 32 and 33 obtained excellent results (no deviation).

Lab 29, two negative deviations for the detection of pig and poultry DNA respectively were recorded with sample 1 (poultry feed containing 0.1 % of ruminant PAP, 0.1 % of pig PAP and 0.1 % of poultry PAP).

4.2.2.2. Assessment of the cut-off values

Labs 32 and 33 have cut-off values that comply with the minimum criteria (9 copies for the ruminant and the poultry PCR tests ; 3 copies for the pig PCR test) set by the EURL-AP.

Concerning Lab 29, the criterion is reached for the ruminant test but data were missing for the pig and the poultry tests.

5. Discussion and conclusions

The results obtained by light microscopy highlighted commonly occurring issues as well as some new ones deserving comments.

As observed from the past, fish detection has to deal with baseline specificity problems. Due to the absence of any fish material within the sample set, this was clearly demonstrated. Explanations for the false positive findings of fish particles rely either on some laboratory carry-over problems or on erroneous interpretation of fragments presenting some visual markers usually attributed to fish material. The later explanation is the most plausible one as supported by the results obtained on the feather meal. In this sample five cases of erroneous findings of fish material were reported. Among those cases, the uncertainty of having fish material is expressed in two comments; there is mention of unusual structures looking very “fishy” but lacking typical features and the absence of other structures relevant for fish material such as scales or gill particles. Such hesitations lead to wrong results. The origin of this confusion may probably be linked to the presence of feather rachis fragments presenting area on which barbs were attached, such fragments may effectively have a “fishy” aspect.

Concerning problems linked to the identification of terrestrial vertebrates, the results point to the difficulty of identifying milk powder (50 %) or interpreting the presence of milk powder (8 %). Effectively, some NRLs mentioned in their comments the presence of milk but erroneously decided to declare the sample as negative for terrestrial vertebrates. The report of the 2021 EURL-AP proficiency test however yet explained how to report in such situation [7]. Among the correct results for this sample, on the exception of one NRL only declaring “bones and muscles” which is obviously an incorrect detailed description, all other NRLs identified and referred to the presence of “milk, milk globules, milk powder, whey powder and lactose crystals”. At last, among the positive answers, one NRLs referred to the presence of blood products (plasma). Such ambiguation can be released by a simple TMB/H_2O_2 test : plasma will immediately turns turquoise while milk powder won’t. This recommendation was also written in the 2021 EURL-AP proficiency test [7].

About the sample consisting of porcine liver meal and not submitted to proficiency assessment, results delivred by the participants were particularly interesting. During the homogeneity study, this material showed a very limited sediment content allowing barely to prepare one slide from this fraction. Within the sediment only a very few bones were detected and from slides prepared from the flotate only two hairs and a single muscle fibre could be identified on the ten entities studied. All other particles were lacking any of the usual morphological markers allowing to identify them which is logical knowing that liver is an organ not belonging to the musculoskeletal system. This means that such material, although being strictly from animal origin, according to the parameters in use for light microscopy could only be declared for animal presence as negative or, at the best, as below the decision limit of the method. Due to this equivocal situation the sample was therefore removed from the proficiency assessment. Compared to the homogeneity study, results of this sample delivered by the participants, at least for the parameter “terrestrial vertebrates” are summarised as follows: 88 % have considered it as positive, 4 % as < LOD and 8 % as negative. Positive declarations were based on the observations illustrated on figure 1.

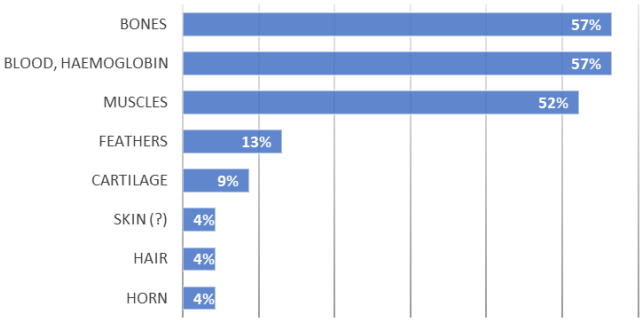


Figure 1: Percentages of declared identified structures in porcine liver meal.

The most reported identified structures were bones, blood (including haemoglobin or blood-like particles) and muscles. Observations that were largely differing from those obtained during the homogeneity study since 56% of the positive results were based on two determinations and thereof more than 10 of these particles were reported by the concerned NRLs. The homogeneity study did not achieve comparable rates of identifiable structures. Among the NRLs having reported blood structures, three mentioned that the observed particles were however not reacting with TMB/H_2O_2 while one confirmed the presence of

haemoglobin by mass-spectrometry. It is worth to mention that one NRL in its comments identified the sample as a liver meal. This type of PAP prepared from soft tissues appears as difficult both in terms of observations and interpretation.

To conclude on the microscopic results, the number of excellent and satisfactory scores obtained within the network of NRLs reached respectively 39 % (34.5 % in 2021) and 42 % (31 % in 2021). The rate of underperforming NRL for the present study reached 19% (34.5 % in 2021). Dedicated follow-up actions will be undertaken for each of them.

Concerning results from non-EU participants, encountered problems were in all comparable. The major source of error was also related to the disclosure of milk powder in sample 2. For these participants the percentage of satisfying results reached 60 % while 40 % were, according to the applied performance criteria, categorised as underperforming.

Looking at the PCR results, the performances of the NRL network are assessed for the first time with the three PCR tests (ruminant, pig and poultry) validated and implemented in the network. It has to be noticed that this year again, all the samples had to be analysed by PCR independently of the light microscopy results. Consequently, the PCR results reflect more objectively the real performances of the participants obtained with these methods.

The PCR skill of the NRLs network specifically with the ruminant PCR method continues to be confirmed with only 1 deviation out of 156 results (0.6 %). The performance of the network is also almost perfect with the pig PCR method. Here again, 1 deviation out of 156 results. More deviations (5 out of 182 results – 2.7 %) were recorded with the poultry PCR method. The rate of false results remains acceptable but needs to be followed during the next proficiency tests.

The individual performances of the participants are also quite encouraging. Twenty NRLs out of 26 (77 %) returned results without any deviation. Five NRLs had only one deviation recorded out of 19 results : four labs obtained a negative deviation with the detection of poultry DNA. For the last lab, it was a positive deviation with the ruminant PCR test. Only one lab had two deviations: one positive deviation with the pig PCR method and 1 negative deviation with the poultry assay.

It has also to be noticed that two results were kept out of assessment by the organisers due to divergent results obtained during the homogeneity study. For the sample type 2 (Pig feed + 2 % milk powder), all the participants came to the same conclusion as the organisers : absence of pig DNA. Concerning the sample type 6 (porcine liver meal), only 3 NRLs reported a presence of ruminant DNA. Fundamentally, the removing of these two parameters did not change the excellent performances of the network.

Acknowledgment

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References

[1] EU. 2017. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation). Official Journal of the European Union L 95, 7/4/2017: 1-142.

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

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

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

[5] Veys P., Fumière O. and Marien A. 2020. Combined microscopy-PCR EURL-AP Proficiency Test 2019: Final version – version 02. CRA-W, Gembloux, Belgium

[6] ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparison.

[7] Veys P., Fumière O. and Marien A. 2022. Combined microscopy-PCR EURL-AP Proficiency Test 2021: Final version. CRA-W, Gembloux, Belgium.

Annex 1

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

Country	Institute Name
Austria	Austrian Agency for Health and Food Safety
Belgium	Federal Agency for the Safety of the Food Chain
Bulgaria	National Diagnostic Research Veterinary Medical Institute
China	*China Agricultural University*
Croatia	Croatian Veterinary Institute
Cyprus	Cyprus Veterinary Services
Czech Republic	State Veterinary Institute Jihlava
Denmark	Ministry of Food, Agriculture and Fisheries Danish Veterinary and Food Admin
Estonia	Veterinary and Food Laboratory
Finland	Finnish Food Safety Authority
France	DG for Fair Trading, Consumer Affairs and Fraud Control-Laboratory Directorate Rennes
Germany	Federal Institute for Risk Assessment
Greece	Feedstuffs Control Laboratory
Hungary	Central Agricultural Office-Directorate Food and Feed Safety-Central Feed Investigation Lab.
Ireland	Department of Agriculture and Food Microscopy Laboratory - Seed Testing Station
Italy	National Reference Centre for the Surveillance and Monitoring of Animal Feed
Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
Lithuania	National Food and Veterinary Risk Assessment Institute
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	Wageningen Food Safety Research
Norway	*Institute of Marine Research*
Peru	*Bureau Veritas*
Poland	National Veterinary Research Institute
Portugal	Instituto Nacional de Investigacao Agraria e Veterinaria
Romania	Hygiene Institute of Veterinary Health
Serbia	*Institute of Veterinary Medicine of Serbia*
Slovakia	State Veterinary and Food Institute
Slovenia	Veterinary faculty - National Veterinary Institute - Institute of Food Safety, Feed and Environment - Department of Environment, Animal Nutrition, Welfare and Hygiene
Spain	Laboratorio Arbitral Agroalimentario
Sweden	National Veterinary Institute, Department of Animal Feed
Thailand	*Bureau of Quality Control of Livestock Products*
United Kingdom	*Animal and Plant Health Agency*

Annex 2

Announcement letter

European Union Reference Laboratory for Animal Proteins in feedingstuffs

Walloon Agricultural Research Centre, Knowledge & valorisation of Agricultural Products Department

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Announcement of the EURL-AP proficiency test 2022/01 for the determination of Processed Animal Proteins (PAPs) in feed

Introduction

The use of processed animal by-products as ingredient for animal feedingstuffs within the European Union is regulated by the TSE Regulation (Regulation EC N°999/2001), as amended. In particular, Article 7 imposes a prohibition to use processed animal proteins in the feeding of farmed animals (extended feed ban).

Commission implementing Regulation (EU) No 2022/893 amending Annex VI of Regulation (EC) No 152/2009, imposes the methods of analysis for the determination of constituents of animal origin for the official control of feed.

Objectives

The objective of the present proficiency test is to assess the performance of the NRLs to detect the presence of PAPs in feed by the reference methods using light microscopy and PCR as stated in Regulation EC 152/2009 as amended by Commission implementing Regulation (EU) No 2022/893 and related SOPs.

The organizer team

The test will be coordinated by the European Union Reference Laboratory for Animal Proteins in feedingstuffs (EURL-AP).

Test material

Samples containing typical compound feed fortified with processed animal proteins (PAPs) will be prepared. The EURL-AP will endorse the homogeneity of the samples. Nevertheless, each laboratory participating to the test is sole responsible to reach appropriate homogeneity for the sample sub-portions taken for analysis.

Each participant will receive a maximum of 7 samples, each of about 40g. Each sample shall be analysed both by light microscopy and PCR.

General outline of the exercise

- The light microscopic and PCR methods to use are described in Annex VI of Commission Regulation EC 152/2009 and related SOPs.
- Parameters that will be assessed are : terrestrial vertebrates and fish presence/absence, DNA presence/absence from ruminants, pig and poultry.
- The EURL-AP will provide participants with an Excel file for reporting the results of the proficiency test analyses.
- Each participating laboratory will be assigned a unique code and only the organizer of the study knows the key to this code. After completing the test each laboratory will get a report including its results and lab code. A final report of the study will be published with anonymised results.
- The participation in this proficiency study is mandatory and free of charge for national reference laboratories within Member States of the European Union.

European Union Reference Laboratory for Animal Proteins in feedingstuffs

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Time schedule

- Official announcement of the study to the NRLs by way of the intranet and e-mail : 9 September 2022
- Sending of the sample boxes and communication of the instructions : 14 October 2022

> ***By default, samples will be sent to the NRL microscopy contact person referred on the intranet. You are asked to check if this person is still your contact and to inform the organizer from any change.***

- Deadline for returning of results to the organizer : 18 November 2022

Further information

- Refer to the address and coordinates mentioned in the heading,

or

- Dr Pascal VEYS
  EURL-AP NRL Network Manager
  ☎32 (0) 81 87 52 28
  32 (0) 81 87 40 19
  E-mail: p.veys@cra.wallonie.be

or

- Dr Olivier FUMIERE
  Head of EURL-AP Molecular biology team
  ☎32 (0) 81 87 52 40
  32 (0) 81 87 40 19
  E-mail: o.fumiere@cra.wallonie.be

Annex 3

Excel result report form

Proficiency Test Microscopy-PCR 2022/01

Laboratory identification

Laboratory code : 1

Responsibility agreement : No

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

Report

Lab code	1	1	1	1	1	1	1
Sample rank	1st	2nd	3th	4th	5th	6th	7th
Sample N°							
Method of analysis	**Light microscopy**						

Light microscopy analyses

Terrestrial vertebrates particles							
details of particles *Only to fill in if in the cell above "present" or "< LOD" is chosen.*							
Fish particles							
details of particles *Only to fill in if in the cell above "present" or "< LOD" is chosen.*							
Analyses performed on							
Number of determinations							
Free comment *Example : presence of unusual fragments,…*							

Method of analysis	**PCR**		
	Ruminant	Poultry	Pig
Cut-off at 15 *(5 for pig)* copies of the PCR platform used (in cycles)			
Copy number at the cut-off of the PCR platform used (in copies)			
Maxter mix used			

PCR analyses

Sample N°							
Ruminant DNA							
Pig DNA							
Poultry DNA							
Dilution 1 (e.g. 1 fold)							
Ct value replicate 1							
Ct value replicate 2							
Dilution 2 (e.g. 10 fold)							
Ct value replicate 1							
Ct value replicate 2							
Comment *Example : PCR inhibition,…*							

Annex 4

Gross results of participants for microscopy (in numerical order of lab ID)

Laboratory identification code : **1**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
7	112	Present	bones, muscles	Absent		Sed. + Flot.	1
4	246	Absent		Absent		Sed. + Flot.	1
2	522	Present	milk	Absent		Sed. + Flot.	1
6	544	Present	bones, blood	Absent		Sed. + Flot.	2
1	660	Present	bones, muscles	Absent		Sed. + Flot.	1
3	748	Present	feathers, keratin parts, bones	Absent		Sed. + Flot.	1
5	752	Present	bones	Absent		Sed. + Flot.	1

Laboratory identification code : **2**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	92	Absent		Absent		Sed. + Flot.	2
7	266	Present	bones	Present	fishbones	Sed. + Flot.	2
2	438	Present	blood products (plasma)	Absent		Sed. + Flot.	2
5	458	Present	bones	Absent		Sed. + Flot.	2
3	608	Present	bones, feathers	Present	fishbones	Sed. + Flot.	2
1	646	Present	bones	Present	fishbones	Sed. + Flot.	2
6	768	Present	bones, blood products	Absent		Sed. + Flot.	2

Laboratory identification code : **3**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	62	Present	Bones, feathers	Absent		Sed. + Flot.	2
5	94	Present	Bones	Absent		Sed. + Flot.	2
2	130	Present	Milk globules	Absent		Sed. + Flot.	2
4	148	Absent		Absent		Sed. + Flot.	1
6	348	Present	Bones, muscle fiber	Absent		Sed. + Flot.	2
7	392	Present	Bones	Absent		Sed. + Flot.	2
1	744	Present	Bones	Absent		Sed. + Flot.	2

Water was used as a mounting medium for detecting milk derivates in flotate in sample 130.

Laboratory identification code : **4**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
7	126	Present	bones	Absent		Sed. + Raw	1
4	288	Absent		Absent		Sed. + Raw	1
5	332	Present	blood, bones, muscles	Absent		Sed. + Raw	1
6	334	Present	muscles, skin?	Absent		Sed. + Raw	1
3	468	Present	feather, bones, muscle	Present	bones	Sed. + Raw	1
2	480	Present	milk	Absent		Sed. + Raw	1
1	506	Present	bomes, muscle	Absent		Sed. + Raw	1

In sample 468 unusual structures look very "fishy" without typical lacunae.

Laboratory identification code : **5**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	260	Absent		Absent		Sed. + Flot.	1
3	342	Present	bones, meat, (hydrolysed) feathers	Absent		Sed. + Flot.	1
5	388	Present	bones	Absent		Sed. + Flot.	1
6	404	Present	bones, meat, pig hair	Absent		Sed. + Flot.	1
1	688	Present	bones, meat	Absent		Sed. + Flot.	1
7	714	Present	bones, meat	Absent		Sed. + Flot.	1
2	760	< LOD	bones	Absent		Sed. + Flot.	2

260: >10 insect particles in flotate.

Laboratory identification code : **6**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	274	Absent		Absent		Sed. + Flot.	2
3	356	Present	Bones, Cartilage, Feathers	Absent		Sed. + Flot.	2
5	444	Present	Bones	Absent		Sed. + Flot.	2
1	576	Present	Bones, muscle fibers	Absent		Sed. + Flot.	2
7	672	Present	Bones, teeth, muscle fibers	Absent		Sed. + Flot.	2
2	746	Absent		Absent		Sed. + Flot.	2
6	754	Present	Bones, muscle fibers	Absent		Sed. + Flot.	2

For samples 274 and 746, two determinations were carried-out, although it would not have been necessary according to the legal protocol. This was done because several people are qualified for this type of analysis in the lab and the entire procedure was performed twice by 2 different people as an exercice.
Sample 754 has delivered only very little sediment.

Laboratory identification code : **7**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
5	10	Absent		Present	fish bones	Sed. + Flot.	2
7	56	Present	bones, muscle	Present	fish bones	Sed. + Flot.	2
3	132	Present	bones, cartilage, muscle, feather, hydrolized feather, blood	Absent		Sed. + Flot.	2
1	212	Present	bones	Present	fish bones	Sed. + Flot.	2
6	320	Present	blood, (muscle <LOD) hydrolized feather	< LOD	fish bones	Sed. + Flot.	2
2	550	Absent		Absent		Sed. + Flot.	2
4	666	Absent		Absent		Sed. + Flot.	2

sample 10: presence of powder milksample 550: presence of powder milk

Laboratory identification code : **8**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	106	Absent		Absent		Sed. + Flot.	
6	264	Present	blood	Present	bones, blood.	Sed. + Flot.	
2	298	Present	bones, muscle	Absent		Sed. + Flot.	
7	462	Present	bones	Absent		Sed. + Flot.	
1	478	Present	bones	Absent		Sed. + Flot.	
3	496	Present	bones	Absent		Sed. + Flot.	
5	626	Present	bones	Absent		Sed. + Flot.	

Laboratory identification code : **9**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	114	Present	bone, cartilage	Absent		Sed. + Raw	2
5	234	Present	bone	Absent		Sed. + Raw	2
2	494	Present	milk powder	Absent		Sed. + Raw	1
3	552	Present	bone, meat, feather, cartilage	Absent		Sed. + Raw	2
6	614	Absent		Absent		Sed. + Raw	1
7	630	Present	bone, cartilage	Absent		Sed. + Raw	2
4	708	Absent		Absent		Sed. + Raw	1

494 had a strange smell that could indicate buttermilk powder

Laboratory identification code : **10**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	22	Absent		Absent		Sed. + Raw	1
3	76	Present	bones, feathers	Absent		Sed. + Raw	2
5	220	Present	bones	Absent		Sed. + Raw	2
6	250	Present	horn	Absent		Sed. + Raw	2
1	492	Present	bones, muscle fibers	Absent		Sed. + Raw	2
2	592	Absent		Absent		Sed. + Raw	1
7	658	Present	bones, muscle fibers	Absent		Sed. + Raw	2

Laboratory identification code : **11**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	282	Present	bones, muscle	Absent		Sed. + Flot.	2
4	372	Absent		Absent		Sed. + Flot.	1
6	390	Present	bones, muscle, hydrolysed feathers	Absent		Sed. + Flot.	2
2	466	Absent		Absent		Sed. + Flot.	1
7	574	Present	bones, muscle	Absent		Sed. + Flot.	2
3	664	Present	bones, cartilage, non-hydrolysed and hydrolysed feathers	Absent		Sed. + Flot.	2
5	738	Present	bones	Absent		Sed. + Flot.	2

TMB negative for sample Nr.390.

Laboratory identification code : **12**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	88	Absent		Absent		Sed. + Flot.	2
4	512	Absent		Absent		Sed. + Flot.	1
7	518	Present	bones	Absent		Sed. + Flot.	1
5	528	Present	bones	Absent		Sed. + Flot.	2
6	558	Present	blood	Absent		Sed. + Flot.	2
1	604	Present	bones	Absent		Sed. + Flot.	1
3	622	Present	bones	Absent		Sed. + Flot.	1

Laboratory identification code : **13**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
6	152	Present	Blood elements	Absent		Sed. + Flot.	1
3	230	Present	bones, muscles, feathers	Present	bones,	Sed. + Flot.	1
1	240	Present	bones, muscles	Absent		Sed. + Flot.	1
7	252	Present	bones, muscles	Absent		Sed. + Flot.	1
2	382	Present	milk (lactose crystals)	Absent		Sed. + Flot.	1
5	486	Present	Bones	Absent		Sed. + Flot.	1
4	498	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **14**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	48	Present	bones, muscles, feathers / hydrolyzed feathers	Absent		Sed. + Flot.	1
1	58	Present	bones, muscles, milk/whey powder	Absent		Sed. + Flot.	1
4	78	Absent		Absent		Sed. + Flot.	1
2	410	Present	milk/whey powder	Absent		Sed. + Flot.	1
6	516	Present	bones, muscles, blood	Absent		Sed. + Flot.	1
5	542	Present	bones	Absent		Sed. + Flot.	1
7	588	Present	bones	Absent		Sed. + Flot.	1

Laboratory identification code : **15**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
6	96	Present	terrestrial bones, cartilage	Absent		Sed. + Raw	2
5	178	Present	terrestrial bones, cartilage	Absent		Sed. + Raw	2
4	218	Absent		Absent		Sed. + Raw	1
2	228	Present	milk globules, lactose crystals	Absent		Sed. + Raw	2
1	436	Present	terrestrial bones,	Absent		Sed. + Raw	2
7	490	Present	terrestrial bones,	Absent		Sed. + Raw	2
3	720	Present	terrestrial bones, cartilage, muscle	Present	fish bones, cartilage, muscle	Sed. + Raw	2

Sample no. 96: small amount of sediment obtained after TCE sedimentation. Raw material stained with TMB+H202, no reaction and no colour development.
Sample no.720: In addition to terrestrial bones, we also noticed the presence of bone particles that we could not identify as terrestrial, they were therefore categorised as fish bones even though we couldn't see the "typical" fish features. We didn't notice the presence of other fish particles (scales, gills, otolith, etc).

Laboratory identification code : **16**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	146	Present	bones, muscles, cartilages, feathers	Absent		Sed. + Raw	1
1	184	Present	bones	Absent		Sed. + Raw	1
7	210	Present	bones	Absent		Sed. + Raw	1
6	222	Present	bones, muscles	Present	fishbones, muscles	Sed. + Raw	1
5	248	Present	bones	Absent		Sed. + Raw	1
4	596	Absent		Absent		Sed. + Raw	1
2	718	Absent		Absent		Sed. + Raw	1

Laboratory identification code : **17**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	36	Absent		Absent		Sed. + Raw	1
7	154	Present	'bones, muscle fibers, cartilages	Absent		Sed. + Raw	1
3	160	Present	'bones, feathers	Absent		Sed. + Raw	1
6	474	Absent		Absent		Sed. + Raw	1
1	674	Present	'bones, muscle fibers, cartilages	Absent		Sed. + Raw	1
5	682	Present	'bones, cartilages	Absent		Sed. + Raw	1
2	704	Absent		Absent		Sed. + Raw	1

Sample 474 : insufficient residue and unsieve . Sample 682 : Determination on 3g.

Laboratory identification code : **18**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	46	Absent		Absent		Sed. + Flot.	2
6	124	Present	Blood	Absent		Sed. + Raw	2
7	182	Present	Bones	Absent		Sed. + Flot.	2
5	430	Present	Bones	Absent		Sed. + Flot.	2
4	526	Absent		Absent		Sed. + Flot.	1
3	538	Present	Bones	Absent		Sed. + Flot.	2
1	702	Present	Bones	Absent		Sed. + Flot.	2

Laboratory identification code : **19**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	116	Present	milk globules	Absent		Sed. + Flot.	1
7	294	Present	bones, muscle fibers	Absent		Sed. + Flot.	1
3	454	Present	bones, muscle fibres, feathers,	Present	fish bones	Sed. + Flot.	1
1	548	Present	bones, muscle fibres, feather	Absent		Sed. + Flot.	1
4	610	Absent		Absent		Sed. + Flot.	1
6	628	Present	blood products, feathers, muscle fiber	Absent		Sed. + Flot.	1
5	668	Present	bones	Absent		Sed. + Flot.	1

Laboratory identification code : **20**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	2	Present	bone, cartilage and muscles	Absent		Sed. + Flot.	1
6	68	Present	bone, cartilage and muscles	Absent		Sed. + Raw	1
2	102	< LOD	bone	Absent		Sed. + Flot.	2
4	232	Absent		Absent		Sed. + Flot.	1
3	272	Present	bone, cartilage, muscles and feathers	Absent		Sed. + Flot.	1
7	322	Present	bone, cartilage and muscles	Absent		Sed. + Flot.	1
5	346	Present	bone and cartilage	Absent		Sed. + Flot.	1

3 samples were ground (no 2, 232 and 322) 4 samples were stained with Alizarin (no 2, 272, 322 and 346)

Laboratory identification code : **21**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
7	28	Present	bones, cartilage, muscle fibres	Absent		Sed. + Flot.	1
1	44	Present	bones, cartilage, muscle fibres	Absent		Sed. + Flot.	1
5	66	Present	bones, cartilage, muscle fibres, feather meal	Absent		Sed. + Flot.	1
6	236	Present	blood meal, bones, muscles fibres	Absent		Sed. + Flot.	1
2	354	Absent		Absent		Sed. + Flot.	1
3	384	Present	feather meal, bones, cartilage, muscle fibres	Absent		Sed. + Flot.	1
4	750	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **22**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
1	16	Present	bones, cartilages, muscle fibers fragments (low presence)	Absent		Sed. + Flot.	1
6	208	< LOD	bones	Absent		Sed. + Flot.	2
2	214	Absent		Absent		Sed. + Flot.	1
5	318	Present	bones, cartilages, blood particles, muscle fibers fragments (low presence)	Absent		Sed. + Flot.	1
7	546	Present	bones, cartilages, muscle fibers fragments (low presence)	Absent		Sed. + Flot.	1
3	636	Present	bones, cartilages, feathers, muscle fibers	Absent		Sed. + Flot.	1
4	764	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **23**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
6	40	Present	Blood	Absent		Sed. + Flot.	1
5	108	Present	Bones, Blood	Absent		Sed. + Flot.	1
2	144	Absent		Present	Bones	Sed. + Flot.	1
1	170	Present	Bones, muscles	Absent		Sed. + Flot.	1
7	504	Present	Bones, muscles	Absent		Sed. + Flot.	1
3	692	Present	Futhers, Bones, muscles	Absent		Sed. + Flot.	1
4	694	Absent		Absent		Sed. + Flot.	1

In the flotate of the sample 144 it was detected milk

Laboratory identification code : **24**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	4	< LOD	Bone	Absent		Sed. + Flot.	2
4	176	Absent		Absent		Sed. + Flot.	1
1	254	Present	Bone, muscle	< LOD	Bone	Sed. + Flot.	2
5	304	Present	Bone	Present	Bone	Sed. + Flot.	2
7	560	Present	Bone, muscle	< LOD	Bone	Sed. + Flot.	2
3	678	Present	Bone, muscle, feathers	Absent		Sed. + Flot.	2
6	740	Present	Bone, muscle	Absent		Sed. + Flot.	2

Laboratory identification code : **25**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
5	38	Present	bones	Absent		Sed. + Flot.	2
7	336	Present	bones, muscle fibres it can't be exluded muscle fibres found only derive from terrestrial vertebrates	Absent		Sed. + Flot.	2
4	344	Absent		Absent		Sed. + Flot.	1
1	394	Present	bones, muscle fibres it can't be exluded muscle fibres found only derive from terrestrial vertebrates	Absent		Sed. + Flot.	2
2	508	Present	milk products	Absent		Sed. + Flot.	2
3	510	Present	bones, feathers, muscle fibres it can't be exluded muscle fibres found only derive from terrestrial vertebrates	Absent		Sed. + Flot.	2
6	698	Present	bones, blood/haemoglobin	Absent		Sed. + Flot.	2

sample 508: Milk products confirmed by MS Sample 698: Haemoglobin confirmed by MS

Laboratory identification code : **26**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	120	Absent		Absent		Sed. + Flot.	2
1	128	Present	bone fragments	Absent		Sed. + Flot.	2
6	166	Present	Blood product-like particles, negative to TMB	Absent		Sed. + Flot.	2
5	262	Present	bone fragments	Absent		Sed. + Flot.	2
2	396	Absent		Absent		Sed. + Flot.	2
7	420	Present	bone fragments	Absent		Sed. + Flot.	2
3	566	Present	bone fragments, feathers	Absent		Sed. + Flot.	2

Sample 166: negative to TMB staining. Looks like liver meal (so expensive). Sample 396: couldn't find any TV particles we were able to detect

Laboratory identification code : **29**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
2	32	Absent		Absent		Sed. + Flot.	1
4	50	Absent		Absent		Sed. + Flot.	1
6	138	Present	bone and blood	Absent		Sed. + Flot.	1
1	296	Present	bone and muscle	Absent		Sed. + Flot.	1
7	308	Present	bone	Absent		Sed. + Flot.	1
3	426	Present	bone, muscle and fearther	Absent		Sed. + Flot.	1
5	514	Present	bone	Absent		Sed. + Flot.	1

Laboratory identification code : **30**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
4	386	Present	blood	Absent		Sed. + Flot.	2
3	314	Present	bones, feathers	Absent		Sed. + Flot.	2
2	270	Absent		Absent		Sed. + Flot.	2
7	476	Present	blood, bones	Absent		Sed. + Flot.	2
1	156	Present	bones	Present	bones	Sed. + Flot.	2
5	584	Present	bones	Absent		Sed. + Flot.	2
6	726	Present	bones	Absent		Sed. + Flot.	2

Laboratory identification code : **32**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
6	54	< LOD	5 bone particles detected in first determination and 2 in second determination. Very litle sediment was recoved and not enough for 2 determinations. Negative on cystine test, Fehling and for lactose crystals. Negative for TMB. Raw material was also investigated due to the low ammounts of sediments.	Absent		Sed. + Flot.	2
7	140	Present	Bone particles and cartilage	Absent		Sed. + Flot.	1
5	360	Present	bone particles > 5. Positive for blood (TMB) in raw samples	Absent		Sed. + Raw	1
3	398	Present	More than 5 bone particles (poultry), cartilage particles, feather like structures, muscle fibers.	Present	Bone particles, more than 5.	Sed. + Flot.	1
2	606	Present	More than five bone fragments, cartilage fragments	Present	Bone and cartilage, possebly gill framents	Sed. + Flot.	1
1	618	Present	> 5 bone particles	Absent		Sed. + Flot.	1
4	638	Absent		Absent		Sed. + Flot.	1

Very litle sediment in sample nr 54. The bone particles in sample number 360 were also a bit different to what we would expect but showed caniculi.

Laboratory identification code : **33**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
5	24	Present	bones, blood	Absent		Sed. + Flot.	1
3	202	Present	bones	Absent		Sed. + Flot.	1
1	324	Present	bones	Absent		Sed. + Flot.	1
7	350	Present	bones	Absent		Sed. + Flot.	1
6	418	Present	muscles	Absent		Sed. + Flot.	1
4	442	Absent		Absent		Sed. + Flot.	1
2	536	Absent		Absent		Sed. + Flot.	1

Laboratory identification code : **36**

Sample type	Sample N°	Terrestrial animal part.	Details of terrestrial part.	Fish part.	Details of fish part.	Fractions used	Number of determinations
3	6	Present	Bones, Muscle fibres, Feathers	Absent		Sed. + Flot.	2
5	122	Absent		Present	Bones	Sed. + Flot.	2
1	268	Present	Bones, Muscle fibres, Feathers	Present	Bones, Muscle fibres	Sed. + Flot.	2
6	306	Present	Muscle fibres, Hairs, Blood drum dried	Absent		Sed. + Flot.	2
4	400	Absent		Absent		Sed. + Flot.	2
7	616	Present	Bones, Muscle fibres, Feathers	Present	Bones, Muscle fibres	Sed. + Flot.	2
2	690	Present	Bones, Muscle fibres, Milk particle	Absent		Sed. + Flot.	2

Annex 5

Gross results of participants for PCR (in numerical order of lab ID)

Laboratory identification code : **1**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,43	43,20	40,48	cycles
Copy number at the cut-off :	10,16	9,05	3,52	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
7	112	Absent	Present	Absent	
4	246	Absent	Absent	Absent	
2	522	Present	Absent	Absent	
6	544	Absent	Present	Present	no inhibition
1	660	Present	Present	Present	
3	748	Absent	Absent	Present	
5	752	Absent	Absent	Absent	

Laboratory identification code : **2**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	37,16	38,16	39,00	cycles
Copy number at the cut-off :	10,29	13,40	4,68	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
4	92	Absent	Absent	Absent	
7	266	Absent	Present	Absent	
2	438	Present	Absent	Absent	
5	458	Absent	Absent	Absent	
3	608	Absent	Absent	Present	
1	646	Present	Present	Present	
6	768	Absent	Present	Absent	

Laboratory identification code : **3**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	34,23	36,59	37,86	cycles
Copy number at the cut-off :	11,08	11,18	3,76	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
3	62	Absent	Absent	Present	Pig DNA: dilution 1 (x5: 38,22; 38,42), dilution 2 (x20: 39,07; 40,95).
5	94	Absent	Absent	Absent	Pig DNA: dilution 1 (x2: 38,37; 38,74), dilution 2 (x20: 40,18; 40,41).
2	130	Present	Absent	Absent	Pig DNA: dilution 1 (5x: 40,97; 41,15), dilution 2 (x20 - undetermined).
4	148	Absent	Absent	Absent	Pig DNA: dilution 1 (x2: 38,14; 38,57), dilution 2 (x20 - undetermined).
6	348	Absent	Present	Present	Pig DNA: dilution 1 (x2: 20,05; 20,21), dilution 2 (x20: 22,96; 23,53)
7	392	Absent	Present	Absent	Pig DNA: dilution 1 (x2: 29,94; 31,18), dilution 2 (x20: 33,32; 35,04).
1	744	Present	Present	Present	Pig DNA: dilution 1 (x2: 30,05; 30,70), dilution 2 (x20: 33,25; 33,87).

Laboratory identification code : **4**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	31,91	35,13	36,10	cycles
Copy number at the cut-off :	10,32	10,13	3,35	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
7	126	Absent	Present	Absent	
4	288	Absent	Absent	Absent	
5	332	Absent	Absent	Absent	
6	334	Absent	Present	Absent	
3	468	Absent	Present	Present	
2	480	Present	Absent	Absent	
1	506	Present	Present	Present	

Laboratory identification code : **5**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,65	38,05	39,60	cycles
Copy number at the cut-off :	10,82	11,10	3,71	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
4	260	Absent	Absent	Absent	
3	342	Absent	Absent	Present	
5	388	Absent	Absent	Absent	
6	404	Absent	Present	Present	
1	688	Present	Present	Present	
7	714	Absent	Present	Absent	
2	760	Present	Absent	Absent	

Laboratory identification code : **6**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	32,37	37,31	38,59	cycles
Copy number at the cut-off :	11,47	9,27	3,58	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
4	274	Absent	Absent	Absent	Above are the Ct values in the following order : Ruminant / Pig /
3	356	Absent	Absent	Present	Above are the Ct values in the following order : Ruminant / Pig /
5	444	Absent	Absent	Absent	Above are the Ct values in the following order : Ruminant / Pig /
1	576	Present	Present	Present	Above are the Ct values in the following order : Ruminant / Pig /
7	672	Absent	Present	Absent	Above are the Ct values in the following order : Ruminant / Pig /
2	746	Present	Absent	Absent	Above are the Ct values in the following order : Ruminant / Pig /
6	754	Absent	Present	Present	Above are the Ct values in the following order : Ruminant / Pig /

Laboratory identification code : **7**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,20	39,46	39,76	cycles
Copy number at the cut-off :	11,48	8,44	3,10	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
5	10	Absent	Absent	Absent	
7	56	Absent	Present	Absent	low inhibition, not affecting the result.
3	132	Absent	Absent	Present	For pig PCR, 1st testing: pos&neg, 2nd testing: pos&neg. Result
1	212	Present	Present	Present	
6	320	Absent	Present	Present	
2	550	Present	Present	Absent	
4	666	Absent	Absent	Absent	

Laboratory identification code : **8**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	32,87	35,12	35,14	cycles
Copy number at the cut-off :	11,89	12,66	4,73	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
4	106	Absent	Absent	Absent	
6	264	Present	Present	Present	
2	298	Present	Absent	Absent	
7	462	Absent	Present	Absent	
1	478	Present	Present	Present	
3	496	Absent	Absent	Present	
5	626	Absent	Absent	Absent	

Laboratory identification code : **9**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	34,98	40,49	38,31	cycles
Copy number at the cut-off :	9,00	7,92	3,32	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
1	114	Present	Present	Present	Values for Ruminant in tabel. Pig 1x and 10x. 34,67, 33,98, 37,83,
5	234	Absent	Absent	Absent	Values for Ruminant in tabel
2	494	Present	Absent	Absent	Values for Ruminant in tabel
3	552	Absent	Absent	Present	Values for Ruminant in tabel
6	614	Absent	Present	Present	Values for Ruminant in tabel
7	630	Absent	Present	Absent	Values for Ruminant in tabel
4	708	Absent	Absent	Absent	Values for Ruminant in tabel

Laboratory identification code : **10**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,66	39,89	41,01	cycles
Copy number at the cut-off :	11,06	9,46	3,27	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
4	22	Absent	Absent	Absent	
3	76	Absent	Absent	Present	
5	220	Absent	Absent	Absent	
6	250	Absent	Present	Present	
1	492	Present	Present	Present	
2	592	Present	Absent	Absent	
7	658	Absent	Present	Absent	

Laboratory identification code : **11**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,57	39,16	41,66	cycles
Copy number at the cut-off :	10,18	9,38	3,22	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
1	282	Present	Present	Present	Pig DNA: 1X- 34.32; 40.93; 10X -38.42; 37.74 (igb). Poultry DNA: 1X -
4	372	Absent	Absent	Absent	
6	390	Absent	Present	Present	Poultry DNA: 1X -35.08;37.72; 10X - 38.43; 37.48 (Ing)
2	466	Present	Absent	Absent	
7	574	Absent	Present	Absent	
3	664	Absent	Absent	Present	
5	738	Absent	Absent	Absent	

Laboratory identification code : **12**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	37,55	39,41	37,74	cycles
Copy number at the cut-off :	10,92	11,10	3,66	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
2	88	Present	Absent	Absent	
4	512	Absent	Absent	Absent	
7	518	Absent	Present	Absent	
5	528	Absent	Absent	Absent	
6	558	Present	Present	Present	
1	604	Present	Present	Present	
3	622	Absent	Absent	Present	

Laboratory identification code : **13**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	34,70	38,20	38,31	cycles
Copy number at the cut-off :	11,73	10,81	3,82	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
6	152	Absent	Present	Present	PIG - 1 fold: 20,96 / 20,62 10 fold: 24,12 / 24,6 POULTRY - 1 fold:
3	230	Absent	Absent	Present	PIG - 1 fold: 0,0 / 0,0 10 fold: 0,0 / 0,0 POULTRY - 1 fold: 34,18 /
1	240	Present	Present	Present	PIG - 1 fold: 27,15 / 29,76 10 fold: 30,83 / 33,02 POULTRY - 1 fold:
7	252	Absent	Present	Absent	PIG - 1 fold: 28,21 / 29,16 10 fold: 30,54 / 31,87 POULTRY - 1 fold:
2	382	Present	Absent	Absent	PIG - 1 fold: 45,6 / 40,97 10 fold: 0,0 / 47,1 POULTRY - 1 fold: 0,0
5	486	Absent	Absent	Absent	PIG - 1 fold: 0,0 / 0,0 10 fold: 0,0 / 0,0 POULTRY - 1 fold: 0,0 / 0,0
4	498	Absent	Absent	Absent	PIG - 1 fold: 48,57 / 43,23 10 fold: 0,0 / 0,0 POULTRY - 1 fold: 0,0

Laboratory identification code : **14**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,66	36,87	38,94	cycles
Copy number at the cut-off :	11,29	11,63	3,99	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
3	48	Absent	Absent	Present	Pig 1x undeter/undeter, 10x undeter/undeter. Poultry 1x
1	58	Present	Present	Present	Pig 1x 31,017/32,694, 10x 34,812/36,161 Poultry 1x 35,385/33,567,
4	78	Absent	Absent	Absent	Pig 1x undeter/undeter, 10x undeter/undeter Poultry 1x
2	410	Present	Absent	Absent	Pig 1x undeter/41,624 10x undeter/undeter Poultry 1x
6	516	Absent	Present	Present	Pig 1x 20,595/20,401 10x 23,228/22,985 Poultry 1x 33,088/32,455
5	542	Absent	Absent	Absent	Pig 1x 43,595/40,759 10x undeter/undeter Poultry 1x undeter/undeter
7	588	Absent	Present	Absent	Pig 1x 30,581/30,745 10x 33,946/34,899 Poultry 1x undeter/undeter

Laboratory identification code : **15**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,09	38,75	38,20	cycles
Copy number at the cut-off :	11,19	11,30	3,72	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
6	96	Absent	Present	Present	undiluted DNA caused PCR inhibition or partial inihibition. 10fold and
5	178	Absent	Absent	Absent	
4	218	Absent	Absent	Absent	
2	228	Present	Absent	Absent	
1	436	Present	Present	Present	
7	490	Absent	Present	Absent	
3	720	Absent	Absent	Present	

Laboratory identification code : **16**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	33,34	35,58	39,28	cycles
Copy number at the cut-off :	10,14	10,03	3,60	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
3	146	Absent	Absent	Present	
1	184	Present	Present	Present	
7	210	Absent	Present	Absent	
6	222	Absent	Present	Present	
5	248	Absent	Absent	Absent	
4	596	Absent	Absent	Absent	
2	718	Present	Absent	Absent	

Laboratory identification code : **17**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,90	40,89	39,58	cycles
Copy number at the cut-off :	9,86	10,29	3,38	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
4	36	Absent	Absent	Absent	2022-90438
7	154	Absent	Present	Absent	2022-90423
3	160	Absent	Absent	Present	2022-90425
6	474	Absent	Present	Present	2022-90426
1	674	Present	Present	Present	2022-90428
5	682	Absent	Absent	Absent	2022-90429 PCR inhibition (new extract 16/11/22)
2	704	Present	Absent	Absent	2022-90431

Laboratory identification code : **18**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,76	37,31	39,32	cycles
Copy number at the cut-off :	9,09	9,26	3,23	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
2	46	Present	Absent	Absent	
6	124	Absent	Present	Present	
7	182	Absent	Present	Absent	
5	430	Absent	Absent	Absent	
4	526	Absent	Absent	Absent	
3	538	Absent	Absent	Present	
1	702	Present	Present	Present	

Laboratory identification code : **19**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,76	37,31	39,32	cycles
Copy number at the cut-off :	9,09	9,26	3,23	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
2	116	Present	Absent	Absent	pig -absent, poultry -absent
7	294	Present	Present	Present	pig dilution 1 ct1-30,79, ct2-32,02, dilution 2-ct134,17, ct2-40,61.
3	454	Absent	Absent	Present	poultry dilution 1 ct1-34,47, ct2-34,16, dilution 2 ct1-38,21 ct2-37,02.
1	548	Present	Present	Present	poultry dilution 1 ct1-35,96, ct2-32,80, dilution 2 ct1-38,84 ct2-
4	610	Absent	Absent	Absent	pig -absent, poultry -absent
6	628	Absent	Present	Present	'poultry dilution 1 ct1-38,38, ct2-39,80, dilution 2 ct1-33,41 ct2-
5	668	Absent	Absent	Absent	pig -absent, poultry -absent

Laboratory identification code : **20**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	34,85	38,51	38,25	cycles
Copy number at the cut-off :	9,84	9,09	3,86	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
1	2	Present	Present	Absent	Ct-values dilutions 1 and 2 for PIG PCR. PCR platform: ABI 7500 Fast
6	68	Present	Present	Present	Ct-values dilutions 1 and 2 for Ruminant PCR
2	102	Present	Absent	Absent	Ct-values dilutions 1 and 2 for Ruminant PCR
4	232	Absent	Absent	Absent	Ct-values dilutions 1 and 2 for Ruminant PCR
3	272	Absent	Absent	Present	Ct-values dilutions 1 and 2 for Poultry PCR
7	322	Absent	Present	Absent	Ct-values dilutions 1 and 2 for PIG PCR
5	346	Absent	Absent	Absent	Ct-values dilutions 1 and 2 for Ruminant PCR

Laboratory identification code : **21**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	37,49	36,99	37,07	cycles
Copy number at the cut-off :	9,34	10,66	3,83	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
7	28	Absent	Present	Absent	
1	44	Present	Present	Present	Ct-values for pig and poultry target upon request
5	66	Absent	Absent	Absent	No Inhibition in spiking test
6	236	Absent	Present	Present	Ct-values for poultry upon request
2	354	Present	Absent	Absent	Unevenly distributed minor traces of pig detected
3	384	Absent	Absent	Present	
4	750	Absent	Absent	Absent	No Inhibition in spiking test

Laboratory identification code : **22**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	37,84	38,00	38,97	cycles
Copy number at the cut-off :	9,37	9,60	3,45	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
1	16	Present	Present	Present	Ct Pig: 33,23/ 16,33 (10 Fold); Ct Poultry: 33,50/ 0 (1 Fold) / 37,34/
6	208	Absent	Present	Present	Ct Poultry: 35,55/ 35,61 (10 Fold)
2	214	Present	Absent	Absent	
5	318	Absent	Absent	Absent	
7	546	Absent	Present	Absent	
3	636	Absent	Absent	Present	
4	764	Absent	Absent	Absent	

Laboratory identification code : **23**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,74	36,36	36,31	cycles
Copy number at the cut-off :	10,17	9,11	3,10	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
6	40	Absent	Present	Present	The given Ct values are those obtained by Ruminant PCR
5	108	Absent	Absent	Absent	The given Ct values are those obtained by Ruminant PCR
2	144	Present	Absent	Absent	The given Ct values are those obtained by Ruminant PCR
1	170	Present	Present	Present	The given Ct values are those obtained by Ruminant PCR
7	504	Absent	Present	Absent	The given Ct values are those obtained by Ruminant PCR
3	692	Absent	Absent	Present	The given Ct values given are those obtained by Ruminant PCR
4	694	Absent	Absent	Absent	The given Ct values are those obtained by Ruminant PCR

Laboratory identification code : **24**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,07	38,63	37,50	cycles
Copy number at the cut-off :	9,00	9,72	3,52	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
2	4	Present	Absent	Absent	Cq values of ruminant were given
4	176	Absent	Absent	Absent	Cq values of ruminant were given (although absent)
1	254	Present	Present	Present	Cq values of ruminant were given. Poultry dilution1 ct1= 35.27 -
5	304	Absent	Absent	Absent	Cq values of ruminant were given (although absent)
7	560	Absent	Present	Absent	Cq values of pig were given
3	678	Absent	Absent	Absent	Cq values of ruminant were given (although absent), DNA was of too
6	740	Absent	Present	Present	Cq values of pig were given. Poultry dilution1 ct1= 35.04 - ct2=35.15

Laboratory identification code : **25**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,89	38,50	41,25	cycles
Copy number at the cut-off :	11,33	9,42	3,56	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
5	38	Absent	Absent	Absent	
7	336	Absent	Present	Absent	
4	344	Absent	Absent	Absent	
1	394	Present	Present	Absent	
2	508	Present	Absent	Absent	
3	510	Absent	Absent	Present	
6	698	Absent	Present	Present	

Laboratory identification code : **26**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	35,30	38,32	39,33	cycles
Copy number at the cut-off :	11,00	9,64	3,76	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
4	120	Absent	Absent	Absent	
1	128	Present	Present	Present	PIG: dil 1x: 32,91; 32,85. Dil 10x: 36,17; 36,35 POULTRY: dil 1x:
6	166	Absent	Present	Present	POULTRY: dil 1x: 32,92; 32,94. Dil 10x: 36,49; 36,18
5	262	Absent	Absent	Absent	
2	396	Present	Absent	Absent	
7	420	Absent	Present	Absent	
3	566	Absent	Absent	Present	

Laboratory identification code : **29**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,46	37,61	34,17	cycles
Copy number at the cut-off :	10,68			copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
2	32	Present	Absent	Absent	No inhibition observed. Poultry and Porcine results from an in-house
4	50	Absent	Absent	Absent	No inhibition observed. Poultry and Porcine results from an in-house
6	138	Absent	Present	Present	No inhibition observed. Poultry and Porcine results from an in-house
1	296	Present	Absent	Absent	No inhibition observed. Poultry and Porcine results from an in-house
7	308	Absent	Present	Absent	No inhibition observed. Poultry and Porcine results from an in-house
3	426	Absent	Absent	Present	No inhibition observed. Poultry and Porcine results from an in-house
5	514	Absent	Absent	Absent	No inhibition observed. Poultry and Porcine results from an in-house

Laboratory identification code : **32**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	37,31	36,47	38,98	cycles
Copy number at the cut-off :	11,17	11,85	4,05	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
6	**54**	Absent	Present	Present	Ct values are presented in this way: Ruminant/Pig/Poultry
7	**140**	Absent	Present	Absent	
5	**360**	Absent	Absent	Absent	
3	**398**	Absent	Absent	Present	
2	**606**	Present	Absent	Absent	
1	**618**	Present	Present	Present	
4	**638**	Absent	Absent	Absent	

Laboratory identification code : **33**

	Ruminant	Poultry	Pig	
Cut-off at 15 (5 for pig) copies :	36,54	37,06	38,93	cycles
Copy number at the cut-off :	10,32	10,23	3,54	copies

Sample type	Sample N°	Ruminant DNA	Pig DNA	Poultry DNA	Comment
5	**24**	Absent	Absent	Absent	
3	**202**	Absent	Absent	Present	
1	**324**	Present	Present	Present	
7	**350**	Absent	Present	Absent	
6	**418**	Absent	Present	Present	
4	**442**	Absent	Absent	Absent	
2	**536**	Present	Absent	Absent	