EURL-AP Poultry PCR Implementation Test 2018

Olivier FUMIÈRE, Aline MARIEN, JULIEN MALJEAN, Gilbert BERBEN

Department Knowledge and Valorisation of Agricultural Products

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European Union Reference Laboratory for Animal Proteins in feedingstuffs
Walloon Agricultural Research Centre
Department Knowledge and Valorisation of Agricultural Products, Henseval Building, Chaussée de Namur 24,
B-5030 GEMBLOUX (BELGIUM)
Phone: +32 (0) 81 87 52 28 - Fax : +32 (0) 81 87 40 19
secretary@eurl.craw.eu – website: https://www.eurl.craw.eu

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Centre wallon de Recherches agronomiques
Service Communication
Rue de Liroux, 9
5030 Gembloux (Belgique)

Table of contents

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 poultry proteins in feed using the PCR test developed and validated by EURL-AP. Total number of participants was 28 NRLs from 26 Member States + Norway and Serbia. The study was based on a set of 8 blind samples. The sample set consisted of 8 feed samples (blanks or feed matrices fortified with poultry processed animal proteins).

One NRL did not provide results in due time (at the deadline of 23 March 2018). Results from the 27 remaining labs 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 poultry PAP are both below 5 % (3.70 % of the false positive results and 1.23 % of negative deviations respectively). With the samples spiked with 0.05 % in mass fraction of poultry PAP, the rate of false negative results reaches 1.85 %.

Keywords :

Processed animal proteins - PAP - Poultry - Chicken - Turkey - 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 validated PCR method for the detection of poultry 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 26 Member States + Norway and Serbia) to detect the presence of poultry processed animal proteins in feed using the poultry PCR method internally evaluated by the EURL-AP and validated through an interlaboratory study [4,5].

3. Material and methods

3.1. Study organisation

In order to have time to order all the necessary reagents, the approached labs received the sequences of the primers and the probe beginning of November 2017. The official announcement of the study was made on the 30th of January 2018 through a letter sent to all participants.

Participants were the 26 NRLs of the EURL-AP network + NRL from Norway and Serbia. A detailed list of the 28 participating labs is included in Annex 1.

The 20th of February 2018, the participants received the protocol by e-mail. One week later (27 February 2018), they received their sample set as well as an additional sample to use as a positive control and the calibrants needed to set the cut-off of their PCR platform. The dedicated Excel file allowing the determination of the cut-off value of a PCR platform was also diffused at that time.

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 23rd of March 2018. A tolerance of few days after this deadline was nevertheless accepted as this inter-laboratory study was not a proficiency test.

3.2. Material

3.2.1. Description of the samples

Three materials containing typical feed ingredients with or without processed animal proteins (PAPs) from poultry origin at concentration levels of 0.1 % or 0.05 % in weight have been prepared as shown in Table 1.

Each participating lab received about 10 g of 8 blind 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 Poultry PCR Implementation Test 2017.

Sample	Material	Intended result
1	Blank feed I	Negative
2	Blank feed I + 0.05 % w/w poultry PAP I	Positive
3	Blank feed I + 0.1 % w/w poultry PAP I	Positive
4	Blank feed II	Negative
5	Blank feed II + 0.05 % w/w poultry PAP II	Positive
6	Blank feed II + 0.1 % w/w poultry PAP II	Positive
7	Blank feed III	Negative
8	Blank feed III + 0.1 % w/w poultry PAP I	Positive
Total		**8 (5 positive and 3 negative results)**

3.2.2. Materials used in the preparation of the samples

The feed matrices used for the preparation of the sample set were a compound feed for poultry: a fattening turkey starter feed (blank feed I), a free range chicken feed (blank feed II) and a laying hen feed (blank feed III). The blank feed I consisted of maize, soyabean oilcake, extruded full-fat soya, sunflower oilcake, limestone, monocalcium phosphate, lysine, vitamins and mineral premix, rapeseed oil, methionine, yeast, sodium chloride, sodium bicarbonate, choline chloride, veterinary drugs, threonine, feed enzymes. The blank feed II was made of maize, wheat, soybean oilcake (GM), wheat by-product, calcium carbonate, soybean oil, premix, sodium chloride, lysine, methionine, essential oils. The composition of blank feed III mentions maize, dehulled soybean oilcake (GM), calcium carbonate, wheat, rice bran, animal fat, roasted soybeans, alfalfa, dicalcium phosphate, sodium chloride, sodium bicarbonate.

Two poultry PAP were used to spike the blank materials. Poultry PAP I was processed according to method 7 (minimum 90°C for 30 minutes on cooking side + drying step 60 minutes at approximately 95°C) and poultry PAP II submitted a heating process according to method 4 (allowing the following possibilities : minimum 100°C during 16 minutes, minimum 110°C during 13 minutes, minimum 120°C during 8 minutes or minimum 130°C during 3 minutes).

3.2.3. Preparation of the samples

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

The final materials at 0.1 % and 0.05 % in mass fraction respectively were obtained by successive dilutions in the feed matrix.

3.3. Qualitative analysis

Qualitative analysis concerned the presence or absence of poultry 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. 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

4.1. Homogeneity study

Ten replicates of each sample (ten vials) were chosen randomly and were analysed using the validated poultry 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 poultry 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	Poultry target	AC
1	Blank feed I	10	-	1.00 (n= 20)
2	Blank feed I + 0.05 % w/w poultry PAP I	10	+	1.00 (n= 20)
3	Blank feed I + 0.1 % w/w poultry PAP I	10	+	1.00 (n= 20)
4	Blank feed II	10	-	1.00 (n= 20)
5	Blank feed II + 0.05 % w/w poultry PAP II	10	+	1.00 (n= 20)
6	Blank feed II + 0.1 % w/w poultry PAP II	10	+	1.00 (n= 20)
7	Blank feed III	10	-	1.00 (n= 20)
8	Blank feed III + 0.1 % w/w poultry PAP I	10	+	1.00 (n= 20)
	Overall results	80		

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

Lab 13 did not return any result. Gross results from the 27 participants are to be found in Annex 4.

4.2.1. Overview of results and performance of the method

Table 3 summarizes the results submitted by 27 NRLs for the eight sample types submitted to qualitative analysis.

Except Lab 25, all participants were able to obtain a cut-off that met the set quality criterion (the corresponding copy number should exceed 9). Notwithstanding this fact, results of Lab 25 were all fine.

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

Sample	Material	Nr of results	AC
1	Blank feed I	27	0.963 (1)
2	Blank feed I + 0.05 % w/w poultry PAP I	27	1.000
3	Blank feed I + 0.1 % w/w poultry PAP I	27	1.000
4	Blank feed II	27	0.963 (1)
5	Blank feed II + 0.05 % w/w poultry PAP II	27	0.963 (1)
6	Blank feed II + 0.1 % w/w poultry PAP II	27	0.963 (1)
7	Blank feed III	27	0.963 (1)
8	Blank feed III + 0.1 % w/w poultry PAP I	27	1.000
	Overall results	216	0.977 (5)

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.

The overall results, expressed in terms of global accuracy (AC), reveal a very good global performance. The rate of false positive results reaches 3.70 % and the rate of false negative results at the target level of 0.1 % w/w of poultry PAP is at 1.23 %. Twenty-four participants out of 27 (89 %) returned perfect results. The 5 deviations out of 216 results (2.3 %) were located only in 3 labs.

4.2.2. Detailed review of results for each sample material

Incorrect results are detailed in this section.

- **Lab 7:** two deviations were recorded: one negative deviation with the sample 6 (blank feed II + 0.1 % of poultry PAP) and one positive deviation with the sample 7 (blank feed III).
- **Lab 15:** two positive deviations were recorded with the sample 1 (blank feed I) and the sample 4 (blank feed II).
- **Lab 18:** the sample 5 (blank feed II + 0.05 % of poultry PAP) was erroneously declared negative for the presence of poultry DNA.

The very limited number of deviations do not allow to find any clear explanation. Moreover, each deviation was obtained with a different sample type.

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 concerning the ability to detect poultry material (Table 4), a majority of the participating NRLs (24 labs out of 27 or 85.7 %) obtained an excellent global performance and had no false

result. One lab (Lab 18) out of the 27 participating NRLs (3.6 %) had only one false negative result but it was at the level of 0.05 % of poultry PAP w/w. The two remaining labs (Lab 7 and 15 - 7.1 %) had 2 false results.

Table 4: NRL proficiencies regarding the detection of poultry 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
8	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
16	1.000	1.000	1.000
17	1.000	1.000	1.000
19	1.000	1.000	1.000
20	1.000	1.000	1.000
21	1.000	1.000	1.000
22	1.000	1.000	1.000
23	1.000	1.000	1.000
24	1.000	1.000	1.000
25	1.000	1.000	1.000
26	1.000	1.000	1.000
27	1.000	1.000	1.000
28	1.000	1.000	1.000
18	0.875	0.800	1.000
7	0.750	0.800	0.667
15	0.750	1.000	0.333

5. Conclusions

This study is the first assessment of the validated poultry PCR method in the NRL network. Out of the 28 participants, only 27 finally participated by submitting results. Looking globally at these results sent to the EURL-AP, 85.71 % of the participating NRLs (24 labs out of 27) obtained an excellent global performance and had no false result. 7.14 % of the participating NRLs (2 labs out of 27) had a false negative result. At the 0.1% poultry PAP level, only one lab (3.57 %) had a false negative result. Two labs (7.14 %) got some false positive results. The overall rate of false negative results is 3.70 % and the overall rate of false positive results is 1.48%, both are below 5%.

Different conclusions can be made:

1. The poultry PCR method is fit for the detection of poultry 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 poultry PCR method appears to be well implemented in a majority of the NRLs (25 labs).

Acknowledgments

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

References

[1] EU. 2004. Commission Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union L 165, 30/04/2004: 1-141.

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[3] The TSE Roadmap 2 - A Strategy paper on Transmissible Spongiform Encephalopathies for 2010-20. Communication from the Commission to the European parliament and the Council. Brussels, 16/07/2010, COM(2010)384 final. http://www.fsai.ie/uploadedFiles/Legislation/FSAI_-_Legislation/2010/07_jul2010/EU_Communication_TSE.pdf

[4] Fumière, O., Marien, A., Maljean, J. and Berben, G. 2023. Evaluation of the PCR method for the detection of poultry DNA. ISBN 978-2-87286-125-5. Available at https://www.eurl.craw.eu/publications/scientific-reports/

[5] Fumière, O., Marien, A., Maljean, J. and Berben, G. 2023. Validation study of a real-time PCR method developed for the detection of poultry DNA in feedingstuffs. ISBN 878-2-87286-128-6. Available at https://www.eurl.craw.eu/publications/scientific-reports/

Annex 1

List 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
Croatia	Croatian Veterinary Institute
Cyprus	Cyprus Veterinary Services
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	Feedingstuffs Control Laboratory of Thessaloniki
Hungary	Central Agricultural Office-Directorate Food and Feed Safety-Central Feed Investigation Lab.
Ireland	Plant Health Laboratory, Department of Agriculture, Food and the Marine
Italy	National Reference Centre for the Surveillance and Monitoring of Animal Feed
Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
Lithuania	National Food and Veterinary Risk Assessment Institute
Luxemburg	Agroscope Liebefeld-Posieux Research Station (Switzerland)
Netherlands	RIKILT Institute of Food Safety, Wageningen UR
Norway	Institute of Marine Research
Poland	National Veterinary Research Institute
Portugal	Laboratorio Nacional de Investigaçao Veterinaria
Romania	Hygiene Institute of Veterinary Health
Serbia	Institute of Veterinary Medicine of Serbia
Slovakia	State Veterinary and Food Institute
Slovenia	Veterinary Faculty-National Veterinary Institute-Unit for pathology of animal nutrition and environmental hygiene
Spain	Laboratorio Arbitral Agroalimentario
Sweden	National Veterinary Institute, Department of Animal Feed
United Kingdom	Animal and Plant Health Agency

Annex 2

Excel result report form

Poultry PCR Implementation Test 2018

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

Lab code	0	0	0	0	0	0	0	0
Sample rank	1st	2nd	3rd	4th	5th	6th	7th	8th
Sample N°								
Qualitative analysis								
Poultry 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,...)*								

Annex 3

Composition of sample sets

	Lab number																											
Sample	**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**	**28**
Blank feed 1 = turkey feed	2487	1191	921	1407	489	705	1137	273	867	381	111	1083	1569	327	1353	435	57	165	1029	2649	2433	2163	651	1947	2217	2595	2325	2109
0.05 % w/w poultry PAP 1 in blank feed 1	1287	1935	1557	801	99	2259	1827	963	153	2313	1125	1503	2205	1179	1773	855	1233	369	2637	2097	423	693	1341	2475	531	1449	2583	909
0.1 % w/w poultry PAP 1 in blank feed 1	1467	1791	1737	2169	927	1251	549	2547	1845	2655	2601	63	1089	765	1197	225	117	981	819	1953	1629	171	2439	2277	1035	9	387	2493
Blank feed 2 = chicken feed	897	195	2571	735	1113	87	951	2301	843	1653	465	2679	1275	1059	681	1545	1005	2409	2355	2247	1599	1923	1707	519	2463	357	1167	1761
0.05 % w/w poultry PAP 2 in blank feed 2	825	1095	2229	1419	771	285	987	15	1041	2283	2391	555	879	1365	2175	663	2445	177	933	231	2067	2607	1203	717	1851	123	339	1149
0.1 % w/w poultry PAP 2 in blank feed 2	2685	1983	1281	93	957	309	1497	1605	1119	39	363	2577	1389	1065	633	2199	2631	255	2415	1335	2091	2469	1551	1659	795	2253	849	417
Blank feed 3 = laying hen feed	615	1209	1317	2127	1371	2451	345	1479	2073	831	993	669	1533	1101	2559	2397	1425	507	1965	183	2181	2505	1857	291	21	453	1155	885
0.1 % w/w poultry PAP 1 in blank feed 3	783	1431	567	2403	405	243	459	1701	837	2187	81	1593	945	513	999	1323	621	1647	1755	2673	675	1053	1917	2133	2457	1161	297	2619

Annex 4

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

Laboratory identification code :	1
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,71 cycles
Copy number at the cut-off :	11,38 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	615	Absent	1 x	50,00	50,00	10 x	50,00	50,00	undet.
2	783	Present	1 x	29,45	32,51	10 x	33,93	35,59	
3	825	Present	1 x	32,07	34,11	10 x	35,82	37,51	
4	897	Absent	1 x	50,00	50,00	10 x	50,00	50,00	undet.
5	1287	Present	1 x	33,72	32,80	10 x	36,81	36,59	
6	1467	Present	1 x	31,72	30,00	10 x	35,47	33,95	
7	2487	Absent	1 x	50,00	50,00	10 x	50,00	50,00	undet.
8	2685	Present	1 x	32,11	34,86	10 x	35,69	40,06	

Laboratory identification code :	2
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,55 cycles
Copy number at the cut-off :	9,91 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	195	Absent	1 x			10 x			
2	1095	Present	1 x	33,70	31,97	10 x	38,93	36,72	
3	1191	Absent	1 x			10 x			
4	1209	Absent	1 x		48,45	10 x			
5	1431	Present	1 x	31,15	30,89	10 x	35,11	35,42	
6	1791	Present	1 x	29,56	30,22	10 x	34,05	34,88	
7	1935	Present	1 x	30,96	31,13	10 x	35,26	36,25	
8	1983	Present	1 x	34,46	34,13	10 x	36,38	36,96	

Laboratory identification code :	3
Responsibility agreement :	Yes
Cut-off at 15 copies :	38,18 cycles
Copy number at the cut-off :	10,49 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	567	Present	1 x	33,54	34,68	10 x	36,08	36,80	
2	921	Absent	1 x			10 x			
3	1281	Present	1 x	34,20	34,11	10 x			10x dilution: no signal detected, although 3 separate runs with 3 separate dilutions were performed
4	1317	Absent	1 x			10 x			
5	1557	Present	1 x	34,02	33,50	10 x	37,03	36,67	
6	1737	Present	1 x	34,10	34,13	10 x	36,68	37,10	
7	2229	Present	1 x	35,31	33,60	10 x		36,21	10x dilution: no signal of replicate 1 detected, although 3 separate runs with 3 separate dilutions were performed
8	2571	Absent	1 x			10 x			

Laboratory identification code :	4
Responsibility agreement :	Yes
Cut-off at 15 copies :	41,90 cycles
Copy number at the cut-off :	10,02 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	93	Present	1 x	36,70	37,30	10 x	42,10	42,00	
2	735	Absent	1 x			10 x			no inhibition (test with calibrant 40 copies)
3	801	Present	1 x	37,40	37,80	10 x	40,90	42,80	
4	1407	Absent	1 x			10 x			no inhibition (test with calibrant 40 copies)
5	1419	Present	1 x	39,00	37,50	10 x		41,70	
6	2127	Absent	1 x			10 x			no inhibition (test with calibrant 40 copies)
7	2169	Present	1 x	35,60	37,00	10 x	39,60	41,20	
8	2403	Present	1 x	35,60	35,90	10 x	41,90	40,70	

Laboratory identification code :	5
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,00 cycles
Copy number at the cut-off :	15,00 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	99	Present	1 x	33,87	33,55	10 x	38,58	36,92	The calculation of cut-off is not satisfying. The number of copies at cut-off is 7.73 copies.
2	405	Present	1 x	31,47	31,68	10 x	34,97	35,93	The R-square for the standard curve is generally very bad (here 0,91)
3	489	Absent	1 x			10 x			The base signal from the probe is generally very high, and we have seen that using two different probes.
4	771	Present	1 x	35,78	35,10	10 x			High base signal can indicate overload of probe on our PCR platform.
5	927	Present	1 x	32,63	31,96	10 x	36,97	37,79	
6	957	Present	1 x	33,77	33,87	10 x	41,82	37,32	
7	1113	Absent	1 x			10 x			
8	1371	Absent	1 x			10 x			

Laboratory identification code :	6
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,03 cycles
Copy number at the cut-off :	11,86 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	1251	Present	1 x	32,85	32,81	10 x	36,62	36,62	
2	2451	Absent	1 x	50,00	50,00	10 x	50,00	50,00	
3	705	Absent	1 x	50,00	50,00	10 x	50,00	50,00	
4	2259	Present	1 x	35,24	34,87	10 x	38,50	38,32	
5	285	Absent	1 x	38,43	37,95	10 x	40,46	42,33	
6	243	Present	1 x	34,31	33,89	10 x	37,89	37,80	
7	309	Present	1 x	35,02	35,35	10 x	40,27	38,72	
8	87	Absent	1 x	50,00	50,00	10 x	50,00	50,00	

Laboratory identification code :	7
Responsibility agreement :	Yes
Cut-off at 15 copies :	39,04 cycles
Copy number at the cut-off :	9,16 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	345	Present	1 x	22,00	20,30	10 x	24,20	26,00	
2	459	Present	1 x	30,00	32,00	10 x	34,90	36,20	
3	549	Present	1 x	29,70	29,60	10 x	33,80	33,70	
4	951	Absent	1 x			10 x			no signal detected in both dilutions in any replicates
5	987	Present	1 x	31,80	29,80	10 x	37,60	38,40	
6	1137	Absent	1 x	41,60		10 x	43,80		no signal detected in 2nd replicate, in both dilutions
7	1497	Absent	1 x	42,70	43,80	10 x	42,80	45,10	
8	1827	Present	1 x	31,30	29,30	10 x	34,80	32,80	

Laboratory identification code :	8
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,46 cycles
Copy number at the cut-off :	10,79 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	15	Present	1 x	33,11	33,31	10 x	36,20	37,21	
2	273	Absent	1 x			10 x			
3	963	Present	1 x	30,52	31,73	10 x	34,91	35,40	
4	1479	Absent	1 x			10 x			
5	1605	Present	1 x	32,06	31,43	10 x	36,61	35,84	
6	1701	Present	1 x	30,62	30,50	10 x	34,10	34,49	
7	2301	Absent	1 x			10 x			
8	2547	Present	1 x	31,20	31,42	10 x	35,85	34,87	

Laboratory identification code :	9
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,59 cycles
Copy number at the cut-off :	10,65 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	153	Present	1 x	34,43	33,77	10 x	37,47	36,75	
2	837	Present	1 x	33,50	32,41	10 x	38,11	37,04	
3	843	Absent	1 x	50,00	50,00	10 x	50,00	50,00	50 - means undetected - the excel did not accept the undet. like result
4	867	Absent	1 x	50,00	50,00	10 x	50,00	50,00	50 - means undetected - the excel did not accept the undet. like result
5	1041	Present	1 x	35,43	35,72	10 x	50,00	42,76	50 - means undetected - the excel did not accept the undet. like result
6	1119	Present	1 x	33,40	33,47	10 x	37,67	37,50	
7	1845	Present	1 x	32,09	31,99	10 x	36,65	35,87	
8	2073	Absent	1 x	50,00	50,00	10 x	50,00	50,00	50 - means undetected - the excel did not accept the undet. like result

Laboratory identification code :	10
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,44 cycles
Copy number at the cut-off :	10,91 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	39	Present	1 x	30,69	31,61	10 x	34,06	35,26	
2	381	Present	1 x	35,93	35,62	10 x	38,96	39,70	
3	831	Absent	1 x	38,86		10 x			
4	1653	Absent	1 x			10 x			
5	2187	Present	1 x	29,57	29,99	10 x	33,07	33,38	
6	2283	Present	1 x	31,23	32,49	10 x	34,98	35,71	
7	2313	Present	1 x	28,48	29,55	10 x	32,82	32,98	
8	2655	Present	1 x	27,45	28,76	10 x	31,02	32,30	

Laboratory identification code :	11
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,67 cycles
Copy number at the cut-off :	10,41 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	81	Present	1 x	30,24	28,97	10 x	33,80	32,63	
2	111	Absent	1 x			10 x			
3	363	Present	1 x	29,14	30,91	10 x	32,29	34,36	
4	465	Absent	1 x			10 x			
5	993	Absent	1 x			10 x			
6	1125	Present	1 x	30,72	30,04	10 x	33,73	33,56	
7	2391	Present	1 x	32,51	32,03	10 x	36,59	35,28	
8	2601	Present	1 x	29,29	29,59	10 x	32,50	32,98	

Laboratory identification code :	13
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,35 cycles
Copy number at the cut-off :	10,16 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	879	Present	1 x	29,00	29,50	10 x	32,10	32,20	
2	945	Present	1 x	29,10	29,80	10 x	32,40	32,60	
3	1089	Present	1 x	30,20	30,40	10 x	33,40	33,50	
4	1275	Absent	1 x			10 x			
5	1389	Present	1 x	30,50	30,60	10 x	33,70	33,80	
6	1533	Absent	1 x			10 x			
7	1569	Absent	1 x			10 x			
8	2205	Present	1 x	30,70	30,80	10 x	33,90	34,10	

Laboratory identification code :	14
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,48 cycles
Copy number at the cut-off :	10,60 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	327	Absent	1 x			10 x			
2	513	Present	1 x	29,04	28,79	10 x	33,05	32,71	
3	765	Present	1 x	29,52	29,39	10 x	33,51	33,35	
4	1059	Absent	1 x			10 x			
5	1065	Present	1 x	30,73	30,54	10 x	35,02	34,09	
6	1101	Absent	1 x			10 x			
7	1179	Present	1 x	30,72	29,34	10 x	34,41	33,63	
8	1365	Present	1 x	32,26	32,32	10 x		42,35	

Laboratory identification code :	15
Responsibility agreement :	Yes
Cut-off at 15 copies :	39,29 cycles
Copy number at the cut-off :	9,17 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	1353	Present		34,68	34,07		38,35	36,33	
2	681	Present		30,59	31,99		41,23	39,51	
3	1197	Present		30,64	30,65		34,42	34,15	
4	2175	Present		31,48	32,06		35,51	36,63	
5	999	Present		31,23	29,62		33,79	33,03	
6	633	Present		31,22	30,90		34,94	34,99	
7	1773	Present		31,42	31,27		36,61	35,92	
8	2559	Absent		39,91	39,54		39,29	40,71	there is an inhibition

Laboratory identification code :	16	
Responsibility agreement :	Yes	
Cut-off at 15 copies :	37,22	cycles
Copy number at the cut-off :	11,17	copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	225	Present	2 x	31,57	31,07	20 x	34,83	34,49	
2	435	Absent	4 x	37,32	37,96	40 x	41,17		
3	663	Present	1 x	33,09	32,82	10 x	36,55	36,33	
4	855	Present	2 x	32,41	31,53	20 x	36,01	35,03	
5	1323	Present	2 x	29,97	31,60	20 x	33,45	35,48	
6	1545	Absent	4 x	37,65	37,88	20 x	40,12	38,53	
7	2199	Present	1 x	32,50	32,02	10 x	36,02	35,60	
8	2397	Absent	4 x	38,69	37,75	40 x	39,34		

Laboratory identification code :	17
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,33 cycles
Copy number at the cut-off :	9,23 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	57	Absent	1 x	36,98	40,00	10 x	40,00	40,00	
2	117	Present	1 x	30,00	28,30	10 x	35,00	31,89	
3	621	Present	1 x	30,72	30,96	10 x	33,67	35,34	
4	1005	Absent	1 x	40,00	40,00	10 x	40,00	40,00	
5	1233	Present	1 x	31,71	31,03	10 x	37,90	34,76	
6	1425	Absent	1 x	40,00	40,00	10 x	40,00	40,00	
7	2445	Present	1 x	34,10	33,57	10 x	40,00	40,00	
8	2631	Present	1 x	30,83	31,83	10 x	35,26	40,00	

Laboratory identification code :	18
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,49 cycles
Copy number at the cut-off :	10,09 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	165	Absent	1 x			10 x			
2	177	Absent	1 x			10 x			
3	255	Present	1 x	33,79	33,98	10 x			10x fold dilutions gave no result, 3x fold dilutions were done; Ct 34,53 and Ct 34,74
4	369	Present	1 x	34,55	34,56	10 x	35,64	36,38	
5	507	Absent	1 x			10 x			
6	981	Present	1 x	33,06	33,33	10 x	35,08	35,48	
7	1647	Present	1 x	34,27	33,87	10 x	36,12	35,76	
8	2409	Absent	1 x			10 x			

Laboratory identification code :	19
Responsibility agreement :	Yes
Cut-off at 15 copies :	39,05 cycles
Copy number at the cut-off :	11,65 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	819	Present	1 x	30,46	30,52	10 x	33,85	33,90	
2	933	Present	1 x	34,61	34,74	10 x	37,95	37,98	
3	1029	Absent	1 x	50,00	50,00	10 x	50,00	50,00	
4	1755	Present	1 x	31,15	31,18	10 x	34,52	34,48	
5	1965	Absent	1 x	50,00	50,00	10 x	50,00	50,00	
6	2355	Absent	1 x	50,00	50,00	10 x	50,00	50,00	
7	2415	Present	1 x	33,62	33,70	10 x	36,92	37,02	
8	2637	Present	1 x	33,50	33,30	10 x	36,84	33,75	

Laboratory identification code :	20
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,13 cycles
Copy number at the cut-off :	10,13 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	183	Absent	1 x		42,48	10 x		43,92	
2	231	Present	1 x	31,18	31,01	10 x	35,03	33,73	
3	1335	Present	1 x	28,78	29,41	10 x	32,05	32,47	
4	1953	Present	1 x	28,04	28,19	10 x	31,78	31,39	
5	2097	Present	1 x	28,30	27,80	10 x	30,84	30,80	
6	2247	Absent	1 x	37,75	45,82	10 x	39,95	45,43	
7	2649	Absent	1 x	35,36		10 x			
8	2673	Present	1 x	27,37	27,42	10 x	30,25	30,84	

Laboratory identification code :	21
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,59 cycles
Copy number at the cut-off :	9,28 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	423	Present	1 x	31,60	32,30	10 x			
2	675	Present	1 x	30,34	29,60	10 x	34,18	32,40	
3	1599	Absent	1 x			10 x			
4	1629	Present	1 x	31,41	31,80	10 x	34,16	34,90	
5	2067	Present	1 x	31,23	33,89	10 x			
6	2091	Present	1 x	30,07	30,90	10 x	33,75	33,71	
7	2181	Absent	1 x			10 x			
8	2433	Absent	1 x			10 x			

Laboratory identification code :	22
Responsibility agreement :	Yes
Cut-off at 15 copies :	38,01 cycles
Copy number at the cut-off :	9,10 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	171	Present		28,42	28,79		31,81	32,08	
2	693	Present		28,39	28,79		31,66	31,46	
3	1053	Present		28,66	28,81		32,88	32,15	
4	1923	Absent							
5	2163	Absent							
6	2469	Present		33,05	33,22		35,89	36,12	
7	2505	Absent							
8	2607	Present		34,13	34,16		37,73	37,57	

Laboratory identification code :	23
Responsibility agreement :	Yes
Cut-off at 15 copies :	36,60 cycles
Copy number at the cut-off :	10,49 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	651	Absent							
2	1203	Present		33,48	38,00		38,56	42,42	
3	1341	Present		30,54	31,26		34,23	34,68	
4	1551	Present		32,89	34,75		38,35	48,07	
5	1707	Absent							
6	1857	Absent							
7	1917	Present		30,36	31,13		33,96	34,70	
8	2439	Present		30,55	29,96		34,02	33,87	

Laboratory identification code :	24
Responsibility agreement :	Yes
Cut-off at 15 copies :	35,98 cycles
Copy number at the cut-off :	9,39 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	291	Absent	1 x			10 x			
2	519	Absent	1 x			10 x			
3	717	Present	1 x	32,42	32,53	10 x			replicate 1, 1:10: Cq 36,35; replicate 2, 1:10: Cq 37,33
4	1659	Present	1 x	31,53	32,34	10 x	35,07	35,91	
5	1947	Absent	1 x			10 x			
6	2133	Present	1 x	29,13	29,58	10 x	32,27	33,01	
7	2277	Present	1 x	31,76	30,81	10 x	35,49	33,61	
8	2475	Present	1 x	31,86	31,20	10 x	34,96	34,40	

Laboratory identification code :	25
Responsibility agreement :	Yes
Cut-off at 15 copies :	42,08 cycles
Copy number at the cut-off :	8,45 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	21	Absent	1 x	50,00	50,00	10 x	50,00	50,00	No inhibition
2	531	Present	1 x	33,93	32,93	10 x	37,37	36,04	
3	795	Present	1 x	33,55	36,19	10 x	36,78	41,98	
4	1035	Present	1 x	33,54	33,57	10 x	36,81	36,98	
5	1851	Present	1 x	38,89	36,85	10 x	43,11	41,48	flat signals
6	2217	Absent	1 x	50,00	50,00	10 x	50,00	50,00	No inhibition
7	2457	Present	1 x	34,98	34,93	10 x	38,37	38,26	
8	2463	Absent	1 x	50,00	50,00	10 x	50,00	50,00	No inhibition

Laboratory identification code :	26
Responsibility agreement :	Yes
Cut-off at 15 copies :	38,32 cycles
Copy number at the cut-off :	11,38 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	9	Present	1 x	31,18	31,00	10 x	34,68	34,64	
2	123	Present	1 x	36,27	35,76	10 x	39,65	38,86	
3	357	Absent	1 x	50,00	50,00	10 x	50,00	50,00	
4	453	Absent	1 x	50,00	50,00	10 x	50,00	50,00	
5	1161	Present	1 x	32,85	34,01	10 x	36,39	37,67	
6	1449	Present	1 x	32,78	32,69	10 x	36,34	36,40	
7	2253	Present	1 x	35,53	34,40	10 x	39,51	38,22	
8	2595	Absent	1 x	50,00	50,00	10 x	50,00	50,00	

Laboratory identification code :	27
Responsibility agreement :	Yes
Cut-off at 15 copies :	38,29 cycles
Copy number at the cut-off :	10,28 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	297	Present	1 x	29,18	30,22	10 x	32,49	34,16	Clear result
2	339	Present	1 x	31,21	32,62	10 x	34,62	36,04	Clear result
3	387	Present	1 x	29,62	29,04	10 x	32,89	32,28	Clear result
4	849	Present	1 x	32,34	32,14	10 x	35,83	35,27	Clear result
5	1155	Absent	1 x			10 x		42,97	Empty cells mean no amplification - flat line. No PCR inhibition.
6	1167	Absent	1 x	43,53		10 x	41,81		Empty cells mean no amplification. Inhibition control with calibrant 40 showed PCR inhibition, but further dilutions (20 and 30 x) had no amplification.
7	2325	Absent	1 x		39,36	10 x	41,82	41,40	Empty cells mean no amplification. Inhibition control with calibrant 40 showed PCR inhibition, but further dilutions (20 and 30 x) had no amplification.
8	2583	Present	1 x	28,68	30,25	10 x	32,17	33,89	Clear result

Laboratory identification code :	28
Responsibility agreement :	Yes
Cut-off at 15 copies :	37,75 cycles
Copy number at the cut-off :	11,27 copies

	Sample N°	Poultry DNA	Dilution 1	Ct value 1	Ct value 2	Dilution 2	Ct value 1	Ct value 2	Comment
1	417	Present	1 x	29,58	31,50	10 x	33,36	35,47	
2	885	Absent	1 x			10 x			
3	909	Present	1 x	31,02	30,84	10 x	34,72	34,18	
4	1149	Present	1 x	33,09	32,20	10 x	36,27	35,71	
5	1761	Absent	1 x			10 x			
6	2109	Absent	1 x			10 x			
7	2493	Present	1 x	31,45	30,22	10 x	34,87	33,81	
8	2619	Present	1 x	30,47	30,72	10 x	34,25	34,11	