



EURL-AP Pig PCR Implementation Test 2015

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Summary

The European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP) organised the present implementation test for assessing the ability of the NRL network with respect to the detection of pig proteins in feed using the PCR test developed and validated by EURL-AP. Total number of participants was 28 NRLs from 27 Member States + Norway. The study was based on a set of 9 blind samples. The sample set consisted of 3 feed samples (blanks or feed matrices fortified with pig processed animal proteins) in triplicate.

Four NRLs did not provide results in due time (at the deadline of 05 February 2016) and one lab reported results that were kept out for question of reliability. Results from the 23 remaining NRLs indicated an excellent performance. These results confirmed what was obtained during the validation study. The rate of false positive results as well as the rate of false negative results for the samples containing 0.1 % w/w of pig PAP are both below 5 % (2.90 % for both type of deviation). With the samples spiked with 0.05 % in mass fraction of pig PAP, the rate of false negative results reaches 8.70 % but 3 of these 6 false negative results come from the same lab which is considered as underperformant.

Keywords :

Processed animal proteins - PAP - Pig - PCR - Polymerase Chain Reaction - Implementation 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://crl.cra.wallonie.be>). 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 implementation of a newly validated PCR method for the detection of pig proteins in feed.

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 [3]. The objective of the present implementation test is to evaluate performances of the network of 28 NRLs (from 27 Member States + Norway) to detect the presence of pig processed animal proteins in feed using the pig PCR method internally evaluated by the EURL-AP and validated through an interlaboratory study [4,5].

3. Material and methods

3.1. Study organisation

Official announcement of the study was made on the 30th of October 2015 through a letter sent to all participants.

Participants were the 27 NRLs of the EURL-AP network + NRL from Norway. A detailed list of the 28 participating labs is included in Annex 1.

Previously (August 2015), the implementation of the method in the NRL network was initiated with the diffusion of the pig PCR protocol and the providing of calibrator sets. The dedicated Excel file allowing the determination of the cut-off value of a PCR platform was also diffused at that time. The 11th of December 2015, the sets of 9 blind samples for the implementation test were provided to the participants by express shipment.

Some general recommendations were delivered to the participants:

- Results had to be encoded by way of an Excel report form (Annex 2). Participants were asked to carefully read the instructions on how to fill in the result form and to testify they did it prior to encoding their results. No other support for communicating the results was accepted.
- A summarized results sheet was automatically generated. Participants were asked to sign the summarized results sheet and to return it by e-mail to the EURL-AP. Only when both the Excel file and the e-mail were received by EURL-AP were results taken into consideration.
- The results had to be sent in both forms concomitantly to the EURL-AP by the 5th of February 2016. A tolerance of a few days after the closing of the results was nevertheless accepted as this inter-laboratory study was not a proficiency test. The reporting was closed once for all with the sending of the feedback tabulation result forms to the participants on the 19th of February 2016.

3.2. Material

3.2.1. Description of the samples

Three materials containing typical feed ingredients with or without processed animal proteins (PAPs) from pig origin at different concentration levels ~ 0.1 % in weight have been prepared as shown in Table 1.

Each participating lab received about 10 g of blind triplicates from the three materials (9 feed samples). A unique random number was assigned to each sample (Annex 3). Details of the samples are indicated in Table 1.

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

Sample	Material	Number of vials and remarks
1	Blank feed 1	1
2	Blank feed 1	1 (replicate of sample #1)
3	Blank feed 1	1 (replicate of sample #1)
4	0.05 % w/w pig PAP in blank feed 1	1
5	0.05 % w/w pig PAP in blank feed 1	1 (replicate of sample #4)
6	0.05 % w/w pig PAP in blank feed 1	1 (replicate of sample #4)
7	0.1 % w/w pig PAP in blank feed 1	1
8	0.1 % w/w pig PAP in blank feed 1	1 (replicate of sample #7)
9	0.1 % w/w pig PAP in blank feed 1	1 (replicate of sample #7)
Total		9

3.2.2. Materials used in the preparation of the samples

The feed matrix used for the preparation of the sample set was a compound feed for pigs bought from an organic feed producer. It consisted of triticale, barley, oat, horse bean, pea, sunflower cake, potato proteins, soybean oil and feed complements (vitamins, salts, minerals). This compound feed was pre-treated by grinding at 2 mm.

The PAP used to spike the blank material was made of porcine material processed according to method 1 (133 °C, 3 bars, 20 min).

3.2.3. Preparation of the samples

The feed matrix used for the blank feed was ground at 2 mm.

To prepare the 2 materials containing porcine PAP, an intermediate mix at 1 % in mass fraction of porcine PAP was prepared. This mix was ground in a ZM 200 meal (Restch) with a sieve at 2 mm. The final materials at 0.1 % and 0.05 % in mass fraction respectively were obtained by dilution in the feed matrix. The two mixes obtained were still ground at 0.5 mm to improve the homogeneity.

3.3. Qualitative analysis

Qualitative analysis concerned the presence or absence of pig 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:

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

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:

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

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

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

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

4. Results

Gross results from all participants are to be found in Annex 4.

4.1. Homogeneity study

Ten replicates of each sample (ten vials) were chosen randomly and were analysed using the validated pig PCR target. Per replicate, 2 DNA extracts were realised according the Promega protocol (<https://www.eurl.craw.eu/legal-sources-and-sops/method-of-reference-and-sops/>). In final, 20 Promega extracts were obtained per sample type to be analyzed.

All Promega extracts were analysed by PCR with the validated pig PCR protocol. All the PCR results were consistent with what was expected.

Table 2: PCR results obtained with feed samples replicates

Sample	Material	Nr of vials	Pig target	AC
1	Blank feed 1	10	-	1.00 (n= 20)
2	0.05 % w/w pig PAP in blank feed 1	10	+	1.00 (n= 20)
3	0.1 % w/w pig PAP in blank feed 1	10	+	1.00 (n= 20)
	Overall results	30		1.00 (n=60)

n = number of results considered

Accuracy (AC) means specificity in case of blank samples and sensitivity in case of spiked samples

4.2. Qualitative analyses from the NRLs

4.2.1. Overview of results and performance of the method

Table 3 summarizes the results submitted by the 24 NRLs for the three sample types submitted to qualitative analysis. The results of one lab were kept out for reliability reasons.

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

Sample	Material	Nr of results	AC
1	Blank feed	69*	0.971 (2)
2	0.05 % w/w pig PAP in blank feed	69*	0.913 (6)
3	0.1 % w/w pig PAP in blank feed	69*	0.971 (2)
	Overall results	207*	0.952 (10)

Accuracy means specificity in case of blank samples and sensitivity in case of spiked samples.

In brackets the number of deviations with what is expected.

*Results of lab 17 were not kept. The reasons are explained in point 4.2.2.

The overall results, expressed in terms of global accuracy (AC), reveal a very good global performance of the test within the network. The numbers of false positive and false negative results at the target level of 0.1 % w/w of pig PAP are both reaching 2.9 %.

4.2.2. Detailed review of results for each sample material

Incorrect results are detailed in this section.

- **Lab 8:** two of the blank triplicates were positive and one of the triplicates at the level of 0.05 % is negative.
- **Lab 13:** the triplicates at the level of 0.05 % and one of the triplicates at the level of 0.1 % were negative. The copy number at the cut-off did not satisfy the minimum quality criterion of 3 copies and the lack of sensitivity is probably due to a too late cut-off.
- **Lab 14:** one of the triplicates at the level of 0.05 % was negative.
- **Lab 17:** only one DNA instead of two was extracted from the samples. Based on the conclusions of the lab, two false negative results (one for both level of pig PAP) are recorded. Nevertheless, looking at the cut-off value and the Ct values reported, all the samples would give positive results and the blank triplicates would give three false positive results.
- **Lab 18:** one of the triplicates at the level of 0.05 % was negative.

Results of Lab 17 cannot be kept as there is a divergence between what the lab declares as result and what it should be with the obtained cut-off. Problems of Lab 13 are partly explained by their determination of the cut-off which does not meet the required quality criterion. Discrepancies for the sample at the level of 0.05% of pig PAP are probably attributable to the fact that this content of pig PAP is very close to the limit of detection.

4.2.3. 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.

As this is an implementation test, the underperformance might be linked to the test itself. However, a majority of the participating NRLs (19 labs out of 24 or 79.2 %) obtained an excellent global performance and had no false result. Two labs (Lab 14 and 18) out of the 24 participating NRLs (8.3 %) had only one false negative result at the level of 0.05 % of pig PAP w/w. This might reflect that this amount of PAP is close to the limit of detection. For the three remaining labs (Lab 8, 13 and 17 - 12.5 %), the observed underperformance is not attributable to the test only, it is also linked to an incorrect implementation of the test in these labs.

**Table 4: NRL proficiencies regarding the detection of pig material starting from feed samples.
Ranking follows AC values.**

Lab code	AC	SE	SP
1	1.000	1.000	1.000
2	1.000	1.000	1.000
3	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
12	1.000	1.000	1.000
15	1.000	1.000	1.000
19	1.000	1.000	1.000
20	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
31	1.000	1.000	1.000
14	0.889	0.833	1.000
18	0.889	0.833	1.000
8	0.667	0.833	0.333
13	0.556	0.333	1.000
17*	-	-	-

*Performance criteria of lab 17 are not determined. The qualitative conclusions (presence or absence of pig DNA) are not in accordance with the decision rules of the method (comparison samples Ct values with the cut-off value in cycles).

5. Conclusions

This study is the first assessment of the validated pig PCR method implementation level in the NRL network. Out of the 28 participants, only 24 finally participated by submitting results. Looking globally at these results sent to the EURL-AP, 79.2 % of the participating NRLs (19 labs out of 24) obtained an excellent global performance and had no false result. 8.3 % of the participating NRLs (2 labs out of 24) had only one false negative result. Two other labs (8.3 %) had 2 or more false results. The results of the last remaining lab were kept out of this study as they were not reliable.

Different conclusions can be made:

1. The pig PCR method is fit for the detection of pig PAP in feed at the level of 0.1 % in weight.
2. The method including the DNA extraction step can be considered as validated.
3. The pig PCR method appears to be well implemented in a majority of the NRLs (21 labs).
4. Three participants will have to improve their performance (Lab 8, 13, 17).
5. Four NRLs (14.3 % of the NRLs all from the EU network) were not able to send results. They have to start/continue the implementation of the method in view of a possible lifting of the pig PAP ban.

Acknowledgments

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

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Annex 1List of 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
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
Norway	National Institute of Nutrition and Seafood Research
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



Annex 2

Excel result report form

Pig PCR Implementation Test 2015

Laboratory identification

Laboratory code :

Responsibility agreement :

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

Report

Lab code	0	0	0	0	0	0	0	0	0
Sample rank	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Sample N°									

Qualitative analysis

Pig DNA

Additional data

Cut-off at 5 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...)

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Annex 3

Composition of sample sets

Sample		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	31		
DQ/15/0758-008 (Feed II)	-	3640	3624	3688	3680	3600	3608	3616	3024	3080	3048	3096	3008	3032	3064	3000	3456	3512	3448	3400	3424	3416	3464	3016	3232	3200	3216	3240	3336		
DQ/15/0758-008 (Feed II)	-	3744	3752	3712	3696	3672	3656	3632	3104	3112	3072	3152	3040	3176	3088	3056	3592	3544	3536	3408	3488	3576	3520	3440	3248	3280	3272	3288	3376		
DQ/15/0758-008 (Feed II)	-	3784	3768	3720	3776	3760	3728	3736	3792	3136	3160	3168	3192	3184	3144	3128	3648	3552	3568	3472	3496	3584	3528	3480	3824	3352	3360	3328	3392		
DQ/15/0758-020 (Feed II + 0.05 % Pig PAP)	+	3234	3122	3106	3322	3098	3018	3586	3002	3338	3090	3130	3258	3410	3210	3218	3314	3178	3570	3050	3010	3042	3074	3394	3082	3250	3026	3546	3442		
DQ/15/0758-020 (Feed II + 0.05 % Pig PAP)	+	3346	3146	3658	3610	3306	3426	3642	3490	3354	3418	3290	3562	3858	3738	3298	3930	3714	3730	3170	3242	3274	3266	3522	3402	3706	3498	3754	3578		
DQ/15/0758-020 (Feed II + 0.05 % Pig PAP)	+	3514	3226	3794	3690	3826	3434	3994	4010	3890	3906	3802	3978	3874	3842	3866	3938	3882	4002	3482	3594	3282	3506	3666	3602	3810	3850	3914	3762		
DQ/15/0758-010 (Feed II + 0.1 % Pig PAP)	+	3004	3276	3172	3012	3052	3220	3092	3308	3332	3044	3084	3244	3228	3068	3324	3020	3060	3260	3148	3428	3212	3484	3100	3076	3116	3700	3132	3500		
DQ/15/0758-010 (Feed II + 0.1 % Pig PAP)	+	3236	3676	3300	3364	3268	3652	3716	3756	3476	3348	3852	3420	3356	3108	3660	3372	3748	3868	3180	3724	3468	3932	3156	3140	3388	3708	3444	3612		
DQ/15/0758-010 (Feed II + 0.1 % Pig PAP)	+	3980	3812	3956	3404	3876	3916	3764	3844	4004	3596	3972	3828	3540	3644	3684	3796	3884	3948	3252	3908	3572	4036	3836	3940	3900	4012	3740	3860		

Annex 4

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

Laboratory identification code :	1
Responsibility agreement :	Yes
Cut-off at 5 copies :	36.98 cycles
Copy number at the cut-off :	3.30 copies



Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3004	Present	1 x	32.92	32.26	10 x	37.17	36.38	
2	3234	Present	1 x	32.99	33.33	10 x	36.27	37.58	
3	3236	Present	1 x	34.58	32.16	10 x	38.55	35.77	
4	3346	Present	1 x	34.38	32.73	10 x	37.84	36.41	
5	3514	Present	1 x	34.48	33.49	10 x	36.52	38.40	
6	3640	Absent	1 x	50.00	37.87	10 x	50.00	50.00	Ct value 50 equals N/A
7	3744	Absent	1 x	50.00	50.00	10 x	50.00	50.00	
8	3784	Absent	1 x	50.00	40.48	10 x	50.00	38.92	
9	3980	Present	1 x	35.63	34.84	10 x	38.15	39.53	

Laboratory identification code :	2
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.32 cycles
Copy number at the cut-off :	3.23 copies



Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3122	Present	1 x	37.10	36.56	10 x	38.73	39.42	
2	3146	Present	1 x	36.92	36.35	10 x	40.79	39.99	
3	3226	Present	1 x	36.16	37.00	10 x	40.05	38.85	
4	3276	Present	1 x	35.39	35.46	10 x	38.84	38.66	
5	3624	Absent	1 x			10 x			
6	3676	Present	1 x	37.20	36.83	10 x	40.26	40.01	
7	3752	Absent	1 x			10 x			
8	3768	Absent	1 x			10 x			
9	3812	Present	1 x	36.03	36.41	10 x	38.45	38.40	

Laboratory identification code :	3
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.12 cycles
Copy number at the cut-off :	3.64 copies



Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3106	Present	1 x	38.32	37.66	10 x	41.66	43.08	
2	3172	Present	1 x	36.12	38.09	10 x	40.27	50.00	
3	3300	Present	1 x	37.32	36.79	10 x	47.15	43.51	
4	3658	Present	1 x	37.12	36.72	10 x	40.48	38.72	
5	3688	Absent	1 x	50.00	50.00	10 x	50.00	50.00	
6	3712	Absent	1 x	45.92	50.00	10 x	50.00	46.91	
7	3720	Absent	1 x	50.00	50.00	10 x	50.00	42.13	
8	3794	Present	1 x	37.87	37.34	10 x	43.51	40.66	
9	3956	Present	1 x	35.42	36.40	10 x	38.96	39.49	



Laboratory identification code :	4
Responsibility agreement :	Yes
Cut-off at 5 copies :	38.86 cycles
Copy number at the cut-off :	4.56 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3012	Present	1 x	32.21	32.27	10 x	35.07	34.93	
2	3322	Present	1 x	31.48	31.13	10 x	35.48	35.85	
3	3364	Present	1 x	31.39	31.39	10 x	35.39	35.53	
4	3404	Present	1 x	30.33	30.28	10 x	34.45	34.51	
5	3610	Present	1 x	30.59	30.51	10 x	35.24	35.40	
6	3680	Absent	1 x	50.00	42.37	10 x	50.00	50.00	*CT value = 50* means Not Detected
7	3690	Present	1 x	33.40	33.08	10 x	37.59	37.49	
8	3696	Absent	1 x	50.00	41.99	10 x	50.00	50.00	*CT value = 50* means Not Detected
9	3776	Absent	1 x	50.00	50.00	10 x	50.00	50.00	*CT value = 50* means Not Detected

Laboratory identification code :	5
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.86 cycles
Copy number at the cut-off :	3.01 copies



Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3052	Present	1 x	33.70	33.60	10 x	38.03	37.33	
2	3098	Present	1 x	33.22	35.35	10 x	37.27	38.13	
3	3268	Present	1 x	33.89	33.72	10 x	38.18	38.73	
4	3306	Present	1 x	35.13	34.94	10 x	39.03	39.21	
5	3600	Absent	1 x			10 x			
6	3672	Absent	1 x			10 x			
7	3760	Absent	1 x			10 x			
8	3826	Present	1 x	36.43	36.29	10 x	40.11		The sample number of the ID and the one of the body of the tube were different (3898, resp. 3826). After clarifying with the EURL-AP, the correct number was 3826.
9	3876	Present	1 x	33.57	33.86	10 x	38.31	37.75	

Laboratory identification code :	6
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.95 cycles
Copy number at the cut-off :	3.15 copies



Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3018	Present	1 x	39.10	38.63	10 x	40.12	40.86	
2	3220	Present	1 x	37.32	37.13	10 x	39.86	39.75	
3	3426	Present	1 x	36.77	37.52	10 x	38.95	39.56	
4	3434	Present	1 x	36.05	36.45	10 x	39.01	39.18	
5	3608	Absent	1 x	39.98	39.96	10 x	40.96	41.58	
6	3652	Present	1 x	35.72	36.61	10 x	37.56	38.42	
7	3656	Absent	1 x	40.52	39.99	10 x	42.83	41.27	
8	3728	Absent	1 x	39.96	40.02	10 x	41.56	42.00	
9	3916	Present	1 x	35.04	36.72	10 x	37.76	38.01	

Laboratory identification code :	7
Responsibility agreement :	Yes
Cut-off at 5 copies :	37.75 cycles
Copy number at the cut-off :	3.54 copies



Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3092	Present	1 x	33.97	33.50	10 x	37.57	37.20	
2	3586	Present	1 x	34.96	34.82	10 x	39.11	38.91	
3	3616	Absent	1 x	48.00	48.00	10 x	49.00	49.00	
4	3632	Absent	1 x	48.00	48.00	10 x	49.00	49.00	
5	3642	Present	1 x	34.56	34.51	10 x	42.18	41.52	
6	3716	Present	1 x	33.88	33.91	10 x	41.06	40.80	
7	3736	Absent	1 x	48.00	48.00	10 x	49.00	49.00	
8	3764	Present	1 x	34.16	33.94	10 x	39.63	39.26	
9	3994	Present	1 x	34.21	34.34	10 x	43.66	42.89	



Laboratory identification code :	8
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.78 cycles
Copy number at the cut-off :	3.11 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3002	Absent	1 x	40.80	41.00	10 x	41.00	43.90	
2	3024	Present	1 x	36.40	35.90	10 x	38.80	39.30	
3	3104	Present	1 x	37.90	38.70	10 x	42.50	44.80	
4	3308	Present	1 x	34.20	33.50	10 x	38.20	37.30	
5	3490	Present	1 x	35.60	35.90	10 x	39.00	39.00	
6	3756	Present	1 x	35.70	35.70	10 x	37.70	37.30	
7	3792	Absent	1 x	40.50	43.80	10 x	43.50	46.80	
8	3844	Present	1 x	34.50	33.50	10 x	39.40	37.80	
9	4010	Present	1 x	35.80	35.60	10 x	37.20	37.50	



Laboratory identification code :	9
Responsibility agreement :	Yes
Cut-off at 5 copies :	38.83 cycles
Copy number at the cut-off :	3.29 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3080	Absent	1 x	N/A	N/A	10 x	44.67	N/A	
2	3112	Absent	1 x	N/A	N/A	10 x	N/A	N/A	
3	3136	Absent	1 x	N/A	N/A	10 x	N/A	40.03	
4	3332	Present	1 x	33.79	33.91	10 x	35.85	37.00	
5	3338	Present	1 x	36.24	34.35	10 x	37.71	37.12	
6	3354	Present	1 x	35.25	34.97	10 x	37.83	38.29	
7	3476	Present	1 x	33.57	34.02	10 x	37.45	N/A	
8	3890	Present	1 x	34.58	32.98	10 x	37.62	38.27	
9	4004	Present	1 x	33.46	33.37	10 x	36.36	36.90	



Laboratory identification code :	10
Responsibility agreement :	Yes
Cut-off at 5 copies :	43.75 cycles
Copy number at the cut-off :	3.61 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3044	Present	1 x	39.81	39.19	10 x	47.16	42.93	
2	3048	Absent	1 x			10 x			
3	3072	Absent	1 x			10 x			
4	3090	Present	1 x	42.51	41.60	10 x			
5	3160	Absent	1 x			10 x			
6	3348	Present	1 x	40.44	41.57	10 x			
7	3418	Present	1 x	41.02	42.87	10 x			
8	3596	Present	1 x	38.83	39.60	40 x	42.14	44.77	
9	3906	Present	1 x	42.50	38.90	10 x		42.50	



Laboratory identification code :	11
Responsibility agreement :	Yes
Cut-off at 5 copies :	38.70 cycles
Copy number at the cut-off :	3.79 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3084	Present	1 x	35.07	33.24	10 x	37.80	34.30	
2	3096	Absent	1 x	No Ct	No Ct	10 x	No Ct	No Ct	
3	3130	Present	1 x	35.02	35.22	10 x	37.62	39.58	
4	3152	Absent	1 x	48.88	No Ct	10 x	No Ct	No Ct	
5	3168	Absent	1 x	No Ct	No Ct	10 x	No Ct	40.29	
6	3290	Present	1 x	34.53	35.10	10 x	36.79	38.92	
7	3802	Present	1 x	35.02	34.92	10 x	39.10	38.43	
8	3852	Present	1 x	36.04	34.03	10 x	No Ct	37.72	
9	3972	Present	1 x	34.99	34.71	10 x	37.65	37.11	



Laboratory identification code :	12
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.45 cycles
Copy number at the cut-off :	3.33 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3008	Absent	1 x	41.95	40.96	10 x	40.98	39.80	
2	3040	Absent	1 x	39.47	41.17	10 x	40.22	41.38	
3	3192	Absent	1 x	39.97	39.16	10 x	40.30	40.34	
4	3244	Present	1 x	35.98	35.74	10 x	38.80	37.71	
5	3258	Present	1 x	37.41	36.38	10 x	39.35	39.39	
6	3420	Present	1 x	35.19	34.98	10 x	36.99	36.94	
7	3562	Present	1 x	35.76	37.71	10 x	37.09	39.16	
8	3828	Present	1 x	35.24	37.28	10 x	37.58	39.23	
9	3978	Present	1 x	36.62	36.72	10 x	39.40	38.26	



Laboratory identification code :	13
Responsibility agreement :	Yes
Cut-off at 5 copies :	41.21 cycles
Copy number at the cut-off :	2.53 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3032	Absent	1 x		10 x				
2	3176	Absent	1 x	42.40	49.87	10 x			
3	3184	Absent	1 x		10 x				
4	3228	Absent	1 x	45.29	43.34	10 x	40.39	43.00	signal in 10x dilution before 1x
5	3356	Present	1 x	37.08	40.92	10 x	39.55	39.32	
6	3410	Absent	1 x	44.68		10 x	41.57	46.39	
7	3540	Present	1 x	35.97	43.29	10 x	40.98	40.76	signal in 10x dilution before 1x
8	3858	Absent	1 x	46.36		10 x		42.28	
9	3874	Absent	1 x	48.29	45.97	10 x	47.05	43.91	signal in 10x dilution before 1x



Laboratory identification code :	14
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.72 cycles
Copy number at the cut-off :	3.76 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3108	Present	1 x	35.68	35.41	10 x	40.84	40.58	
2	3842	Present	1 x	36.60	35.43	10 x	40.28	40.40	
3	3068	Present	1 x	35.09	35.41	10 x	39.69	40.16	
4	3738	Absent	1 x	41.35	42.21	5 x	43.67	50.00	
5	3144	Absent	1 x	50.00	50.00	5 x	50.00	50.00	no amplification in all tested dilutions, but inhibition controls were positive
6	3644	Present	1 x	35.60	34.24	10 x	39.40	37.94	
7	3064	Absent	1 x	50.00	50.00	5 x	50.00	50.00	no amplification in all tested dilutions, but inhibition controls were positive
8	3210	Present	1 x	38.01	38.57	10 x	43.50	42.52	
9	3088	Absent	1 x	50.00	41.38	5 x	50.00	50.00	



Laboratory identification code :	15
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.99 cycles
Copy number at the cut-off :	3.68 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3000	Absent	1 x	50.00	50.00	10 x	50.00	50.00	No PCR inhibition
2	3056	Absent	1 x	50.00	50.00	10 x	50.00	50.00	No PCR inhibition
3	3128	Absent	1 x	50.00	50.00	10 x	50.00	50.00	No PCR inhibition
4	3218	Present	1 x	37.98	38.51	10 x	50.00	50.00	No PCR inhibition
5	3298	Present	1 x	38.91	38.06	10 x	50.00	50.00	No PCR inhibition
6	3324	Present	1 x	36.79	37.83	10 x	50.00	50.00	No PCR inhibition
7	3660	Present	1 x	36.81	37.21	10 x	50.00	50.00	No PCR inhibition
8	3684	Present	1 x	37.83	38.47	10 x	50.00	50.00	No PCR inhibition
9	3866	Present	1 x	38.19	37.57	10 x	50.00	50.00	No PCR inhibition



Laboratory identification code :	17
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.45 cycles
Copy number at the cut-off :	3.02 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	3060	Absent	1 x	37.03	20 x	38.50		nont:36,9 negk:38,07 pozk:30,71
2	3178	Absent	1 x	37.70	20 x	37.80		
3	3512	Absent	1 x	36.90	20 x	37.89		
4	3544	Absent	1 x	37.56	20 x	37.85		
5	3552	Absent	1 x	37.91	20 x	38.13		
6	3714	Present	1 x	35.32	20 x	37.11		
7	3748	Present	1 x	36.00	20 x	37.44		
8	3882	Present	1 x	36.34	20 x	37.31		
9	3884	Present	1 x	36.14	20 x	36.80		



Laboratory identification code :	18
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.96 cycles
Copy number at the cut-off :	3.33 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	3260	Present	1 x	38.55	10 x	42.01		also 3X and 30X analysed
2	3448	Absent	1 x		10 x			also 3X and 30X analysed
3	3536	Absent	1 x		10 x			also 3X and 30X analysed
4	3568	Absent	1 x		10 x			also 3X and 30X analysed
5	3570	Absent	1 x	42.44	10 x	41.50		also 3X and 30X analysed
6	3730	Present	1 x	39.02	10 x	39.24		also 3X analysed
7	3868	Present	1 x	39.23	10 x	39.62		also 3X and 30X analysed
8	3948	Present	1 x	37.99	10 x	37.40	40.57	41.73 also 3X analysed
9	4002	Present	1 x	39.23	10 x	39.16		also 3X and 30X analysed



Laboratory identification code :	19
Responsibility agreement :	Yes
Cut-off at 5 copies :	38.01 cycles
Copy number at the cut-off :	3.70 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	3050	Present	1 x	33.85	10 x	33.67	37.42	36.45
2	3148	Present	1 x	33.02	10 x	33.03	35.93	36.00
3	3170	Present	1 x	35.51	10 x	34.07	38.32	36.59
4	3180	Present	1 x	34.16	10 x	33.08	35.93	36.05
5	3252	Present	1 x	33.86	10 x	33.16	37.41	36.47
6	3400	Absent	1 x		10 x			
7	3408	Absent	1 x		10 x			
8	3472	Absent	1 x		10 x			
9	3482	Present	1 x	33.80	10 x	33.50	39.91	36.43



Laboratory identification code :	20
Responsibility agreement :	Yes
Cut-off at 5 copies :	40.95 cycles
Copy number at the cut-off :	3.27 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3010	Present	1 x	37.83	10 x	39.23	41.59	45.05	15.2372
2	3242	Present	1 x	37.62	10 x	37.21			15.2373
3	3424	Absent	1 x		10 x				15.2374
4	3428	Present	1 x	35.09	10 x	37.14	39.98	42.53	15.2375
5	3488	Absent	1 x		10 x				15.2376
6	3496	Absent	1 x		10 x				15.2377
7	3594	Present	1 x	37.74	10 x	39.01	40.54	42.13	15.2378
8	3724	Present	1 x	37.38	10 x	36.85	48.79	41.26	15.2379
9	3908	Present	1 x	38.07	10 x	36.90	40.49	42.46	15.2380



Laboratory identification code :	22
Responsibility agreement :	Yes
Cut-off at 5 copies :	39.67 cycles
Copy number at the cut-off :	3.07 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	3074	Present	34 x	33.91	34.32	36 x	36.25	36.58
2	3266	Present	34 x	34.40	34.09	38 x	37.35	38.27
3	3464	Absent						ND
4	3484	Present	33 x	33.09	33.32	36 x	35.62	35.67
5	3506	Present	35 x	35.30	35.20	37 x	37.10	37.08
6	3520	Absent						ND
7	3528	Absent						ND
8	3932	Present	33 x	32.83	32.22	35 x	34.87	34.81
9	4036	Present	34 x	33.65	33.86	36 x	36.31	36.05



Laboratory identification code :	24
Responsibility agreement :	Yes
Cut-off at 5 copies :	40.82 cycles
Copy number at the cut-off :	3.65 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	3076	Present	1 x	35.44	35.99	10 x	36.94	37.82
2	3082	Present	1 x	37.44	36.30	10 x	40.20	38.79
3	3140	Present	1 x	35.21	35.53	37 x	37.01	37.27
4	3232	Absent	1 x			10 x		
5	3248	Absent	1 x			10 x		
6	3402	Present	1 x	37.50	34.53	10 x	38.99	36.56
7	3602	Present	1 x	36.31	37.34	10 x	39.18	39.85
8	3824	Absent	1 x			10 x		
9	3940	Present	1 x	35.27	34.96	10 x	38.13	37.70



Laboratory identification code :	25
Responsibility agreement :	Yes
Cut-off at 5 copies :	37.87 cycles
Copy number at the cut-off :	3.76 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	3116	Present	1 x	33.24	33.38	10 x	36.70	37.14
2	3200	Absent	1 x	50.00	50.00	10 x	50.00	50.00
3	3250	Present	1 x	35.51	35.12	10 x	38.62	38.92
4	3280	Absent	1 x	50.00	50.00	10 x	50.00	50.00
5	3352	Absent	1 x	50.00	50.00	10 x	50.00	50.00
6	3388	Present	1 x	33.50	33.53	10 x	36.82	37.08
7	3706	Present	1 x	36.04	36.00	10 x	38.32	38.60
8	3810	Present	1 x	35.00	34.76	10 x	38.64	38.86
9	3900	Present	1 x	33.79	33.76	10 x	36.81	37.00



Laboratory identification code :	27
Responsibility agreement :	Yes
Cut-off at 5 copies :	38.62 cycles
Copy number at the cut-off :	3.19 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment	
1	3132	Present	1 x	33.43	33.94	10 x	41.81	45.44	Delta Ct between 1-fold and 10-fold dilution unusual high (> 8) and not as expected
2	3240	Absent	1 x			10 x		all undetermined	
3	3288	Absent	1 x			10 x		all undetermined	
4	3328	Absent	1 x			10 x		all undetermined	
5	3444	Present	1 x	33.57	33.59	10 x	38.23	36.43	
6	3546	Present	1 x	35.16	36.38	10 x	41.19		Repl. 2/10fold undetermined
7	3740	Present	1 x	32.93	32.29	10 x	39.77	36.18	
8	3754	Present	1 x	30.87	34.65	10 x	35.20	39.78	
9	3914	Present	1 x	36.38	36.07	10 x	39.72	44.59	Delta Ct for Repl. 2 between 1-fold and 10-fold dilution unusual high (> 8)



Laboratory identification code :	31
Responsibility agreement :	Yes
Cut-off at 5 copies :	41.43 cycles
Copy number at the cut-off :	3.27 copies

Sample N°	Pig DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	3336	Absent	3 x	42.71	44.41	30 x	46.66	
2	3376	Absent	3 x			30 x		
3	3392	Absent	3 x	43.67	42.48	30 x		
4	3442	Present	3 x	38.99	38.14	30 x	44.51 42.94	
5	3500	Present	3 x	36.62	36.08	30 x	41.53 38.37	
6	3578	Present	3 x	38.53	37.35	30 x	44.15 42.22	
7	3612	Present	3 x	36.51	36.04	30 x	40.00 38.98	
8	3762	Present	3 x	38.11	37.70	30 x	41.73 42.00	
9	3860	Present	3 x	36.99	36.16	30 x	40.32 40.44	