EURL-AP PCR Proficiency Test 2014

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 1.0 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 feed samples (blanks, feed matrices fortified with terrestrial processed animal proteins or contaminated feed) sent to the participants the 21st of April 2014. In order to be in line with the reintroduction of non-ruminant PAPs in aquafeed, five out of the 6 samples were aquafeed.

All NRLs provided results in due time (deadline: 28th of April 2014). As an innovation for this year, all the participants received after the closure of the results (29th of April 2014) an individual table giving them a feedback of their results.

Five labs reported one false result out of 6 analyses to be carried out per lab (3 labs with one false positive result and 2 labs with one false negative result) and 3 labs had 2 false results (gathering the three possible combinations for 2 false results) on their six analyses. Corrective actions are taken with the participants having 2 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 the network of 27 NRLs to detect the presence of ruminant processed animal proteins 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 21st of February 2014 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 21st of March 2014, the material for the test (a set of 6 blind samples) was provided to the participants by express shipment. The 24th of March 2014 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 28th of April 2014.

The 27 NRLs participants delivered their results in due time.

Concerning the non NRL-AP participants, one lab sent the results only the 06th of May but it was in agreement with the EURL-AP (due to administrative problems, this lab received its set of samples only the 07th of April).

3.2. Material

3.2.1. Description of the samples

Five samples of aquafeed with or without processed animal proteins (PAPs) from ruminant (cattle) origin at a concentration level ~ 0.1 % in mass fraction have been prepared as shown in Table 1 and provided to the participants. A sixth sample of feed adulterated with a sheep PAP was also included in all the sets distributed to the participants. Two samples (one positive sample and a negative one) among the six were already distributed in the sets for the PCR proficiency test 2013 and one sample (sample 6) was already present in the sets of the implementation test of 2012. 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). Details of the samples are indicated in Table 1. The composition of the aquafeed is described in Annex 5.

Table 1 : Composition of the blind sample set used in the EURL-AP PCR Proficiency Test 2014.

Sample	Material	Quantity/lab	Remark
1	Aquafeed 1 (containing ruminant DNA)	1	Negative with microscopy but positive with PCR
2	Aquafeed 2 free of ruminant PAP	1	Already in duplicate in the sample set of PT PCR 2013
3	Aquafeed 3 free of ruminant PAP	1	
4	Aquafeed 4 free of ruminant PAP + 5 % pig blood meal	1	
5	Aquafeed 2 free of ruminant PAP + 0.1 % w/w cattle PAP in	1	Already in duplicate in the sample set of PT PCR 2013
6	Feed free of ruminant PAP + 0.1 % w/w sheep PAP	1	Already in the sample set of the implementation test 2012
Total		6	

3.2.2. Materials used in the preparation of the samples

- The aquafeed matrices were selected among the EURL-AP sample bank.
  They had not to be too fatty to allow a grinding and a good homogenization. They also had to be free of any traces of ruminant DNA (this parameter was checked by PCR and microscopy).
- The PAPs used to spike the blank aquafeed material were the following ones:
  - a cattle PAP heat treated at 137 °C (used in sample #5)
  - a sheep PAP heat treated at 133 °C (used in sample #6).

3.2.3. Homogeneity study

Two samples were already prepared for the PT PCR 2013 (samples #2 and #5 of Table 1) and a third one was realised for the implementation test in 2012 (sample #6 of Table 1). Their homogeneity was already checked in 2013 and 2012 respectively. One replicate of these three samples were successfully analysed during the preparation of this study.

Concerning the three other samples (samples #1, #3 and #4 of Table 1), ten replicates 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 1.0. In final, 20 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 1.0 and another target corresponding to the species present in the sample. The results are detailed in Table 2.

Table 2 : PCR results obtained with sample replicates

Sample type	Material	Nr of samples analysed	Nr of PCR results	Detection with		
				Ruminant target	Pig target	Fish target
1	Aquafeed 1	10	20	20x positive	NT[2]	NT[2]
2	Aquafeed 2	1	2	2x negative	NT[2]	2x positive
3	Aquafeed 3	10	20	20x negative	NT[2]	20x positive
4	Aquafeed 4 + 5 % w/w pig blood meal	10	20	20x negative[1]	20x positive	NT[2]
5	Aquafeed 2 + 0.1 % w/w cattle PAP	1	2	2x positive	NT[2]	NT[2]
6	Feed + 0.1 % w/w sheep PAP	1	2	2x positive	NT[2]	NT[2]

[1] However, 1 extraction replicate of the sample gave one positive result which was not confirmed by the second PCR analysis.

[2] NT : not tested

The homogeneity study showed that positive samples for ruminant detection are continuously positive when analysed. Similarly the negative samples all led to negative results even if one of the 10 test portion replicates of sample #4 gave once an ambiguous result. The quality of the DNAs giving the negative results is successfully controlled with other DNA targets (pig and fish assay) that must be present.

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 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 27 NRLs for the six sample types submitted to qualitative analysis.

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

Sample	Material	Nr of results	AC
1	Aquafeed 1	27	0.963 (1)
2	Aquafeed 2	27	0.963 (1)
3	Aquafeed 3	27	0.963 (1)
4	Aquafeed 4 + 5 % w/w pig blood meal	27	0.852 (4)
5	Aquafeed 2 + 0.1 % w/w cattle PAP	27	0.889 (3)
6	Feed + 0.1 % w/w sheep PAP	27	1.000 (0)

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

For samples #2 and #5, the overall results, expressed in terms of global accuracy (AC), are not as good as in 2013 (0.981 for the 2 samples) but as these 2 samples were in duplicate last year, one false result had less impact on the global accuracy.

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	1	1.000	1.000	1.000
	2	1.000	1.000	1.000
	4	1.000	1.000	1.000
	5	1.000	1.000	1.000
	6	1.000	1.000	1.000
	7	1.000	1.000	1.000
	9	1.000	1.000	1.000
	10	1.000	1.000	1.000
	11	1.000	1.000	1.000
	13	1.000	1.000	1.000
	14	1.000	1.000	1.000
	15	1.000	1.000	1.000
	16	1.000	1.000	1.000
	18	1.000	1.000	1.000
	19	1.000	1.000	1.000
	22	1.000	1.000	1.000
	24	1.000	1.000	1.000
	25	1.000	1.000	1.000
	27	1.000	1.000	1.000
20	17	0.833	1.000	0.667
	20	0.833	1.000	0.667
	23	0.833	1.000	0.667
23	8	0.833	0.667	1.000
	12	0.833	0.667	1.000
25	26	0.667	1.000	0.334
	3	0.667	0.667	0.667
	21	0.667	0.334	1.000

Table 4 illustrates the very good level of global performance for 19 labs out of 27 NRLs (70.4 % of the NRLs) having no false result. Nevertheless, 5 labs out of the 27 (18.5 %) provided 1 incorrect result:

- PD : labs 17, 20 and 23 ;
- ND : labs 8 and 12.

In addition, 3 labs (11.1 %) had 2 incorrect results:

- 2 PD : lab 26 ;
- 1 PD and 1 ND : lab 3 ;
- 2 ND : lab 21.

4.2.3. Assessment of the usefulness of the 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 6, 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 6 : 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)
1	11.85	36.46	No
8	11.59	35.90	Yes
2	11.37	35.56	No
21	11.37	36.44	Yes
23	11.35	36.03	Yes
20	11.30	34.27	Yes
24	11.25	36.52	No
5	11.17	34.30	No
22	11.02	36.43	No
26	11.00	37.82	Yes
13	10.94	35.77	No
16	10.93	37.25	No
25	10.88	34.30	No
9	10.76	35.92	No
7	10.65	34.69	No
10	10.60	37.47	No
4	10.55	37.34	No
15	10.17	31.93	No
27	10.02	34.94	No
17	9.72	38.02	Yes
14	9.50	36.20	No
19	9.48	40.33	No
12	9.38	36.63	Yes
6	9.16	37.30	No
11	9.09	36.35	No
3	8.00	36.15	Yes
18	7.79	34.48	No

Two participants (labs 3 and 18) out of the 27 were unable to reach the minimum criterion of 9.00 copies.

In 2013, the introduction of this quality control on the cut-off value improved the standardisation of the method. In 2014, the use of the calibrants provided by the JRC-IRMM globally improved the values obtained by the participants : 1. The range in copies shifts from 6.28-11.18 to 7.79-11.85; 2. This time one can also observe a higher percentage of the labs with

a cut-off corresponding to a number of copies > 10 (70.4 % instead of 55.6 % during the PT 2013).

Except for Lab 3, the false results cannot be attributed to a wrongly set cut-off anymore.

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

4.3.1. Overview of results

Table 7 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 7 : Global results expressed as accuracy (AC) for the six sample types

Sample	Material	Nr of results	AC
1	Aquafeed 1	4	1.000
2	Aquafeed 2	4	1.000
3	Aquafeed 3	4	1.000
4	Aquafeed 4 + 5 % w/w pig blood meal	4	1.000
5	Aquafeed 2 + 0.1 % w/w cattle PAP	4	1.000
6	Feed + 0.1 % w/w sheep PAP	4	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 perfect results are to be found in Table 8.

Table 8 : 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	28	1.000	1.000	1.000
	29	1.000	1.000	1.000
	30	1.000	1.000	1.000
	32	1.000	1.000	1.000

Table 8 illustrates the very good level of these 4 labs participating for the first time to an interlaboratory study organized by the EURL-AP and having no false result.

4.3.3. Assessment of the cut-off values

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

Table 9 : 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)
28	10.92	33.25	No
29	9.30	35.24	No
30	10.00	36.00	No
32	10.61	35.15	No

5. Conclusions

This study is the first assessment of the proficiency level in PCR methods for the detection of the ruminant PAP of the NRL network since the reintroduction of non-ruminant PAP in aquafeed which occured the 1st of June 2013. The 27 NRLs submitted results before the deadline. Looking globally at these results sent to the EURL-AP, 70.4 % of the participating NRLs (19 labs out of 27) were able to detect correctly the presence of ruminant PAPs in aquafeed and had no false result. Three NRLs (Labs 17, 20 and 23) had one false positive result and two other NRLs (Lab 8 and 12) had one false negative result. Three NRLs had two false results (Labs 3, 21 and 26). These 3 labs were considered as under-performing and received a new set of samples as corrective and follow-up action.

The detection of ruminant PAPs in feeds in general by PCR is well implemented in 19 NRLs out of 27 (70.4 % of the NRLs) having no false result. The occurrence of false negative results is of 6.17 % (5 results out of 81 analyses) and comes from 4 NRLs. The rate of false positive results (6 results out of 81 analyses) is of 7.41 % and is a little bit too high. Nevertheless, it does not reach the level claimed by the sector.

The determination of the cut-off is now well implemented by almost all the NRLs that reached the minimum quality criterion set by the EURL-AP. Moreover an improvement of the values of this quality criterion is observed in a majority of the NRLs (20 NRLs out of the 27). This tendency is probably due to the use of the calibrants produced and provided by the JRC-IRMM. The data collected demonstrate the high quality of this reference material and remind why their use is now mandatory. Except for one false positive result of Lab 3, it seems that false results are no longer linked to a not correctly set cut-off. The false negative result provided by Lab 12 with the sample 14-169 can also be explained by a PCR inhibition not totally removed in the DNA dilution rates tested (1x and 10x). Additional dilution rates should have been tested before any conclusion.

It must be underlined that the experience of a majority of the network labs is still small and to improve to avoid most of the other false results. As with the microscopy, a regular practice of the PCR will be the only way to get a better proficiency.

Concerning the 4 labs external to the NRLs network, they obtained perfect results for their first participation thanks most probably to their large experience in PCR.

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 2014 for the detection of ruminant DNA in feed using the validated PCR assay

Walloon Agricultural Research Centre

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.

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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 : **21 February 2014**
- Sending of the sample boxes and communication of the instructions : **21 March 2014**

> ***Samples will be sent to the NRL 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 changes.***

- Deadline for returning of results to the organizer : **28 April 2014**

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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Annex 2

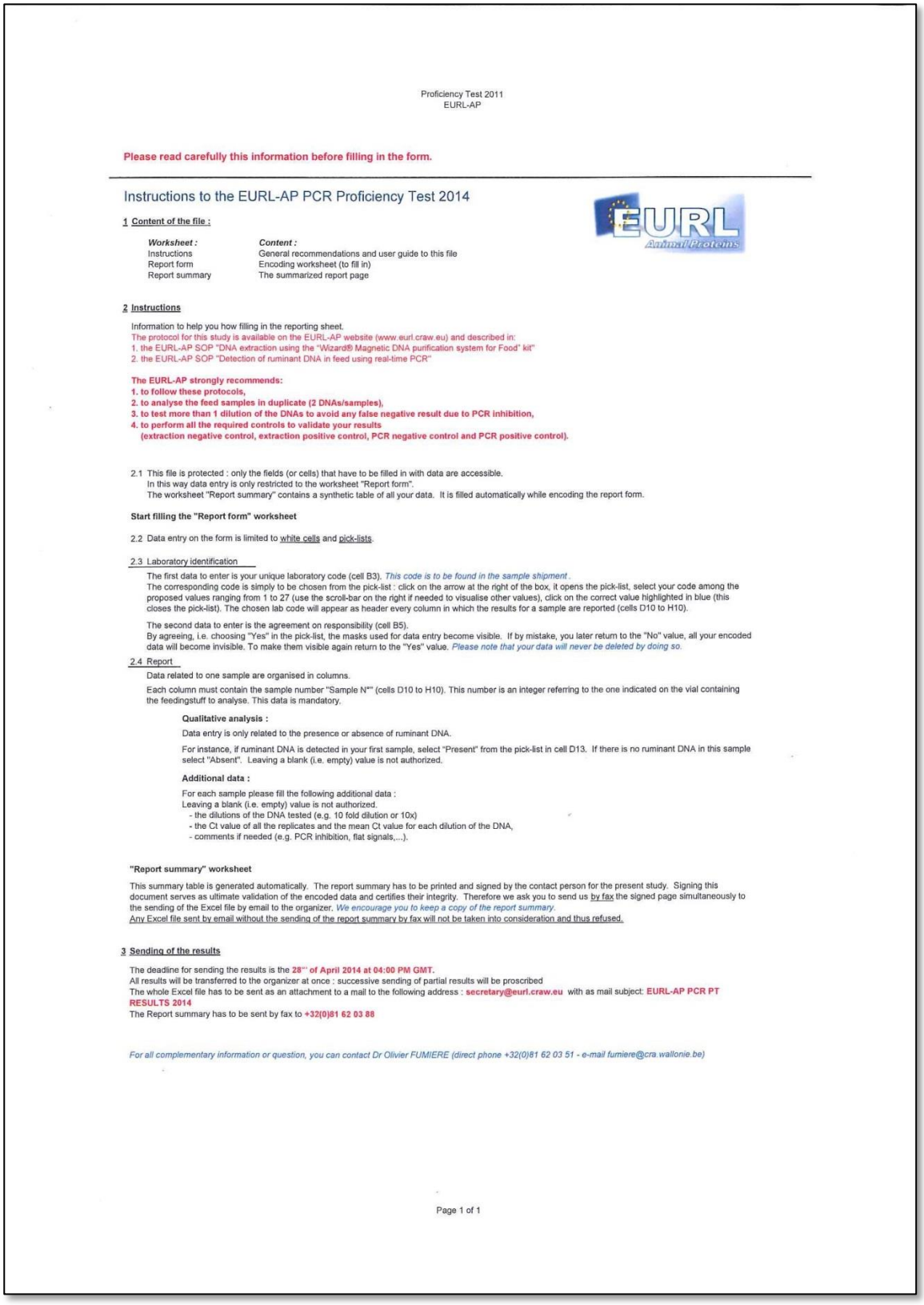
List of participants

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**
France	Eurofins Analytics
Netherlands	NutriControl
Netherlands	TNO Triskelion bv
Norway	National Institute of Nutrition and Seafood Research

Annex 3

Excel result report file

a. Instruction sheet



Proficiency Test 2011
EURL-AP

Please read carefully this information before filling in the form.

Instructions to the EURL-AP PCR Proficiency Test 2014

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 **28th of April 2014 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 2014**
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 51 - e-mail fumiere@cra.wallonie.be)

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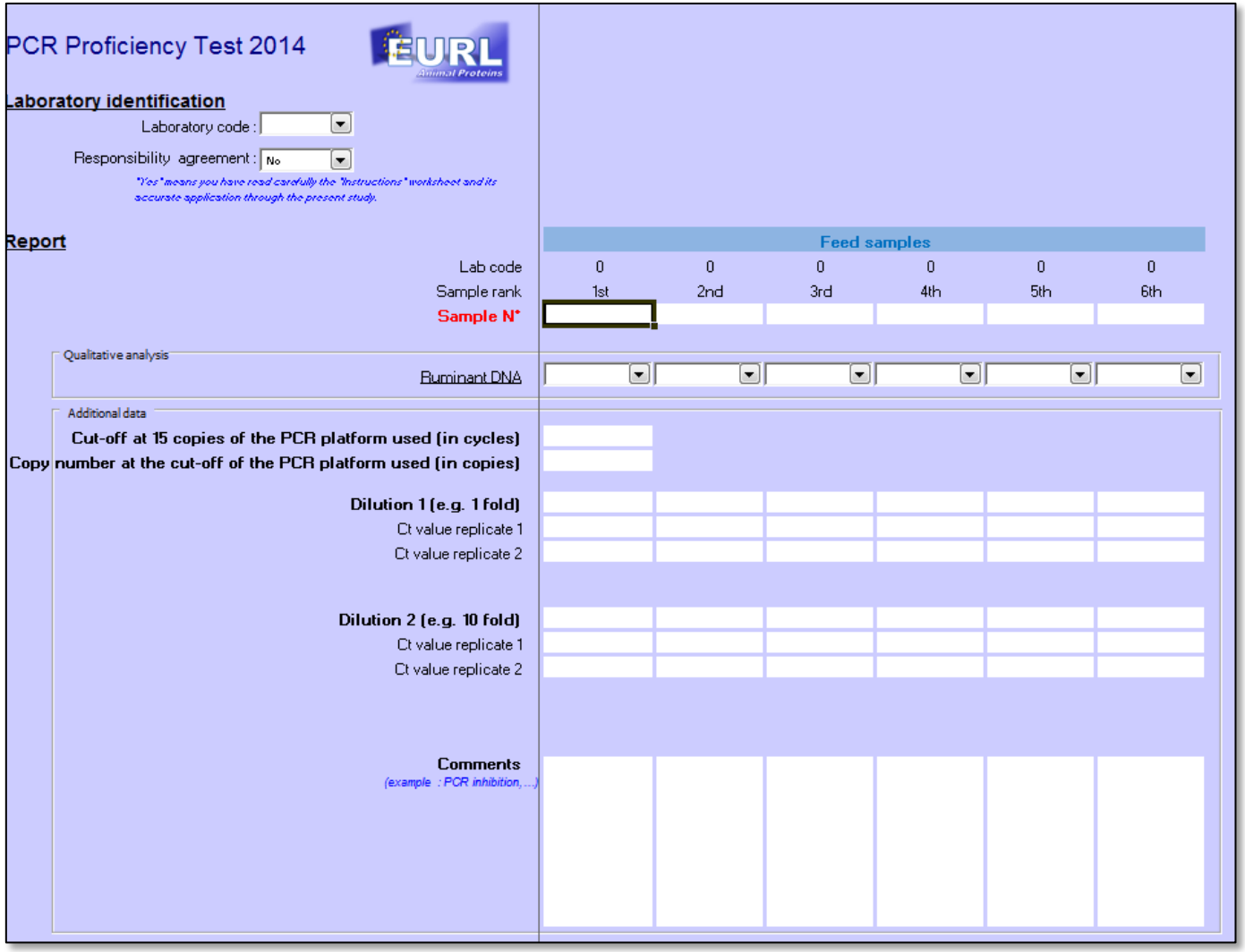
b. Recording sheet



PCR Proficiency Test 2014

EURL Animal Proteins

Laboratory identification

Laboratory code :

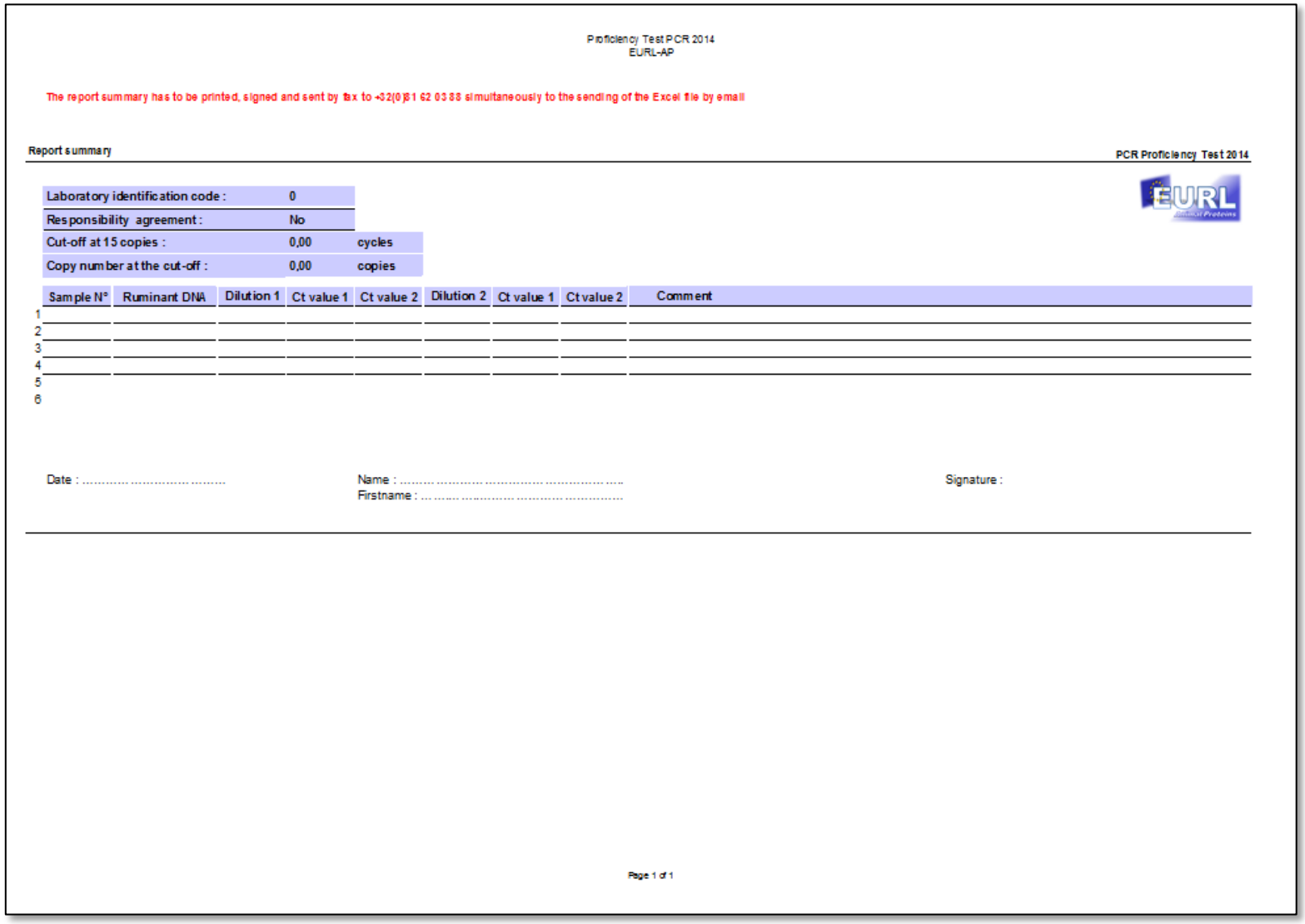
Responsibility agreement : No

"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



Proficiency Test PCR 2014
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 2014

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 :

Page 1 of 1

Annex 4

Composition of sample sets

1. EURL-AP network participants

		Lab number																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Samples	**Intended results**																											
Aquafeed 2 + 0.1 % w/w cattle PAP	+	14-001	14-012	14-016	14-019	14-026	14-033	14-037	14-048	14-052	14-055	14-062	14-069	14-073	14-084	14-088	14-091	14-098	14-105	14-109	14-120	14-124	14-127	14-134	14-141	14-145	14-156	14-160
Feed + 0.1 % w/w sheep PAP	+	14-002	14-007	14-018	14-021	14-025	14-034	14-038	14-043	14-054	14-057	14-061	14-070	14-074	14-079	14-090	14-093	14-097	14-106	14-110	14-115	14-126	14-129	14-133	14-142	14-146	14-151	14-162
Aquafeed 1	+	14-003	14-008	14-013	14-023	14-029	14-031	14-039	14-044	14-049	14-059	14-065	14-067	14-075	14-080	14-085	14-095	14-101	14-103	14-111	14-116	14-121	14-131	14-137	14-139	14-147	14-152	14-157
Aquafeed 2	-	14-004	14-009	14-014	14-020	14-030	14-035	14-040	14-045	14-050	14-056	14-066	14-071	14-076	14-081	14-086	14-092	14-102	14-107	14-112	14-117	14-122	14-128	14-138	14-143	14-148	14-153	14-158
Aquafeed 3	-	14-005	14-010	14-015	14-022	14-027	14-036	14-041	14-046	14-051	14-058	14-063	14-072	14-077	14-082	14-087	14-094	14-099	14-108	14-113	14-118	14-123	14-130	14-135	14-144	14-149	14-154	14-159
Aquafeed 4 + 5 % pig blood meal	-	14-006	14-011	14-017	14-024	14-028	14-032	14-042	14-047	14-053	14-060	14-064	14-068	14-078	14-083	14-089	14-096	14-100	14-104	14-114	14-119	14-125	14-132	14-136	14-140	14-150	14-155	14-161

* Red cells correspond to false results submitted by the participants

2. Non EURL-AP network participants

		Lab number			
		28	29	30	32
Samples	**Intended results**				
Aquafeed 2 + 0.1 % w/w cattle PAP	+	14-163	14-170	14-177	14-192
Feed + 0.1 % w/w sheep PAP	+	14-165	14-169	14-178	14-187
Aquafeed 1	+	14-167	14-173	14-175	14-188
Aquafeed 2	-	14-164	14-174	14-179	14-189
Aquafeed 3	-	14-166	14-171	14-180	14-190
Aquafeed 4 + 5 % pig blood meal	-	14-168	14-172	14-176	14-191

Annex 5

Aquafeed formulations

AQUAFEED 1

Composition

Fish meal 19.3%, fish oil 11.0%, faba beans 5.9%, lineseed oil 3.6%, maize gluten 22.4%, soya meal 30.4%, soya oil 3.6%, wheat flower 2.6%, premix oligo vitamins 0.8%, amino acids 0.4%.

Remark: The origin of the ruminant material in the sample is unknown but is most probably due to a contamination with ruminant blood.

AQUAFEED 2 (CARP FEED)

Composition

Soya meal, wheat, fish meal, sunflower, maize gluten, fish oil, rapeseed, mono ammonium phosphate, yeasts, algae.

AQUAFEED 3 (COMPLETE FEEDINGSTUFF FOR FISH)

Analytical constituents

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

AQUAFEED 4 (40% COMPLETE FEED FOR SALMON + 60 % FISHFEED)

1. COMPLETE FEED FOR SALMON

Analytical constituents
Oils & fats 36.0%
Calcium 0.7%
Protein 33.0%
Phosphorus 0.8%
Ash 5.3%
Sodium 0.1%
Fibre 2.9%

Composition
Fish meal, fish oil, sunflower meal, wheat, soya bean meal, wheat gluten, beans, vitamins, minerals, L-lysine, DL-methionine, single cell pigment (Panaferd).

Additives (per kg)

E1	Iron (Ferrous sulphate)	50 mg
E2	Iodine (Calcium iodate)	3 mg
E4	Copper (Cupric sulphate)	6 mg
E5	Manganese (Manganese sulphate)	36 mg
E6	Zinc (Zinc sulphate)	120 mg
3b8-12	Selenium (selenised yeast)	0.1 mg
E671	Vitamin D3	1500 IU

2. FISH FEED

Composition
Rapeseed oil 20.2%, sunflower expeller 16.6%, North Atlantic fish meal 15%, wheat 10%, soya protein concentrate 9.7%, fish oil 8.7%, South American fish meal 5%, corn gluten meal 5%, wheat gluten 5%, additives 2.8%, recycled material 2%.

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 2014 for the detection of ruminant PAPs

TABULATION RESULTS

NAME OF LABORATORY:
LABORATORY CODE:
CONTACT PERSON:
MATERIAL SET:

Samples	Composition	Intended result	Reported result
# 14-XXX	Aquafeed I	Positive	
# 14-XXX	Aquafeed II	Negative	
# 14-XXX	Aquafeed III	Negative	
# 14-XXX	Aquafeed II + 0.1 % cattle PAP	Positive	
# 14-XXX	Aquafeed IV + 5 % pig blood meal	Negative	
# 14-XXX	Feed + 0.1 % sheep PAP	Positive	

Walloon Agricultural Research Centre

Further information

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  e-mail: berben@cra.wallonie.be
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  🖷 +32 (0)81 62 03 88
  e-mail: fumiere@cra.wallonie.be

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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 :	36,46 cycles
Copy number at the cut-off :	11,62 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	1	Present	1 x	33,89	31,28	10 x	36,38	34,72	
2	2	Present	1 x	32,67	31,84	10 x	32,78	31,97	
3	3	Present	1 x	33,55	34,35	10 x	34,91	35,68	
4	4	Absent	1 x	46,81	42,65	10 x	47,46	47,64	
5	5	Absent	1 x	42,95	43,81	10 x	41,96	41,47	
6	6	Absent	1 x	43,07	49,04	10 x	43,25	41,11	

Laboratory identification code :	2
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,56 cycles
Copy number at the cut-off :	11,37 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	7	Present	1 x	30,99	29,90	10 x	33,46	31,54	
2	8	Present	1 x	33,89	33,45	10 x	37,13	36,45	
3	9	Absent	1 x	42,21	42,50	10 x	44,21	44,98	
4	10	Absent	1 x	40,85	39,88	10 x	41,01	40,57	Possible PCR inhibition.Ct values on 2x dilution: 39.857 and 38.516. No Inhibition was observed in other dilutions (5x and 10x).
5	11	Absent	1 x	38,98	38,01	10 x	41,43	41,23	
6	12	Present	1 x	31,62	34,25	10 x	33,25	35,94	High standard deviation between two test portion (however lower than 3ct).

Laboratory identification code :	3
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,15 cycles
Copy number at the cut-off :	8,00 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	13	Absent	1 x	36,43	36,33	10 x	36,58	36,68	
2	14	Absent	1 x	38,10	39,00	10 x	40,00	40,00	
3	15	Absent	1 x	40,00	40,00	10 x	40,00	40,00	
4	16	Present	1 x	35,34	35,14	10 x	37,83	37,52	
5	17	Present	1 x	35,74	36,24	10 x	35,86	36,57	
6	18	Present	1 x	34,26	33,52	10 x	36,44	37,52	

Laboratory identification code :	4
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,34 cycles
Copy number at the cut-off :	10,55 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	19	Present	1 x	32,24	33,13	10 x	33,03	34,48	
2	20	Absent	1 x	44,89		10 x	44,78	42,48	
3	21	Present	1 x	31,10	29,89	10 x	33,25	31,87	
4	22	Absent	1 x	41,87	41,15	10 x	39,88	39,15	
5	23	Present	1 x	32,46	31,97	10 x	35,77	35,24	
6	24	Absent	1 x	39,89	40,78	10 x	42,06	42,82	

Laboratory identification code :	5
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	25	Present	1 x	30,29	31,35	10 x	31,04	32,43	
2	26	Present	1 x	33,57	34,04	10 x	34,76	34,57	
3	27	Absent	1 x	47,99	48,03	10 x	41,75	40,57	
4	28	Absent	1 x	40,57	39,40	10 x	41,09	40,08	
5	29	Present	1 x	32,44	32,16	10 x	34,81	34,36	
6	30	Absent	1 x	45,52	42,20	10 x	48,79	43,53	

Laboratory identification code :	6
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,30 cycles
Copy number at the cut-off :	9,16 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	31	Present	1 x	31,21	30,90	10 x	33,70	33,80	
2	32	Absent	1 x	38,17	39,76	10 x	38,51	39,63	
3	33	Present	1 x	31,85	32,64	10 x	32,73	33,00	
4	34	Present	1 x	29,48	27,19	10 x	31,71	29,11	
5	35	Absent	1 x	41,08	38,87	10 x	40,02	40,25	
6	36	Absent	1 x	39,56	41,41	10 x	38,30	39,35	

Laboratory identification code :	7
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	37	Present	10 x	28,23	31,04	40 x	30,48	33,17	
2	38	Present	10 x	29,80	25,71	40 x	30,52	24,80	Inhibition
3	39	Present	10 x	32,77	32,07	40 x	32,47	33,42	Inhibition
4	10	Absent	10 x	40,15	39,76	40 x	38,57	38,39	Repetition run because of ambiguous results in the first run
5	41	Absent	10 x	39,26	39,29	40 x	37,10	38,95	Repetition run because of ambiguous results in the first run
6	42	Absent	10 x	37,95	38,12	40 x	38,61	38,95	Repetition run because of ambiguous results in the first run

Laboratory identification code :	8
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,90 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	14-043	Present	1 x	33,04	31,88	10 x	35,01	33,92	
2	14-044	Present	1 x	34,12	33,90	10 x	36,59	37,10	
3	14-045	Absent	1 x	47,00	46,16	10 x	42,61	43,46	
4	14-046	Absent	1 x	43,22	40,96	10 x	42,09	41,38	
5	14-047	Absent	1 x	40,00	40,15	10 x	41,12	41,78	
6	14-048	Absent	1 x	37,53	36,56	10 x	38,36	38,09	

Laboratory identification code :	9
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	49	Present	1 x	32,20	32,49	10 x	35,93	35,25	
2	50	Absent	1 x	41,11	40,67	10 x	40,74	44,28	
3	51	Absent	1 x	39,52	39,94	10 x	39,50	39,77	
4	52	Present	1 x	30,36	29,74	10 x	33,16	32,31	
5	53	Absent	1 x	39,34	40,30	10 x	41,26	40,50	
6	54	Present	1 x	29,17	28,97	10 x	31,23	31,03	

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

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	55	Present	1 x	34,27	35,37	10 x	34,28	34,65	
2	56	Absent	1 x	40,52	45,56	10 x	42,08	39,34	
3	57	Present	1 x	30,34	30,35	10 x	28,35	29,23	PCR inhibition
4	58	Absent	1 x	47,89	45,86	10 x	40,92	40,68	PCR inhibition
5	59	Present	1 x	35,59	34,81	10 x	35,46	36,19	
6	60	Absent	1 x	43,36		10 x	41,40	44,96	

Laboratory identification code :	11
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,35 cycles
Copy number at the cut-off :	9,09 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	61	Present	3 x	31,15	29,48	30 x	33,32	31,85	
2	62	Present	3 x	30,45	33,25	30 x	33,29	35,47	
3	63	Absent	3 x	39,93	38,01	30 x	44,21	41,26	
4	64	Absent	3 x	38,21	37,91	30 x	41,20	47,64	
5	65	Present	3 x	34,30	34,67	30 x	37,07	37,33	
6	66	Absent	3 x	37,62	38,64	30 x	39,43	41,22	

Laboratory identification code :	12
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,63 cycles
Copy number at the cut-off :	9,38 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	67	Present	1 x	36,26	36,56	10 x	37,80	37,90	PCR inhibition
2	68	Absent	1 x	44,96	42,60	10 x	41,41	39,98	
3	69	Absent	1 x	36,78	37,89	10 x	37,01	37,23	PCR inhibition
4	70	Present	1 x	34,10	34,56	10 x	35,65	35,78	
5	71	Absent	1 x	40,94	40,70	10 x	42,00	41,97	
6	72	Absent	1 x	40,39	39,36	10 x	42,08	40,36	

Laboratory identification code :	13
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,77 cycles
Copy number at the cut-off :	10,94 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	73	Present	1 x	34,78	34,59	10 x	37,26	37,63	
2	74	Present	1 x	33,18	35,01	10 x	36,94	39,06	
3	75	Present	1 x	34,89	33,91	10 x	37,26	35,89	
4	76	Absent	1 x		41,92	10 x		45,00	
5	77	Absent	1 x	38,72	42,51	10 x	40,39	45,00	
6	78	Absent	1 x	45,00	41,32	10 x		45,00	

Laboratory identification code :	14
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,20 cycles
Copy number at the cut-off :	9,50 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	79	Present	1 x	25,62	26,34	10 x	28,85	29,11	
2	80	Present	1 x	29,41	28,88	10 x	33,05	34,28	
3	81	Absent	1 x	37,55	37,69	10 x	41,22	42,19	
4	82	Absent	1 x	36,30	36,46	10 x	37,25	36,97	
5	83	Absent	1 x	36,80	36,95	10 x	38,21	37,94	
6	84	Present	1 x	31,52	32,46	10 x	35,17	36,18	

Laboratory identification code :	15
Responsibility agreement :	Yes
Cut-off at 15 copies :	31,93 cycles
Copy number at the cut-off :	10,17 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	85	Present	1 x	30,49	30,28	10 x	32,01	30,98	
2	86	Absent	1 x	40,68	42,17	10 x	38,42	37,47	DNA quality confirmed by 18S PCR
3	87	Absent	1 x	39,35	41,01	10 x	37,85	37,86	DNA quality confirmed by 18S PCR
4	88	Present	1 x	30,26	30,97	10 x	31,23	31,41	
5	89	Absent	1 x	37,41	37,18	10 x	37,47	37,83	DNA quality confirmed by 18S PCR
6	90	Present	1 x	27,19	28,29	10 x	28,72	30,11	

Laboratory identification code :	16
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	91	Present	1 x	33,05	33,26	10 x	33,22	33,57	
2	92	Absent	1 x	39,53	45,00	10 x	38,57	41,30	
3	93	Present	1 x	30,95	30,84	10 x	31,75	31,66	
4	94	Absent	1 x	45,00	43,72	10 x	38,83	38,25	
5	95	Present	1 x	29,66	30,20	10 x	30,02	29,78	
6	96	Absent	1 x	40,49	45,00	10 x	38,94	40,46	

Laboratory identification code :	17
Responsibility agreement :	Yes
Cut-off at 15 copies :	38,02 cycles
Copy number at the cut-off :	9,72 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	97	Present	1 x	32,44	33,39	10 x	34,74	35,00	
2	98	Present	1 x	32,96	33,28	10 x	37,33	37,45	
3	99	Absent	1 x	40,40	39,01	10 x	39,15	39,77	
4	100	Absent	1 x	40,42	39,73	10 x	38,80	38,69	
5	101	Present	1 x	34,03	33,78	10 x	37,18	36,62	
6	102	Present	1 x	35,47	35,80	10 x	37,58	37,36	

Laboratory identification code :	18
Responsibility agreement :	Yes
Cut-off at 15 copies :	34,48 cycles
Copy number at the cut-off :	7,79 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	103	Present	1 x	29,54	29,14	10 x	32,05	32,50	2 extr. per sample, 2 PCRs per extr., hence the Ct value repl. is the mean of 2 PCRs. The # of cps at the cut-off is below 9 cps.
2	104	Absent	1 x	37,13	36,56	10 x	36,90	36,62	2 extr. per sample, 2 PCRs per extr., hence the Ct value repl. is the mean of 2 PCRs. The # of cps at the cut-off is below 9 cps.
3	105	Present	1 x	29,62	27,59	10 x	31,08	29,06	2 extr. per sample, 2 PCRs per extr., hence the Ct value repl. is the mean of 2 PCRs. The # of cps at the cut-off is below 9 cps.
4	106	Present	1 x	25,43	27,89	10 x	27,65	29,73	2 extr. per sample, 2 PCRs per extr., hence the Ct value repl. is the mean of 2 PCRs. The # of cps at the cut-off is below 9 cps.
5	107	Absent	1 x	37,23	38,54	10 x	36,87	36,36	2 extr. per sample, 2 PCRs per extr., hence the Ct value repl. is the mean of 2 PCRs. The # of cps at the cut-off is below 9 cps.
6	108	Absent	1 x	39,43	39,59	10 x	36,86	37,03	2 extr. per sample, 2 PCRs per extr., hence the Ct value repl. is the mean of 2 PCRs. The # of cps at the cut-off is below 9 cps.

Laboratory identification code :	19
Responsibility agreement :	Yes
Cut-off at 15 copies :	40,33 cycles
Copy number at the cut-off :	9,48 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	109	Present	1 x	33,79	33,30	10 x	33,83	33,87	
2	110	Present	1 x	29,97	29,26	10 x	31,56	30,61	
3	111	Present	1 x	32,19	31,38	10 x	34,30	35,15	
4	112	Absent	1 x	43,49	42,05	10 x	41,68	42,50	
5	113	Absent	1 x	42,75	43,48	10 x	40,99	41,60	
6	114	Absent	1 x	43,98	41,35	10 x	42,38	42,50	

Laboratory identification code :	20
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	115	Present	1 x	24,79	24,81	10 x	27,79	27,79	
2	116	Present	1 x	28,83	28,78	10 x	32,74	33,05	
3	117	Absent	1 x	37,83	37,63	10 x	37,24	44,30	
4	118	Absent	1 x	36,24	35,99	10 x	37,01	38,40	
5	119	Present	1 x	33,80	33,84	10 x	36,61	35,15	
6	120	Present	1 x	29,87	29,63	10 x	33,15	33,22	

Laboratory identification code :	21
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,44 cycles
Copy number at the cut-off :	11,37 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	121	Absent	1 x	39,71	39,16	10 x	39,08	40,03	No inhibition, C+ inh= 32copies
2	122	Absent	1 x	40,11	39,70	10 x	39,43	42,15	No inhibition, C+ inh= 32copies
3	123	Absent	1 x	47,28	39,15	10 x	42,53	43,43	No inhibition, C+ inh= 32copies
4	124	Absent	1 x	37,42	36,47	10 x	37,59	36,73	No inhibition. 1st extr, dilut 1: inconsistent. 2nd extr, both dilut: NEG. Data shown: 2nd extr.
5	125	Absent	1 x	41,82	47,49	10 x	42,20	44,38	No inhibition, C+ inh= 32copies
6	126	Present	1 x	34,49	36,15	10 x	33,81	36,51	

Laboratory identification code :	22
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,43 cycles
Copy number at the cut-off :	11,02 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	127	Present	1 x	33,08	32,83	10 x	34,50	34,18	
2	128	Absent	1 x	39,33	37,02	10 x	38,35	37,38	Further testing showed no PCR inhibtion in this sample.
3	129	Present	1 x	28,72	28,88	10 x	31,20	30,58	
4	130	Absent	1 x	40,34	41,90	10 x	38,43	39,57	Further testing showed no PCR inhibtion in this sample
5	131	Present	1 x	30,50	30,25	10 x	32,28	31,91	
6	132	Absent	1 x	35,58	38,49	10 x	37,53	38,89	Further testing showed no PCR inhibtion in this sample

Laboratory identification code :	23
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,03 cycles
Copy number at the cut-off :	11,35 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	133	Present	1 x	30,19	30,22	10 x	31,18	30,94	
2	134	Present	1 x	32,58	32,59	10 x	32,95	32,81	
3	135	Absent	1 x	39,07	39,66	10 x	38,78	38,84	
4	136	Present	1 x	35,69	35,72	10 x	36,55	36,75	
5	137	Present	1 x	31,62	31,72	10 x	33,19	33,63	
6	138	Absent	1 x	37,22	37,55	10 x	37,75	38,28	

Laboratory identification code :	24
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,52 cycles
Copy number at the cut-off :	11,25 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	139	Present	1 x	29,74	29,96	10 x	31,79	31,69	
2	140	Absent	1 x	40,67	38,07	10 x	39,11	39,76	
3	141	Present	1 x	33,29	33,50	10 x	36,02	35,68	
4	142	Present	1 x	29,01	30,96	10 x	32,07	33,83	
5	143	Absent	1 x	39,84	42,08	10 x	39,94	41,48	
6	144	Absent	1 x	43,75	45,00	10 x	38,51	38,74	

Laboratory identification code :	25
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	145	Present	20 x	32,15	31,87	100 x	33,57	34,23	PCR inhibition
2	146	Present	20 x	29,51	29,97	100 x	31,91	32,71	
3	147	Present	20 x	29,93	28,94	100 x	31,86	31,06	
4	148	Absent	20 x	39,00	39,24	100 x	39,85	39,00	
5	149	Absent	20 x	39,48	37,73	100 x	38,33	39,05	
6	150	Absent	20 x	35,36	36,36	100 x	36,76	36,00	

Laboratory identification code :	26
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,82 cycles
Copy number at the cut-off :	11,00 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	151	Present	1 x	28,70	26,11	10 x	31,55	27,85	
2	152	Present	1 x	30,61	34,38	10 x	33,96	37,74	
3	153	Absent	1 x	38,52	39,28	10 x	40,86	50,00	
4	154	Present	1 x	36,55	36,78	10 x	38,33	38,63	
5	155	Present	1 x	36,44	36,37	10 x	39,47	39,92	
6	156	Present	1 x	29,29	29,14	10 x	30,61	32,18	

Laboratory identification code :	27
Responsibility agreement :	Yes
Cut-off at 15 copies :	34,94 cycles
Copy number at the cut-off :	10,02 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	57	Present	1 x	30,12	29,55	10 x	33,03	30,96	
2	58	Absent	1 x	39,14	40,21	10 x			Flat signal in 10 fold dilution
3	59	Absent	1 x	36,62	37,03	10 x	36,41	37,96	
4	60	Present	1 x	31,81	26,73	10 x	33,07	27,87	
5	61	Absent	1 x	35,43	36,69	10 x	37,72	38,48	
6	62	Present	1 x	28,03	27,00	10 x	29,89	29,03	

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Laboratory identification code :	28
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	163	Present	3 x	32,57	32,37	30 x	33,78	33,82	
2	164	Absent	3 x	41,40	41,05	30 x	39,66	39,00	
3	165	Present	3 x	31,38	30,49	30 x	32,08	31,09	
4	166	Absent	3 x	40,24	40,97	30 x	39,23	39,27	
5	167	Present	3 x	31,08	31,36	30 x	33,02	33,71	
6	168	Absent	3 x	41,00	42,63	30 x	40,60	39,66	

Laboratory identification code :	29
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,24 cycles
Copy number at the cut-off :	9,30 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	169	Present		30,30	31,95	10 x	32,11	33,37	no inhibition
2	170	Present		33,37	33,35	10 x	35,22	34,95	no inhibition
3	171	Absent		40,65	40,64	10 x	38,55	39,72	no inhibition
4	172	Absent		39,37	37,09	10 x	38,91	37,90	no inhibition
5	173	Present		31,47	33,26	10 x	34,19	36,63	no inhibition
6	174	Absent		39,93	38,43	10 x	39,31	38,75	no inhibition

Laboratory identification code :	30
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,00 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	175	Present	160 x	32,32	32,65	640 x	34,04	34,61	
2	176	Absent	160 x		37,18	640 x	38,58	38,82	undetermined
3	177	Present	160 x	32,95	33,60	640 x	34,24	34,98	
4	178	Present	160 x	31,11	29,51	640 x	32,62	31,21	
5	179	Absent	160 x		38,99	640 x	39,80		undetermined
6	180	Absent	160 x		39,00	640 x			undetermined

Laboratory identification code :	32
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,15 cycles
Copy number at the cut-off :	10,61 copies

	Sample N°	Ruminant DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	187	Present	2 x	31,00	30,90				Internal method of DNA extraction
2	188	Present	2 x	33,40	33,50				Internal method of DNA extraction
3	189	Absent	2 x	40,00	40,00				Internal method of DNA extraction
4	190	Absent	2 x	40,00	40,00	3 x	40,00	40,00	- Internal method of DNA extraction - Presence of inhibition with dilution 1/2
5	191	Absent	2 x	40,00	39,50				Internal method of DNA extraction
6	192	Present	2 x	35,00	34,60				Internal method of DNA extraction