EURL-AP PCR Proficiency Test 2015

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 ruminant proteins in feed using PCR according the Commission Regulation n°51/2013 and the version 1.1 of the EURL-AP SOPs "DNA extraction using the Wizard® Magnetic DNA purification system for Food kit" and "Detection of ruminant DNA in feed using real-time PCR". Total number of participants was 31 (27 NRLs and 4 labs outside the NRL network). The study was based on a set of 6 blind samples consisting of 4 feed samples (blanks, feed matrices fortified with terrestrial processed animal proteins or contaminated feed – 2 samples are in duplicate) sent to the participants the 24th of April 2015. In order to be in line with the reintroduction of non-ruminant PAPs in aquafeed, the 6 samples were fishfeed or fishmeal.

Twenty six of the 27 NRLs provided results in due time (deadline: 29th of May 2015). Like in 2014, all the participants received after the closure of the results (12th of June 2015) an individual table giving them a rapid feedback on their results.

This year, more challenging samples giving results closer to the cut-off were included in the sample sets to analyse. Seven labs out of 31 participants provided excellent results. Five labs reported one false positive result out of 6 analyses to be carried out per lab. Seventeen labs had one false negative result. The performances of these 22 labs were considered as satisfying as their only false result was obtained with one of the 2 challenging samples. Two labs had 2 false results on their six analyses. For one of them, the errors are clearly due to a mistake during the reporting. Finally, one lab was unable to detect any of the 3 positive samples in its set. Corrective actions were taken with the under-performant participants having 2 or 3 false results.

Keywords :

Processed animal proteins – Aquafeed – Ruminant – PCR – Polymerase Chain Reaction – Proficiency test – Qualitative analysis

1. Foreword

European Union Reference Laboratories (EURL) – formerly referred to as Community Reference Laboratories (CRL) – were created in order to ensure a high level of quality and a uniformity of the results provided by European control laboratories. On 29 April 2004, the European Parliament and the Council adopted the Regulation EC/882/2004 [1], improving the effectiveness of the official food and feed controls while redefining the obligations of the relevant authorities and their obligations in the organization of these controls.

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

(i) To provide National Reference Laboratories (NRLs) with detailed analytical methods, including reference methods for the network of Member State NRLs;
(ii) To coordinate application by NRLs of the methods by organizing interlaboratory studies;
(iii) To develop new analytical methods for the detection of animal proteins in feedingstuffs (light microscopy, near infrared microscopy, PCR, immunology …);
(iv) To conduct training courses for the benefit of NRL staffs from Member States and future Member States;
(v) To provide scientific and technical assistance to the European Commission, especially in cases of disputed results between Member States.

In this framework, the EURL-AP organised this PCR interlaboratory study for the assessment of the NRL proficiency with respect to the detection of ruminant proteins in feed using the PCR method as indicated in the new Commission Regulation n°51/2013 [3].

2. Introduction

According to the TSE Roadmap II, alternative analytical methods to the classical microscopy able to detect and identify the species of processed animal proteins (PAPs) in animal feed are the main condition for a possible lifting of the extended feed ban [4]. Commission Regulations n° 51/2013 and 56/2013 [5] give to PCR the status of official method for the detection of PAP in feed. The objective of the present proficiency test is to evaluate performances of 27 NRLs to detect the presence of ruminant DNA in feed using the ruminant PCR method [6]. Due to the reintroduction of the non-ruminant PAPs in aquafeed since the 1st of June 2013, the study focussed on the analyses of aquafeed samples.

3. Material and methods

3.1. Study organisation

Official announcement of the study was made on the 9th of April 2015 through a letter sent to the 27 NRLs of the EURL-AP network (Annex 1). A detailed list of the 31 participating labs (4 labs external to the EURL-AP network also participated to the study) is included in Annex 2.

On the 24th of April 2015, the material for the test (a set of 6 blind samples) was provided to the participants by express shipment. The 29th of April 2015 all the participants had their set of samples. On the same date the Excel result report file containing the instructions, a recording sheet and a report summary (Annex 3) was posted on the intranet part of the EURL-AP website.

Some general recommendations were delivered to the participants:

- Results had to be encoded by way of the Excel result report file (Annex 3b). Participants were asked to carefully read the instructions on how to fill in the result form and to testify they did it prior to encoding their results. No other support for communicating the results was accepted.
- A summarized results sheet was automatically generated (Annex 3c). Participants were asked to sign the summarized results sheet and to return it by fax and/or e-mail to the EURL-AP. The results were taken into consideration only when both the Excel file and the signed version were received by EURL-AP.
- The results had to be sent in both forms concomitantly to the EURL-AP by the 29th of May 2015.

Twenty-six out of the 27 NRLs participants delivered their results in due time. Lab 27 reported its results one week after the deadline and therefore these results are not considered in this report.

Concerning the non NRL-AP participants, all the labs sent their results before the deadline.

3.2. Material

3.2.1. Description of the samples

The sample set consisted of two samples of fishmeal and 2 samples of fishfeed with or without processed animal proteins (PAPs) from terrestrial origin at a concentration level ~ 0.1 % in mass fraction as shown in Table 1 and provided to the participants. Two samples (one fishmeal and one fishfeed) among the six were in duplicates in the sets. The composition of the samples is described in Annex 5. Fishmeal II and fishfeed II were not fortified with ruminant material but are real world sample contaminated at low level by ruminant material.

Each participating lab received about 10 g of the six feed samples to extract their DNA according to the protocol imposed by the EURL–AP.

A unique random number was assigned to each sample (Annex 4).

Table 1 : Composition of the blind sample set used in the EURL-AP PCR Proficiency Test 2015.

Sample	Material	Quantity/lab	Intended result with the ruminant target
1	Fishmeal I	2	Negative
2	Fishmeal II	1	Positive
3	Fishfeed I + 0.1 % porcine PAP	1	Negative
4	Fishfeed II	2	Positive
Total		6	

3.2.2. Materials used in the preparation of the samples

- The aquafeed matrices were selected among the EURL-AP sample bank. The presence of ruminant DNA was checked by PCR.
- The PAPs used to spike the blank aquafeed material was the following one:
  - a pig PAP (used in sample #3).

3.2.3. Homogeneity study

During the period between end 2014 beginning 2015, 10 replicates of the four samples (Table 1) were chosen randomly. Per sample replicate, 2 DNA extracts were realised according the EURL-AP Standard Operating Procedure DNA extraction using the "Wizard® Magnetic DNA purification system for Food" kit version 1.1. In the framework of a future accreditation to the ISO 17043 standard, 10 additional replicates of three samples (Fishmeal II, Fishfeed I + 0.1 % porcine PAP and Fishfeed II) were analysed according the same protocol to assess the stability of the samples (July-August 2015). In final, 20 (Fishmeal I) and 40 (the 3 remaining samples) Promega extracts were obtained per sample type to be analyzed. They were all analysed using the ruminant PCR target according the Standard Operating Procedure Detection of ruminant DNA in feed using real-time PCR version 1.1 and another target corresponding to the species present in the sample. The results are detailed in Table 2.

Table 2 : Results of the homogeneity study obtained with sample replicates

Sample type	Material	Nr of samples analysed	Nr of PCR results	Detection with				
				Ruminant target[1]			Pig target	Fish target
				Ct_{mean}[2]	Ct_{min}[2]	Ct_{max}[2]		
1	Fishmeal I	10	20	NA[4]	20x negative 40.17	NS[5]	NT[3]	20x positive
2	Fishmeal II	20	40	34.78	40x positive 33.55	35.24	NT[3]	40x positive
3	Fishfeed I + 0.1 % porcine PAP	20	40	38.74	40x negative 37.56	40.35	40x positive	40x positive
4	Fishfeed II	20	40	31.65	40x positive 29.79	32.84	NT[3]	40x positive

(1) EURL-AP Cut-off for the ruminant target = 36.34 cycles
(2) Ct values in cycles
(3) NT : not tested
(4) NA : not applicable
(5) NS : no signal (or Ct = 50.00 cycles)

The analyses showed that positive samples for ruminant detection are continuously positive when analysed. Similarly the negative samples all led to negative results. The quality of the DNAs giving the negative results is successfully controlled with other DNA targets (pig and fish assay) that must be present. As can be seen from the Ct results provided in Table 2, samples #2 and #3 are more challenging because they are somewhat closer to the cut-off (here 36.34 cycles). All the results were positive and confirmed the ones obtained previously.

The composition of the samples is described in Annex 5. The variability of the copy numbers extracted from the samples during the homogeneity study showed that the ruminant-positive sample #2 should remain positive in more than 99% (with the approximation of a normal distribution of the copy numbers) of the cases whatever the sample taken from the prepared batch for this sample type. Similarly for the ruminant-negative sample #3, the results should remain negative in more than 99% (with the approximation of a normal distribution of the copy numbers) of the cases whatever sample of the batch is chosen.

3.3. Expression of results

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

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

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

With :

PA : positive agreement (i.e. number of times detection was done when expected)
NA : negative agreement (i.e. number of times there was no detection when expected)
PD : positive deviation (i.e. number of times detection was done even though detection was not expected)
ND : negative deviation (i.e. number of times there was no detection even though detection was expected)

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

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

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

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

The *AC, SE* and *SP* were calculated separately for each laboratory for the estimation of its proficiency.

4. Results

4.1. Gross results

Gross results from all participants replying within the set deadline are to be found in Annex 7.

4.2. Qualitative analyses from the NRLs

4.2.1. Overview of results and global performance of the test

Table 3 summarizes the results provided by the 26 NRLs for the four sample types submitted to qualitative analysis.

Table 3 : Global results expressed as accuracy (AC) for the four sample types

Sample	Material	Intended result	Nr of results	AC
1	Fishmeal I	Negative	52	0.962 (2)
2	Fishmeal II	Positive	26	0.462 (14)
3	Fishfeed I + 0.1 % porcine PAP	Negative	26	0.808 (5)
4	Fishfeed II	Positive	52	0.942 (3)

Accuracy means sensitivity in case of ND and specificity in case of PD.
In brackets the number of false results.

For sample #2, the overall results, expressed in terms of global accuracy (AC), are not good whereas for sample #3, the occurrence of false positive results is less frequent. The use of a cut-off value as implemented according to the SOP is efficient to limit the occurrence of false positive results. Depending the laboratories, the detection of low copy number of ruminant target can be affected but without calling into question the proficiency of the labs to detect the presence of 0.1 % of ruminant PAP in a feed.

4.2.2. Individual performances of NRLs in qualitative analysis

Individual performances were assessed for each participant by calculating the accuracy, sensitivity and specificity over the blind samples. A ranking of the labs was prepared based on the accuracy. Results are to be found in Table 4 that summarizes the results obtained by the participants for the six samples.

Table 4 : NRL proficiencies regarding the detection of ruminant material starting from the six samples. Ranking follows AC values

Ranking	Lab code	AC	SE	SP
1	3	1.000	1.000	1.000
	12	1.000	1.000	1.000
	15	1.000	1.000	1.000
	20	1.000	1.000	1.000
	22	1.000	1.000	1.000
	26	1.000	1.000	1.000
7	14	0.833	1.000	0.750
	16	0.833	1.000	0.750
	17	0.833	1.000	0.750
	28	0.833	1.000	0.750
11	1	0.833	0.667	1.000
	2	0.833	0.667	1.000
	4	0.833	0.667	1.000
	6	0.833	0.667	1.000
	7	0.833	0.667	1.000
	8	0.833	0.667	1.000
	10	0.833	0.667	1.000
	11	0.833	0.667	1.000
	13	0.833	0.667	1.000
	19	0.833	0.667	1.000
	25	0.833	0.667	1.000
	30	0.833	0.667	1.000
	31	0.833	0.667	1.000
24	9	0.667	0.667	0.667
25	5	0.667	1.000	0.333
26	29	0.500	0.000	0.500

Table 4 illustrates the excellent level of global performance for 6 labs (in green) out of 26 NRLs (23.1 % of the NRLs) having no false result. Seventeen labs (in black) out of the 26 (65.4 %) obtained satisfactory level of performance by providing 1 incorrect result:

- PD : labs 14, 16, 17 and 28 ;
- ND : labs 1, 2, 4, 6, 7, 8, 10, 11, 13, 19,25, 30 and 31.

Three labs (11.5 %) had 2 incorrect results or more and are considered as under-performant (in orange and red) :

- 2 PD : lab 5 ;
- 1 PD and 1 ND : lab 9 ;
- 3 ND : lab 29.

Concerning lab 9, the 2 false results are due to errors during the reporting as confirmed by the lab. Lab 29 received a new set of 4 samples as corrective action.

4.2.3. Cut-off quality control

A quality control for the number of copies of the ruminant 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 was required. Indeed, depending on the variability of the lab (PCR platform + operator), the cut-off value can correspond to a too low number of copies.

In Table 5, the participants are ranked by decreasing number of copies at the cut-off. The occurrence of false result is mentioned in the last column of the Table.

Table 5 : Number of copies at the cut-off value, cut-off value in cycles and false results

Lab code	Number of copies at the cut-off	Cut-off (cycles)	False result(s)
4	11.60	34.29	1 ND
9	11.59	35.07	1 ND + 1 PD [1]
29	11.59	35.87	3 ND
8	11.30	34.27	1 ND
10	11.27	35.58	1 ND
13	11.22	35.74	1 ND
31	11.17	34.30	1 ND
20	10.99	36.76	No deviation
26	10.97	36.75	No deviation
28	10.93	37.25	1 PD
22	10.90	34.96	No deviation
16	10.88	34.30	1 PD
1	10.76	35.92	1 ND
30	10.74	35.61	1 ND
15	10.71	36.74	No deviation
17	10.70	36.00	1 PD
25	10.65	34.69	1 ND
14	10.60	37.47	1 PD
11	10.48	36.49	1 ND
6	10.13	36.33	1 ND
5	10.08	37.19	2 PD
2	9.98	36.98	1 ND
7	9.92	34.36	1 ND
12	9.25	36.27	No deviation
3	9.11	35.75	No deviation
19	9.05	37.28	1 ND

[1] Reporting errors

All the participants reached the minimum criterion of 9.00 copies. The percentage of the labs with a cut-off corresponding to a number of copies > 10 continues to increase (80.7 % instead of 70.4 % in 2014 and 55.6 % in 2013). It is not possible anymore to link any deviation to a cut-off problem.

4.3. Qualitative analyses from the non EURL-AP network participants

4.3.1. Overview of results

Table 6 summarizes the results provided by the 4 non-NRL participants for the six sample types submitted to qualitative analysis. No false result was noticed.

Table 6 : Global results expressed as accuracy (AC) for the six sample types

Sample	Material	Nr of results	AC
1	Fishmeal I	8	1.000
2	Fishmeal II	4	0.250 (3)
3	Fishfeed I + 0.1 % porcine PAP	4	1.000
4	Fishfeed II	8	1.000

Accuracy means sensitivity in case of ND and specificity in case of PD.

4.3.2. Individual performances

Individual performances were assessed for each of these four participants by calculating the accuracy, sensitivity and specificity over the blind samples. Their results are to be found in Table 7.

Table 7 : Non NRL participant proficiencies regarding the detection of ruminant material starting from the six samples. Ranking follows AC values

Ranking	Lab code	AC	SE	SP
1	23	1.000	1.000	1.000
2	18	0.833	0.667	1.000
	21	0.833	0.667	1.000
	24	0.833	0.667	1.000

Table 7 illustrates the good level of these 4 labs. Lab 23 gave excellent results (in green) and none of them is underperforming.

4.3.3. Assessment of the cut-off values

In Table 8, the number of copies at the cut-off determined by each participant is mentioned. All of them comply with the minimum criterion of 9 copies set by the EURL-AP.

Table 8 : Number of copies at the cut-off value, cut-off value in cycles and false results

Lab code	Number of copies at the cut-off	Cut-off (cycles)	False result(s)
24	10.92	33.25	1 ND
21	10.30	35.17	1 ND
23	10.00	35.90	No deviation
18	10.14	36.62	1 ND

5. Conclusions

This study is the second assessment of the proficiency level in PCR methods for the detection of ruminant PAPs of the NRL network since the reintroduction of non-ruminant PAP in aquafeed which occurred the 1st of June 2013. Twenty-six NRLs out of the 27 submitted results in due time. Looking globally at these results sent to the EURL-AP, 23.1 % of the participating NRLs (6 labs out of 26) were able to detect correctly the presence of ruminant DNA in aquafeed and had no false result. Four NRLs (Labs 14, 16, 17 and 28) had one false positive result and thirteen NRLs had one false negative result. Three NRLs had more than one false result (Labs 5, 9 and 29). These 3 labs were considered as under-performing. The case of one NRL (Lab 9) was easily closed and a reporting error was rapidly identified. Lab 29 received a new set of samples as corrective and follow-up action. The results were improved with this 2nd set of samples.

These results could give the impression of a global NRL proficiency degradation. Nevertheless, it must be pointed that the set was clearly more challenging than the ones used during the previous studies: 1) if three out of the six samples are systematically detected as positive by the EURL-AP none of them are reputed to contain ruminant PAP and the source of ruminant DNA is unknown ; 2) two samples out of the six give Ct values closer to the cut-off even if the EURL-AP obtains consistent and reproducible results. These 2 characteristics of the sample set have certainly increased the risk of false result.

The occurrence of false positive results (7 results out of 73 analyses) remains however limited (9.59 %). Quite surprisingly when we remember the main critics about the too high sensitivity of the method, the number of false negative results is more frequent (17 results out of 78 analyses – 21.8 %) but comes essentially from one of the challenging samples (14 false negative results out of the 17).

The results obtained are most probably not due to the cut-off values used by the participants. On the contrary, all the NRLs reached or exceed largely the minimum quality criterion set by the EURL-AP. Moreover no link with the occurrence of false results can be observed.

Concerning the 4 labs external to the NRLs network, they all obtained excellent or satisfactory results. Three of them had one false negative result with the same sample #2 that was difficult also for the NRL network.

As more than half of the participants came to the conclusion of a negative result for sample #2, this sample was reanalysed by digital PCR to check independently the copy number of the ruminant targets obtained in the DNA extract.

This confirmed that the sample has to be considered as positive but also provided a hint from where the problem encountered within the network might come. Digital PCR clearly showed that the DNA extract of this sample is affected by PCR inhibition that results in a later signal (with the risk in some cases to be after the cut-off value for the Ct). Therefore it is very probable that the false negative outcome of a majority of laboratories arises from a difficulty to master the inhibition. Of course one might argue that the quality of the extract might have an influence but we experienced that sometimes even by exchanging the extracts the results remain the same (positive result at EURL-AP whatever the extract used and negative result by the other lab whatever the extract used) because the way the DNA extraction is done is already very harmonized. The EURL-AP will analyse this more in depth, in order to improve the results of the network.

Acknowledgments

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

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Annex 1

Official announcement

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Announcement of the EURL-AP PCR proficiency test 2015 for the detection of ruminant DNA in feed using the validated PCR assay

Gembloux, 09 April 2015

Introduction

Use of non-ruminant processed animal proteins was reauthorized in aquafeed since 1st June 2013. The EURL-AP organizes a proficiency test with all the NRL's of the EURL-AP network in relation to the application of the ruminant PCR test.

In view of this proficiency test you are asked to take the required measures to be ready with all the reagents that are needed: e.g. the primers, probe, master mix as well as the DNA extraction kit that has to be used in combination with this assay. **As NRL, your participation to this proficiency test is mandatory according to regulation 882/2004.**

Objective

The objective of the present proficiency test is to assess the performance of the NRLs to detect the presence of ruminant DNA in feed by the PCR method as stated in Regulation EC 51/2013.

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 laboratory will have :

1. To grind and homogenize 6 samples before to weigh the test portions ;
2. To extract the DNA ;
3. To perform PCR on the extracts ;
4. To submit the results to the EURL-AP.

General outline of the exercise

- The method protocol to use is described in Commission Regulation EC 51/2013 and in EURL-AP SOPs "DNA extraction using the "Wizard® Magnetic DNA purification system for Food" kit" and "Detection of ruminant DNA in feed using real-time PCR" available on the EURL-AP website.
- We will provide you with an Excel sheet for reporting the results of the analyses.
- Each laboratory will be assigned a unique code and only the coordinator 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.
- The participation in this proficiency study is free of charge for national reference laboratories within Member States of the European Union.

Walloon Agricultural Research Centre

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Wallonie

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Time schedule

- Official announcement of the study to the NRLs by way of the intranet and e-mail : **9 April 2015**
- Sending of the sample boxes and communication of the instructions : **27 April 2015**

> ***Samples will be sent to the PCR contact person referred during the inquiry for the database. You are asked to check if this person is still your contact (not available yet on intranet) and to inform the organizer from any changes if needed.***

- Deadline for returning of results to the organizer : **29 May 2015**

Further information

- **Reminder** : Calibrants are now provided by the JRC-IRMM. The EURL-AP recommends to switch to this new calibrants of top quality as soon as possible. Further information are available at http://eurl.craw.eu/en/24/news/26
- Refer to the address and coordinates mentioned in the heading,

or

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Dr Gilbert Berben
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Wallonie

Annex 2

List of participants

EURL-AP network participants	
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
Croatia	Croatian Veterinary Institute
Cyprus	Cyprus Veterinary Services
Czech Republic	Central Institute of sampling and testing in Agriculture
Denmark	Danish Veterinary and Food Administration
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 Veterinary Laboratory
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Poland	National Veterinary Research Institute
Portugal	Laboratorio Nacional de Investigaçao Veterinaria
Romania	Hygiene Institute of Veterinary Health
Slovakia	State Veterinary and Food Institute
Slovenia	Veterinary Faculty-National Veterinary Institute-Unit for pathology of animal nutrition and environmental hygiene
Spain	Laboratorio Arbitral Agroalimentario
Sweden	National Veterinary Institute, Department of Animal Feed
United Kingdom	Animal Health and Veterinary Laboratories Agency
Non EURL-AP network participants	
Country	**Institute Name**
Netherlands	Darling Ingredients
Netherlands	NutriControl
Netherlands	TNO Triskelion bv
Norway	National Institute of Nutrition and Seafood Research

Annex 3

Excel result report file

a. Instruction sheet

Please read carefully this information before filling in the form.

Instructions to the EURL-AP PCR Proficiency Test 2015

1 Content of the file :

Worksheet :	*Content :*
Instructions	General recommendations and user guide to this file
Report form	Encoding worksheet (to fill in)
Report summary	The summarized report page

2 Instructions

Information to help you how filling in the reporting sheet.
The protocol for this study is available on the EURL-AP website (www.eurl.craw.eu) and described in:
1. the EURL-AP SOP "DNA extraction using the "Wizard® Magnetic DNA purification system for Food" kit"
2. the EURL-AP SOP "Detection of ruminant DNA in feed using real-time PCR"

The EURL-AP strongly recommends:
1. to follow these protocols,
2. to analyse the feed samples in duplicate (2 DNAs/samples),
3. to test more than 1 dilution of the DNAs to avoid any false negative result due to PCR inhibition,
4. to perform all the required controls to validate your results
(extraction negative control, extraction positive control, PCR negative control and PCR positive control).

2.1 This file is protected : only the fields (or cells) that have to be filled in with data are accessible.
In this way data entry is only restricted to the worksheet "Report form".
The worksheet "Report summary" contains a synthetic table of all your data. It is filled automatically while encoding the report form.

Start filling the "Report form" worksheet

2.2 Data entry on the form is limited to white cells and pick-lists.

2.3 Laboratory identification

The first data to enter is your unique laboratory code (cell B3). *This code is to be found in the sample shipment.*
The corresponding code is simply to be chosen from the pick-list : click on the arrow at the right of the box, it opens the pick-list, select your code among the proposed values ranging from 1 to 27 (use the scroll-bar on the right if needed to visualise other values), click on the correct value highlighted in blue (this closes the pick-list). The chosen lab code will appear as header every column in which the results for a sample are reported (cells D10 to H10).

The second data to enter is the agreement on responsibility (cell B5).
By agreeing, i.e. choosing "Yes" in the pick-list, the masks used for data entry become visible. If by mistake, you later return to the "No" value, all your encoded data will become invisible. To make them visible again return to the "Yes" value. *Please note that your data will never be deleted by doing so.*

2.4 Report

Data related to one sample are organised in columns.
Each column must contain the sample number "Sample N°" (cells D10 to H10). This number is an integer referring to the one indicated on the vial containing the feedingstuff to analyse. This data is mandatory.

Qualitative analysis :

Data entry is only related to the presence or absence of ruminant DNA.

For instance, if ruminant DNA is detected in your first sample, select "Present" from the pick-list in cell D13. If there is no ruminant DNA in this sample select "Absent". Leaving a blank (i.e. empty) value is not authorized.

Additional data :

For each sample please fill the following additional data :
Leaving a blank (i.e. empty) value is not authorized.
- the dilutions of the DNA tested (e.g. 10 fold dilution or 10x)
- the Ct value of all the replicates and the mean Ct value for each dilution of the DNA,
- comments if needed (e.g. PCR inhibition, flat signals,...).

"Report summary" worksheet

This summary table is generated automatically. The report summary has to be printed and signed by the contact person for the present study. Signing this document serves as ultimate validation of the encoded data and certifies their integrity. Therefore we ask you to send us by fax the signed page simultaneously to the sending of the Excel file by email to the organizer. *We encourage you to keep a copy of the report summary.*
Any Excel file sent by email without the sending of the report summary by fax will not be taken into consideration and thus refused.

3 Sending of the results

The deadline for sending the results is the **29th of May 2015 at 04:00 PM GMT.**
All results will be transferred to the organizer at once : successive sending of partial results will be proscribed
The whole Excel file has to be sent as an attachment to a mail to the following address : **secretary@eurl.craw.eu** with as mail subject: **EURL-AP PCR PT RESULTS 2015**
The Report summary has to be sent by fax to **+32(0)81 62 03 88**

For all complementary information or question, you can contact Dr Olivier FUMIERE (direct phone +32(0)81 62 03 61 - e-mail fumiere@cra.wallonie.be)

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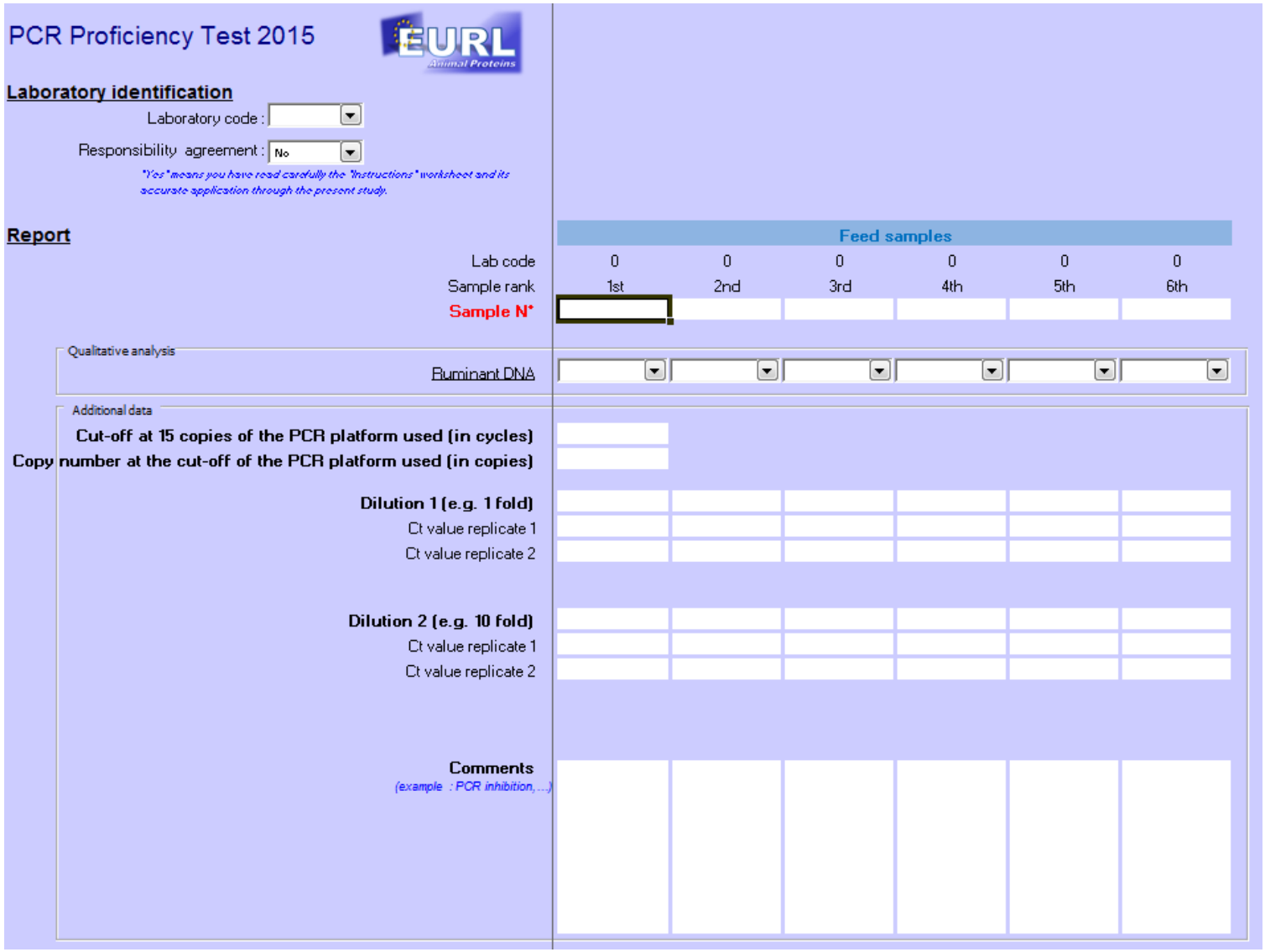
b. Recording sheet



PCR Proficiency Test 2015

EURL Animal Proteins

Laboratory identification

Laboratory code :

Responsibility agreement : No

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

Report

	Feed samples					
Lab code	0	0	0	0	0	0
Sample rank	1st	2nd	3rd	4th	5th	6th
Sample N°						
Qualitative analysis – Ruminant DNA						
Additional data – Cut-off at 15 copies of the PCR platform used (in cycles)						
Copy number at the cut-off of the PCR platform used (in copies)						
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						
Comments (example : PCR inhibition,...)						

c. Report summary sheet

Centre wallon de Recherches agronomiques

Proficiency Test PCR 2015
EURL-AP

The report summary has to be printed, signed and sent by fax to +32(0)81 62 03 88 simultaneously to the sending of the Excel file by email

Report summary **PCR Proficiency Test 2015**

Laboratory identification code :	0	
Responsibility agreement :	No	
Cut-off at 15 copies :	0,00	cycles
Copy number at the cut-off :	0,00	copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1									
2									
3									
4									
5									
6									

Date : .....

Name : .....
Firstname : .....

Signature :

PT-MSCBM-DONNEES-EA-DE02 Result report form BM Version 1 31/07/2014 Page 1 of 1

Annex 4

Composition of sample sets received by the participants with the correspondence of the sample numbers

1. EURL-AP network participants

Samples	Intended result	Lab number																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	22	25	26	28	29	30	31
Fishmeal I	-	467	311	101	899	992	521	89	17	404	689	836	374	458	26	395	530	68	563	1004	857	110	572	38	584	803	416
Fishmeal I	-	941	1025	227	1013	920	752	878	815	698	794	950	656	887	782	677	845	59	824	236	971	383	710	122	647	761	614
Fishmeal II	+	893	431	305	935	662	221	977	536	200	326	557	74	263	1103	1229	389	1124	767	998	704	809	410	11	95	620	347
Fishfeed I + 0.1 % porcine PAP	-	329	581	1001	224	413	938	1274	980	833	602	728	1064	1085	1400	707	1127	1358	161	686	1295	1022	98	1463	35	14	560
Fishfeed II	+	869	806	428	575	680	71	1499	701	533	974	932	260	1919	8	155	1961	50	764	911	554	218	407	1247	365	386	239
Fishfeed II	+	1142	596	1835	1604	848	1226	1730	1667	2066	512	1478	2045	638	449	1268	617	995	176	1415	1205	953	785	1352	2087	722	1100

* Red cells correspond to false results submitted by the participants

2. Non EURL-AP network participants

Samples	Intended result	Lab number			
		18	21	23	24
Fishmeal I	-	983	1034	185	1046
Fishmeal I	-	551	773	626	248
Fishmeal II	+	641	683	851	242
Fishfeed I + 0.1 % porcine PAP	-	959	1043	770	812
Fishfeed II	+	1877	1751	1079	1646
Fishfeed II	+	323	827	344	29

* Red cells correspond to false results submitted by the participants

Annex 5

Aquafeed formulations

FISHMEAL 1

Mix of 2 fishmeals

Composition

Pure fishmeal from Denmark.

Iceland fishmeal 91 % Hering, 6% Capelin and 3 % Blue whiting.

FISHMEAL 2 (CARP FEED)

Composition

Pure fishmeal from Peru.

Remark: This fishmeal is contaminated by ruminant material with unknown origin.

FISHFEED 1 + 0.1 % W/W PORCINE PAP

Analytical constituents of the fishfeed

Oils & fats 18.0%
Calcium 2.0%
Protein 54.0%
Phosphorus 1.4%
Ash 10.0%
Sodium 0.6%
Fibre 1.0%

Composition

Fish meal, Vital wheat gluten, fish oil, soya (bean) meal, maize gluten, wheat, fish protein hydrolysed, horse beans dehulled, vitamins, yeasts, minerals, permitted flavour, lysine, methionine.
Also contains a natural source of selenium.

Additives (per kg)

E1	Iron (Ferrous sulphate monohydrate)	40 mg
E2	Iodine (Calcium iodate anhydrous)	2 mg
E4	Copper (Cupric sulphate pentahydrate)	5 mg
E5	Manganese (Manganese sulphate monohydrate)	15 mg
E6	Zinc (Zinc sulphate monohydrate)	100 mg
E310	Propyl gallate	5 mg
E320	BHA (Butylated hydroxyanisole)	5 mg
E321	BHT (Butylated hydroxytoluene)	147 mg
E671	Vitamin D3	3000 IU
E672	Vitamin A	4000 IU

FISHFEED 2 (40% COMPLETE FEED FOR SALMON + 60 % FISHFEED)

Composition

AA, faba beans, fish meal, fish oil, lineseed oil, maize gluten, premix oligo vitamins, soya meal, soya oil, wheat flower.

Remark: This fishfeed is contaminated by ruminant material with unknown origin.

Annex 6

Individual tabulation results

European Union Reference Laboratory for Animal Proteins in feedingstuffs

Walloon Agricultural Research Centre, Valorisation of Agricultural Products Department
Henseval Building, Chaussée de Namur 24, B – 5030 GEMBLOUX

☎32 (0) 81 62 03 50 🖷32 (0) 81 62 03 88
e-mail: secretary@eurl.craw.eu Internet : http://eurl.craw.eu

PCR proficiency test 2015
for the detection of ruminant PAPs

TABULATION RESULTS

NAME OF LABORATORY:

LABORATORY CODE:

CONTACT PERSON:

MATERIAL SET:

Samples	Composition	Intended result	Reported result
# ---	Fishmeal I	Negative	
# ---	Fishmeal I	Negative	
# ---	Fishmeal II	Positive	
# ---	Fishfeed I + 0.1 % porcine PAP	Negative	
# ---	Fishfeed II	Positive	
# ---	Fishfeed II	Positive	

Further information

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Walloon Agricultural Research Centre

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Wallonie

Annex 7

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

1. EURL-AP network participants

Laboratory identification code :	1
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,92 cycles
Copy number at the cut-off :	10,76 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	329	Absent	1 x	38,26	39,26	10 x	38,18	38,00	
2	467	Absent	1 x	42,00	42,37	10 x	43,31	40,56	
3	869	Present	1 x	31,97	32,29	10 x	35,73	36,18	
4	893	Absent	1 x	35,97	36,56	10 x	38,26	38,26	
5	941	Absent	1 x	42,26	44,69	10 x	42,12	48,58	
6	1142	Present	1 x	32,34	32,61	10 x	36,43	36,22	

Laboratory identification code :	2
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,98 cycles
Copy number at the cut-off :	9,98 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	311	Absent	3 x	41,21	45,22	30 x	43,98	41,91	
2	431	Absent	3 x	38,33	38,56	30 x	39,56	38,48	one of four DNA purifications has been positive in 3x dilution
3	581	Absent	3 x	38,51	37,70	30 x	39,40	39,06	
4	596	Present	3 x	30,91	32,59	30 x	33,35	34,70	
5	806	Present	3 x	35,32	33,95	30 x	37,94	37,51	
6	1025	Absent	3 x	40,79	39,81	30 x	41,08	40,84	

Laboratory identification code :	3
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,75 cycles
Copy number at the cut-off :	9,11 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	101	Absent	1 x	36,79	36,55	10 x	38,82	39,10	
2	227	Absent	1 x	37,35	38,20	10 x	41,20		
3	305	Present	1 x	35,65	35,50	10 x	37,64	37,86	
4	428	Present	1 x	34,36	33,98	10 x	36,02	36,23	
5	1001	Absent	1 x	37,59	38,14	10 x		42,13	
6	1835	Present	1 x	34,62	34,95	10 x	37,11	36,97	

Laboratory identification code :	4
Responsibility agreement :	Yes
Cut-off at 15 copies :	34,29 cycles
Copy number at the cut-off :	11,60 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	224	Absent	1 x	38,32	38,33	10 x	38,73	37,10	
2	575	Present	1 x	30,48	30,83	10 x	33,28	33,49	
3	899	Absent	1 x	43,07	39,44	10 x	40,75	40,47	
4	935	Absent	1 x	34,88	35,20	10 x	36,16	37,52	
5	1013	Absent	1 x	43,82	40,64	10 x	40,73	38,53	possible inhibition (10x before 1x), but still after cut-off
6	1604	Present	1 x	31,48	30,17	10 x	34,29	32,15	

Laboratory identification code :	5
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,19 cycles
Copy number at the cut-off :	10,08 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	413	Present	1 x	34,35	34,38	10 x	36,90	36,97	
2	662	Present	1 x	33,72	33,68	10 x	35,15	35,20	
3	680	Present	1 x	33,79	34,10	10 x	35,35	35,40	
4	848	Present	1 x	35,30	35,00	10 x	37,09	37,07	
5	920	Absent	1 x	37,09	37,20	10 x	38,95	38,69	
6	992	Present	1 x	35,26	35,20	10 x	37,00	37,08	

Laboratory identification code :	6
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,33 cycles
Copy number at the cut-off :	10,13 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	71	Present	1 x	32,56	32,95	10 x	33,09	35,06	Partial inhibition
2	221	Absent	1 x	38,39	37,45	10 x	37,96	37,96	Partial inhibition. Repeated extraction with identical results
3	521	Absent	1 x	44,03	40,89	10 x	43,94	43,80	Partial inhibition
4	752	Absent	1 x	45,52	40,23	10 x	44,55	39,84	Partial inhibition
5	938	Absent	1 x	40,32	44,12	10 x	38,53	41,16	Partial inhibition
6	1226	Present	1 x	34,26	34,00	10 x	36,06	36,40	Partial inhibition

Laboratory identification code :	7
Responsibility agreement :	Yes
Cut-off at 15 copies :	34,36 cycles
Copy number at the cut-off :	9,92 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	68	Absent	1 x	39,01	40,00	20 x	45,00	40,00	
2	878	Absent	1 x	36,30	35,96	20 x	45,00	40,00	
3	977	Absent	1 x	35,00	37,79	20 x	45,00	34,47	inhibition
4	1274	Absent	1 x	40,30	36,78	20 x	45,00	40,00	
5	1499	Present	1 x	30,69	29,60	20 x	35,56	31,90	
6	1731	Present	1 x	28,70	29,90	20 x	29,43	34,26	

Laboratory identification code :	8
Responsibility agreement :	Yes
Cut-off at 15 copies :	34,27 cycles
Copy number at the cut-off :	11,30 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	17	Absent	1 x	41,06	41,38	10 x	37,11	37,46	PCR inhibition
2	536	Absent	1 x	34,87	34,81	10 x	37,05	37,05	
3	701	Present	1 x	32,53	32,20	10 x	35,51	35,50	
4	815	Absent	1 x	43,23	42,34	10 x	38,47	37,31	PCR inhibition
5	980	Absent	1 x	37,58	37,29	10 x	35,43	35,53	PCR inhibition
6	1667	Present	1 x	32,86	32,23	10 x	35,30	35,13	

Laboratory identification code :	9
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,07 cycles
Copy number at the cut-off :	11,59 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	200	Present	1 x	34,46	34,95	10 x	35,41	35,25	The result of the positive non-diluted result is beneath but very close to the cut-off value, which to me is a questionable result.
2	404	Absent	1 x	45,89	47,12	10 x	39,33	35,88	
3	533	Present	1 x	29,96	29,67	10 x	32,64	32,50	
4	833	Absent	1 x	46,11	45,95	10 x	39,10	40,58	
5	2066	Absent	1 x	41,27	41,49	10 x	36,91	36,85	
6	698	Present	1 x	29,77	29,63	10 x	32,57	32,64	

Laboratory identification code :	10
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,58 cycles
Copy number at the cut-off :	11,27 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	326	Absent	1 x	38,68	38,40	2 x	40,49	39,25	Amplification plots were detected in both replicates, however Ct values were under cut-off value
2	512	Present	1 x	33,01	32,15	10 x	35,99	34,81	
3	602	Absent	1 x	40,36	39,73	2 x	41,78	40,45	Amplification plots were detected in both replicates, however Ct values were under cut-off value
4	689	Absent	1 x	50,00	48,58	2 x	50,00	50,00	"Ct value = 50" means Not Detected
5	794	Absent	1 x	47,53	43,24	2 x	50,00	50,00	"Ct value = 50" means Not Detected
6	974	Present	1 x	31,36	31,95	10 x	34,96	34,94	

Laboratory identification code :	11
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,49 cycles
Copy number at the cut-off :	10,48 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	557	Absent	1 x	38,16	38,36	10 x	37,79	37,51	Further testing showed no PCR inhibtion in this sample.
2	728	Absent	1 x	44,23	44,39	10 x	38,88	42,25	Further testing showed no PCR inhibtion in this sample.
3	836	Absent	1 x	49,90	46,96	10 x	41,72	41,36	Further testing showed no PCR inhibtion in this sample.
4	932	Present	1 x	34,47	34,35	10 x	36,34	36,23	
5	950	Absent	1 x		47,85	10 x	41,68	39,19	Further testing showed no PCR inhibtion in this sample.
6	1478	Present	1 x	34,26	33,50	10 x	35,66	35,90	

Laboratory identification code :	12
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,27 cycles
Copy number at the cut-off :	9,25 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	74	Present	1 x	35,61	40,03	10 x	35,98	36,95	
2	260	Present	1 x	32,84	35,49	10 x	31,98	34,36	
3	374	Absent	1 x	48,34	41,32	10 x			
4	656	Absent	1 x	45,05	43,23	10 x	45,31		
5	1064	Absent	1 x	38,38	36,63	10 x	37,97	37,48	
6	2045	Present	1 x	32,25	34,98	10 x	31,99	34,56	

Laboratory identification code :	13
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,74 cycles
Copy number at the cut-off :	11,22 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	263	Absent	1 x	38,55	37,16	10 x	38,32	36,86	
2	458	Absent	1 x		43,47	10 x	39,86	38,18	
3	638	Present	1 x	31,71	31,96	10 x	33,62	34,08	
4	887	Absent	1 x	41,77	41,93	10 x	39,43	41,16	
5	1085	Absent	1 x	40,10	40,75	10 x	36,79	36,90	
6	1919	Present	1 x	32,13	32,38	10 x	32,51	33,85	

Laboratory identification code :	14
Responsibility agreement :	Yes
Cut-off at 15 copies :	10,60 cycles
Copy number at the cut-off :	37,47 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	8	Present	1 x	35,26	34,01	10 x	32,96	32,18	
2	26	Absent	1 x	44,16	39,45	10 x	38,24	37,43	
3	449	Present	1 x	34,86	32,56	10 x	32,10	31,04	
4	782	Absent	1 x	46,29	40,73	10 x	39,76	38,85	
5	1103	Present	1 x	35,99	35,64	10 x	35,85	36,49	
6	1400	Present	1 x	37,34	37,22	10 x	35,96	36,44	

Laboratory identification code :	15
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,74 cycles
Copy number at the cut-off :	10,71 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	155	Present	1 x	29,72	29,36	10 x	32,06	30,11	also 3 fold and 30 fold dilutions
2	395	Absent	1 x	45,00		10 x	41,94	39,82	also 3 fold and 30 fold dilutions
3	677	Absent	1 x			10 x	43,79	45,00	also 3 fold and 30 fold dilutions
4	707	Absent	1 x	38,33	37,37	10 x	37,22	37,88	also 3 fold and 30 fold dilutions
5	1229	Present	1 x	36,16	36,15	10 x	36,34	36,49	also 3 fold and 30 fold dilutions
6	1268	Present	1 x	29,60	29,47	10 x	30,47	30,74	also 3 fold and 30 fold dilutions

Laboratory identification code :	16	
Responsibility agreement :	Yes	
Cut-off at 15 copies :	34,30	cycles
Copy number at the cut-off :	10,88	copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	389	Present	20 x	29,97	30,32	200 x	32,84	32,59	
2	530	Absent	20 x		39,14	200 x			
3	617	Present	20 x	27,01	26,84	200 x	30,40	30,08	
4	845	Absent	20 x			200 x		37,96	
5	1127	Present	20 x	33,30	30,64	200 x	36,42	33,64	
6	1961	Present	20 x	27,29	26,13	200 x	30,71	29,46	

Laboratory identification code :	17
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,00 cycles
Copy number at the cut-off :	10,70 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	50	Present	1 x	33,81	34,25	10 x	37,02	37,56	
2	59	Absent	1 x	50,00	39,52	10 x	49,57	39,97	
3	68	Absent	1 x	41,23	40,85	10 x	50,00	39,79	
4	995	Present	1 x	33,36	32,72	10 x	36,30	36,25	
5	1124	Present	1 x	34,29	35,26	10 x	37,54	38,87	
6	1358	Present	1 x	34,67	35,13	10 x	35,42	34,98	

Laboratory identification code :	19
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,28 cycles
Copy number at the cut-off :	9,05 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	161	Absent	41 x	41,31	39,97	40 x	40,28	38,92	
2	176	Present	35 x	35,03	34,86	38 x	38,29	37,96	
3	563	Absent				48 x		48,26	
4	764	Present	34 x	33,99	33,41	36 x	35,96	35,74	
5	767	Absent	38 x	37,75	37,44	39 x	37,80	39,25	
6	824	Absent				42 x	42,35		

Laboratory identification code :	20
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,76 cycles
Copy number at the cut-off :	10,99 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	236	Absent	1 x	40,10	41,08	10 x	38,65	39,22	
2	686	Absent	1 x	38,63	39,34	10 x	37,48	37,12	
3	911	Present	1 x	32,57	32,98	10 x	35,39	36,12	
4	998	Present	1 x	34,36	34,08	10 x	35,91	34,13	
5	1004	Absent	1 x	41,73	40,96	10 x	39,08	38,69	
6	1415	Present	1 x	34,04	33,96	10 x	36,75	36,43	

Laboratory identification code :	22
Responsibility agreement :	Yes
Cut-off at 15 copies :	34,96 cycles
Copy number at the cut-off :	10,90 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	554	Present	1 x	29,22	29,30	10 x	35,12	35,25	
2	704	Present	1 x	32,86	32,77	10 x	35,87	35,92	
3	857	Absent	1 x	42,93	43,50	10 x	45,80	46,50	
4	971	Absent	1 x	41,19	42,50	10 x	45,10	45,90	
5	1205	Present	1 x	30,08	30,20	10 x	33,10	33,50	
6	1295	Absent	1 x	35,10	35,00	10 x	38,90	39,10	

Laboratory identification code :	25
Responsibility agreement :	Yes
Cut-off at 15 copies :	34,69 cycles
Copy number at the cut-off :	10,65 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	110	Absent	1 x	44,11	43,55	10 x	39,90	39,93	
2	218	Present	1 x	33,03	33,67	10 x	36,23	36,50	
3	383	Absent	1 x	41,33	43,86	10 x	38,70	39,50	
4	809	Absent	1 x	35,82	35,99	10 x	36,81	36,68	
5	953	Present	1 x	33,99	32,25	10 x	37,45	35,47	
6	1022	Absent	1 x	37,72	37,23	10 x	36,93	36,76	

Laboratory identification code :	26
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,75 cycles
Copy number at the cut-off :	10,97 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	98	Absent	1 x	39,81	39,27	10 x	38,55	38,70	num lab 2015-548
2	407	Present	1 x	32,60	31,92	10 x	35,47	35,51	num lab 2015-549
3	410	Present	1 x	36,62	36,16	10 x	37,03	36,92	num lab 2015-550
4	572	Absent	1 x	44,81		10 x	43,01		num lab 2015-551
5	710	Absent	1 x	43,94		10 x			num lab 2015-552
6	785	Present	1 x	31,89	32,04	10 x	35,53	35,28	num lab 2015-553

Laboratory identification code :	28
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,25 cycles
Copy number at the cut-off :	10,93 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	11	Present	1 x	34,54	34,84	10 x	36,54	36,05	
2	38	Absent	1 x		45,00	10 x	41,52		
3	122	Absent	1 x			10 x	39,89		
4	1247	Present	1 x	31,25	31,36	10 x	34,45	34,96	
5	1352	Present	1 x	31,71	31,63	10 x	35,03	35,04	
6	1463	Present	1 x	38,74	38,74	10 x	36,30	36,69	

Laboratory identification code :	29
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,87 cycles
Copy number at the cut-off :	11,59 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	35	Absent	1 x	41,55	42,88	10 x	41,66	45,69	PCR Inhibition control ran but no Inhibition present
2	95	Absent	1 x	40,23	40,54	10 x	39,19	43,21	PCR Inhibition control ran but no Inhibition present
3	365	Absent	1 x	37,08	36,53	10 x	39,32	40,87	PCR Inhibition control ran but no Inhibition present
4	584	Absent	1 x	47,00	47,00	10 x	46,34	46,82	PCR Inhibition control ran but no Inhibition present
5	647	Absent	1 x	45,31	47,00	10 x	45,92	44,23	PCR Inhibition control ran but no Inhibition present
6	2087	Absent	1 x	37,17	36,34	10 x	41,21	40,38	PCR Inhibition control ran but no Inhibition present

Laboratory identification code :	30
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,61 cycles
Copy number at the cut-off :	10,74 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	14	Absent	1 x	42,91	43,46	10 x	40,28	39,92	
2	386	Present	1 x	32,75	31,90	10 x	35,39	34,44	
3	620	Absent	1 x	38,13	38,90	10 x	38,25	38,63	
4	722	Present	1 x	32,83	32,62	10 x	34,49	34,93	
5	761	Absent	1 x	48,88	48,33	10 x	46,20	43,44	
6	803	Absent	1 x	47,57	48,25	10 x	44,54	46,58	

Laboratory identification code :	**31**	
Responsibility agreement :	**Yes**	
Cut-off at 15 copies :	**34,30**	**cycles**
Copy number at the cut-off :	**11,17**	**copies**

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	239	Present	1 x	32,16	31,20	10 x	35,70	34,30	
2	347	Absent	1 x	35,30	36,80	10 x	38,00	37,20	
3	416	Absent	1 x		48,70	10 x	45,00	41,76	
4	560	Absent	1 x	40,10	39,60	10 x	38,00	38,00	
5	614	Absent	1 x	44,00	48,00	10 x	44,20	43,70	
6	1100	Present	1 x	32,54	32,78	10 x	36,00	36,50	

2. Non EURL-AP network participants

Laboratory identification code :	**18**	
Responsibility agreement :	**Yes**	
Cut-off at 15 copies :	**36,62**	**cycles**
Copy number at the cut-off :	**10,14**	**copies**

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	323	Present	1 x	33,59	33,65	10 x	34,87	35,22	1- fold is undiluted
2	551	Absent	1 x	48,44	49,12	10 x	47,34	41,63	
3	641	Absent	1 x	35,62	37,83	10 x	36,19	38,51	inconsistant result: PCR repeated. 1x ct values:35,73 / 38,33 10x ct values:36,55 / 39,60.2 times inconsistant is absent
4	959	Absent	1 x	40,13	38,10	10 x	37,93	37,49	pcr inhibition: extra dilution 100x ct values 40,38 / 41,78
5	983	Absent	1 x	45,78	50,00	10 x	41,87	50,00	ct value 50: no amplification (N/A) was detected (flat signal)
6	1877	Present	1 x	32,49	32,65	10 x	33,72	33,90	

Laboratory identification code :	**21**	
Responsibility agreement :	**Yes**	
Cut-off at 15 copies :	**35,17**	**cycles**
Copy number at the cut-off :	**11,30**	**copies**

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	683	Absent		38,81	38,10		38,91	40,22	no inhibition
2	773	Absent		45,99			43,08	41,65	no inhibition
3	827	Present		34,57	34,66		36,84	36,27	no inhibition
4	1034	Absent					40,18	42,29	no inhibition
5	1043	Absent		41,44	40,86		41,44	39,91	no inhibition
6	1751	Present		34,08	33,80		36,39	35,68	no inhibition

Laboratory identification code :	**23**	
Responsibility agreement :	**Yes**	
Cut-off at 15 copies :	**35,90**	**cycles**
Copy number at the cut-off :	**10,00**	**copies**

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	185	Absent	160 x	39,31	42,88	640 x	38,95	39,58	
2	344	Present	160 x	31,28	31,67	640 x	32,92	33,33	
3	626	Absent	160 x	45,98	42,06	640 x	45,47	41,98	
4	770	Absent	160 x	38,12	37,08	640 x	37,18	38,76	
5	851	Present	160 x	35,32	35,36	640 x	35,70	35,92	PCR inhibition observed going from dilution 160 to 640 without an expected (=2) increase of Ct value
6	1079	Present	160 x	31,64	30,98	640 x	33,29	32,85	

Laboratory identification code :	**24**	
Responsibility agreement :	**Yes**	
Cut-off at 15 copies :	**33,25**	**cycles**
Copy number at the cut-off :	**10,92**	**copies**

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	29	Present	3 x	32,87	32,40	30 x	36,03	35,35	
2	242	Absent	3 x	36,75	36,67	30 x	39,66	38,05	
3	248	Absent	3 x	44,28	45,00	30 x	41,78	42,86	
4	812	Absent	3 x	39,24	42,39	30 x	39,42	38,22	
5	1046	Absent	3 x	43,65	39,87	30 x	41,14	38,41	
6	1646	Present	3 x	32,09	32,01	30 x	34,73	34,47	