

Interlaboratory study for the evaluation of NRLs PCR methods

O. Fumière, A. Marien and G. Berben

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FINAL REPORT

European Union Reference Laboratory for Animal Proteins in feedingstuffs

Walloon Agricultural Research Centre

Valorisation of Agricultural Products Department

Bât. Henseval Chaussée de Namur, 24 – B-5030 GEMBLoux - BELGIUM

Tél : ++ 32 (0)81 62 03 50 - Fax : ++ 32 (0)81 62 03 88

secretary@eurl.craw.eu - <http://www.eurl.craw.be>



Wallonie

Contact information

Olivier Fumière
Authentication and Traceability Unit (U16)
Valorisation of Agricultural Products Department
Walloon Agricultural Research Centre - CRA-W
European Union Reference Laboratory for Animal Protein in feedingstuffs – EURL-AP
Building "Henseval"
Chaussée de Namur, 24
5030 Gembloux (Belgium)
Tél : +32(0)81 62 03 51
Fax : +32(0)81 62 03 88
Mail : fumiere@cra.wallonie.be
Website : <http://www.cra.wallonie.be>
<http://eurl.craw.eu>

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Walloon Agricultural Research Centre
Communications Service
Rue de Liroux, 9
5030 Gembloux (Belgium)
cra@cra.wallonie.be
<http://www.cra.wallonie.be>

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Summary

Polymerase Chain Reaction (PCR) could be helpful for the control of the species origin of PAP and a possible lifting of the ban on the use of non-ruminant PAP in non-ruminant feed without the lifting of the existing prohibition on intra-species recycling as considered by the Commission in the TSE roadmap II¹.

The present inter-laboratory study aimed 1) to evaluate the potential of PCR targets present in the NRLs for the detection of PAPs according to the information collected through the 2010 EURL-AP survey about PCR capacities of the NRLs and 2) to identify assays that would be of interest for a future validation.

The results show that the PCR tests used by some NRLs are fully reliable. More than 15 targets gave interesting results to be considered by the EURL-AP for further investigations on their fitness for the detection of PAPs. Nevertheless, a majority of the assays is not fit for the purpose or is not sensitive enough to be used as such in routine analysis.

¹ **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

1. Introduction

In the TSE roadmap II², the Commission considers a possible lifting of the ban on the use of non-ruminant PAP in non-ruminant feed without the lifting of the existing prohibition on intra-species recycling. Such a measure would however be acceptable only if validated analytical techniques to determine the species origin of PAP are available. Polymerase Chain Reaction (PCR) could be helpful for that purpose.

The 2010 EURL-AP survey about PCR capacities of the NRLs indicated that some NRLs developed and used PCR tests focussed on animal targets. The present inter-laboratory study would aim to evaluate the potential of PCR targets present in the NRLs for the detection of PAPs and to identify assays that would be of interest for a future validation.

2. Organizer team

The study was conducted and coordinated by the EURL-AP (Department Valorisation of Agricultural Products of the CRA-W).

3. Participants

Eleven National Reference Laboratories (NRLs) were contacted through an invitation letter (Annex I) and agreed to participate.

Table 1. List of participating National Reference Laboratories (NRLs)

Organization name	Country
Agroscope Liebefeld Posieux - HARAS	Posieux, Switzerland
Bundesinstitut für Risikobewertung (BfR)	Berlin, Germany
Central Agricultural Office	Budapest, Hungary
Central Institute for Supervising and Testing in Agriculture	Prague, Czech Republic
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSTO – CreAA)	Torino, Italy
Laboratorio Arbitral Agroalimentario	Madrid, Spain
National Food and Veterinary Risk Assessment Institute	Vilnius, Lithuania
National Veterinary Research Institute	Pulawy, Poland
Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES)	Linz, Austria
RIKILT-Institute of Food Safety	Wageningen, The Netherlands
Veterinary Laboratory Agency (VLA)	Penrith, Cumbria, UK

² **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. Time schedule of the study

The 17th of January 2011, an invitation letter (Annex I) was sent to the NRLs having reported to use PCR methods for the detection of PAPs to know whether they were interested to participate in the study. The document described the following points:

- ✓ objective of the study,
- ✓ organizer team,
- ✓ material provided,
- ✓ general outline of the exercise,
- ✓ time schedule of the study.

The laboratories had to confirm their participation by the 31st of January 2011 through a reply form (Annex II) indicating the targets that they accepted to include in the study as these targets could be shared within the EURL-AP network in case of convenient results.

The 14th of February 2011, the experimental material was sent to all the participating laboratories which received the material in good conditions between the 15th and the 17th of February 2011 except for the NRL #4 which received defrosted vials of the provided DNA extracts.

The results were collected between the 25th of February and the 29th of March 2011 (official deadline: 1st to 4th of March).

The participants received an Excel file made of three sheets: 1) the instructions (Annex III), 2) the form for encoding of the results (Annex IV), 3) the report summary which is automatically generated by filling results in sheet 2 (Annex V).

5. Purpose of the study

The objective of this study was to evaluate the potential of PCR targets present in the NRLs for the detection of PAPs and to identify assays that would be of interest for a future full validation through an interlaboratory study leading to a sharing of the tests within the EURL-AP network if the validation is successful.

6. Design of the study

The task of the participating laboratories consisted to analyse 17 blind DNA samples with all the targets they accepted to evaluate. As the DNAs were extracted according the protocol of the CRA-W (semi-automatic extraction protocol using the Wizard[®] Magnetic DNA Purification System for Food -Promega- and a KingFisher extractor -Thermo), an additional labelled sample containing a DNA extracted from a sample contaminated with 0.1% of cattle MBM was also provided to the participants in order to adapt their PCR protocols to the samples.

7. Description and preparation of test materials

A set of 17 samples to be analysed by the participants was prepared. They all consisted of DNA extracts. The composition of the samples is presented in Annex VI.

Nine samples were prepared: one blank A (consisting of soybean), five mixes containing 0.2% in weight of cattle MBM, pig MBM, sheep MBM, chicken MBM or fishmeal respectively in blank A and three mixes containing 1 % in weight of pig MBM, chicken MBM or fishmeal in blank A. The entire samples were submitted to the DNA extraction protocol in use at the CRA-W (see point 6). The DNAs were then mixed to obtain the fifteen samples containing one or two animal species. The samples were prepared as described in the Figure 1.

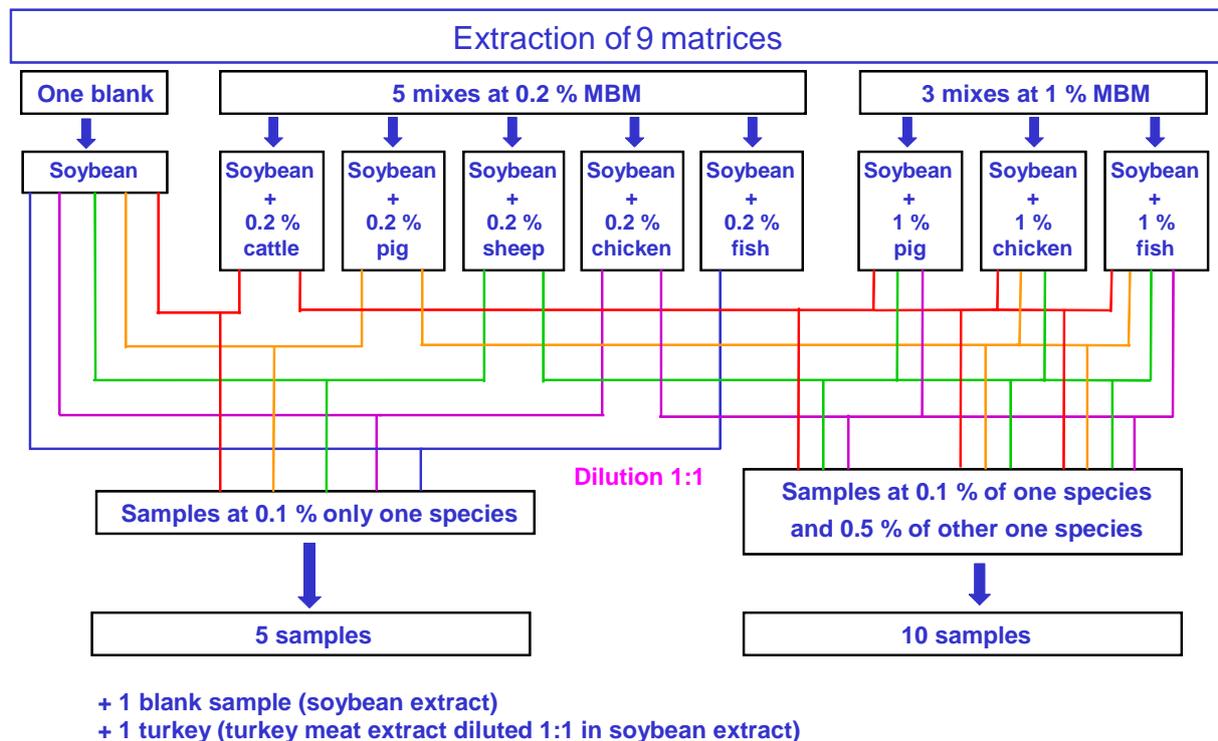


Figure 1. Preparation of the samples

8. Tests performed to check the samples

The composition of all the samples was checked with the targets present at EURL-AP (cattle, pig, sheep, chicken and fish) and all results were as expected.

Possible presence of turkey material in chicken MBM was outsourced to an external laboratory as the EURL-AP does not have such a target. The results were inconclusive as turkey was apparently also found in the blank sample which is impossible with respect to all the care taken to prepare this sample.

9. Results

Among the participants, one lab (NRL #6) did not send any result nor explanation for this. NRL #7 sent an e-mail explaining that they were unable to send reliable results.

The results are compiled in Annex VII and summarised in Table 2.

Table 2. Results

Lab	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish
NRL # 1	1 false pos. result	NT	✓	NT	✓	✓	✓	✓	NT	NT	NT	NT	NT	NT	✓
NRL # 2	6 false neg. results	2 false pos. results	3 false neg. results	✓*	NT	3 false neg. results	1 false neg. result	✓	NT	NT	NT	NT	NT	NT	NT
NRL # 3	NT	4 false neg. results	4 false neg. results	NT	NT	6 false neg. results	3 false neg. results	✓	✓*	✓*	NT	NT	NT	NT	NT
NRL # 4	NT	NT	NT	NT	6 false neg. results + 1 false pos. result	✓	NT	NT	NT	NT	1 false pos. result + 1 false neg. result ³	NT	NT	NT	NT
NRL # 5	5 false neg. results	4 false neg. results	4 false neg. results	NT	NT	6 false neg. results	NT	NT	NT	NT	NT	✓	NT	NT	NT
NRL # 6	No result reported														
NRL # 7	No reliable result obtained by the lab														
NRL # 8	14 false neg. results	5 false neg. results	NT	NT	NT	NT	6 false neg. results + 1 false pos. result	NT	NT	NT	NT	NT	NT	NT	NT
NRL # 9	NT	4 false neg. results	4 false neg. results	✓*	✓	3 false neg. results	✓	✓	NT	✓*	NT	NT	NT	NT	NT
NRL # 10	NT	✓ ¹	1 false pos. result ²	NT	1 false pos. result + 1 false neg. result ¹	1 false neg. result ^{1,2}	1 false pos. result ¹	NT	NT	NT	✓ ²	NT	NT	NT	NT
NRL # 11	NT	✓	NT	NT	NT	1 false neg. result	✓	NT	NT	NT	NT	NT	NT	NT	1 false pos. result

Legend : ✓ = no false result
 ✓* = not really evaluated – no aspecificity observed

¹ Method developed by NRL #10

² Kit used by NRL #10

³ Turkey not detected

NT = not tested

Looking at these results, the following comments can be done :

1. Fifteen targets used in 8 NRLs gave excellent results. They cover the cattle, sheep, ruminant, pig, chicken, turkey, avian and fish taxons.
2. Five targets developed for the detection of goat, goose and duck DNA show no aspecificity with the species present in the study. Their sensitivity was nevertheless not evaluated.
3. Nineteen targets gave only false negative results due to a lack of sensitivity. Looking at the Ct values provided by the participants, the results could be improved for 6 targets (cattle, sheep, pig, chicken targets of NRL #3; pig target of NRL #5; pig target of NRL #9) by setting more adequately the cut-off value of the methods.
4. The remaining targets gave poor results and are not fit for the detection of PAPs.
5. Even if samples of NRL #4 arrived defrosted, one may conclude that it did not affect the results because all the samples analysed with the pig target of NRL #4 were correctly identified (even those at 0.1% of pig MBM).

10. Conclusions

The results showed that PCR is already used in some NRLs. The results obtained by NRL #1 prove that they can obtain reliable results except for what was claimed with the animal target being finally an eukaryotic target (so plants do react as well). Nevertheless, a lot of targets are not fit for the purpose or don't have a good sensitivity to be used as such in routine analysis.

More than 15 targets gave interesting results to be considered by the EURL-AP for further investigations on their fitness for the detection of PAPs.

11. Acknowledgements

The EURL-AP would like to thank the National Reference Laboratories which participated in this study. The authors are also grateful to Cécile Ancion, Gaëlle Antoine, Julie Hulin and Denis Roulez for their efficient technical assistance.

12. Annexes

Annex I: Invitation letter



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 74 ☎ 32 (0) 81 62 03 88
e-mail: secretary@crl.cra.wallonie.be Internet : <http://crl.cra.wallonie.be>



Gembloux, 17 January 2011

Invitation to participate to an inter-laboratory study for the evaluation of NRLs PCR methods

Introduction and objective of the study

The ban on processed animal proteins (PAPs) in feed for farmed animals led to a significant reduction of the number of bovine spongiform encephalopathy (BSE) cases. Presently, optical microscopy remains the only reference method for the detection of PAPs to be applied for official control (Regulation EC/152/2009). The legislation also foresees that other methods which provide information about the origin of the animal constituents may be applied in addition to classical microscopy. Polymerase Chain Reaction (PCR) could be helpful for that purpose.

In the TSE roadmap II¹, the Commission considers a possible lifting of the ban on the use of PAP from non-ruminant in non-ruminant feed without the lifting of the existing prohibition on intra-species recycling. Such a measure would however be acceptable only if validated analytical techniques to determine the species origin of PAP are available.

The 2010 EURL-AP survey about PCR capacities of the NRLs indicated that some NRLs developed and used animal PCR targets. The present inter-laboratory study would aim to evaluate the potential of PCR targets present in the NRLs for the detection of PAPs. This would be a kind of first proficiency test as the participating NRLs will use their own PCR assays and it is a way to identify assays that would be of interest for a future validation. **As a prerequisite to your participation, DG SANCO and EURL-AP have decided that the participating NRLs agree 1°) to communicate to the EURL-AP the complete protocols of the assays used in this inter-laboratory study for further evaluation ; 2°) to share with the EURL-AP network the tests that will be validated at the end of the process.**

The organizer team

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

E-mail: secretary@crl.cra.wallonie.be

Material provided

The organisers want to evaluate the PCR targets of the participants. For that reason, the organisers will provide DNA samples extracted according to the EURL-AP protocol using the Wizard[®] Magnetic DNA Purification System for Food (Promega). The participants will receive enough amounts of DNA extracts to adapt their PCR step to the DNA samples provided (PCR inhibition, amount of DNA in the PCR, ...).

¹ The TSE Road map 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



General outline of the exercise

The study will focus on the detection of **ruminant (cattle, sheep, goat,...), poultry (chicken, turkey, duck, goose, ...)** and **pig PAPs**.

The set of samples (DNA extracts) to analyse is composed of 16 blind DNA samples.

One DNA extracted from a feed sample containing 0.1 % of a cattle MBM will be also provided to the participants for possible adaptation of the protocol to our DNA extracts.

Time schedule

- The study will take place in **February and March 2011**
- The samples will be sent to the participants between the **15th of February and the 1st of March**
- The deadline for returning of results to organizers is **2 weeks (10 working days) after reception of the samples**
- EURL-AP expects to present the first results of the study during the annual EURL-AP workshop in Vienna (6-7 April 2011)

Further information

- Dr Gilbert BERBEN
☎ +32 (0)81 62 03 63
☎ +32 (0)81 62 03 88
e-mail: berben@cra.wallonie.be
- Dr Olivier FUMIERE
☎ +32 (0)81 62 03 51
☎ +32 (0)81 62 03 88
e-mail: fumiere@cra.wallonie.be

We would very much appreciate a confirmation of your interest to participate by returning your signed reply form via e-mail to secretary@crl.cra.wallonie.be and/or Fax (+32 (0)81 62 03 88) by **31th of January 2011 at noon** as well as its original hard copy by normal mail.

Please indicate in your reply form (see reply form file):

- your interest to participate.
- the targets that you accept to include in the study.
- the name(s) of the person(s) to whom the material should be directed as well as the detailed shipping address plus phone number and e-mail.

We thank you very much in advance for your support in this task.

Yours sincerely,



Dr Gilbert Berben
Community Reference Laboratory for Animal Proteins in Feedingstuffs



European Union Reference Laboratory for Animal Proteins in feedingstuffs

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

☎ +32 (0) 81 62 03 74 ☎ +32 (0) 81 62 03 88
e-mail: secretary@crl.cra.wallonie.be Internet: <http://crl.cra.wallonie.be>



**Invitation to participate to an inter-laboratory study
for the evaluation of NRLs PCR methods**

Reply form
to send via e-mail to secretary@crl.cra.wallonie.be
and/or Fax (+32 (0)81 62 03 88) by **31st of January 2011 at noon**

Name and address of the participant

e.g. Walloon Agricultural Research Centre (CRA-W)
Department Quality of Agricultural Products
Chaussée de Namur 21
B-5030 Gembloux
Belgium

Are you interested to participate to the study ?

Yes

No

Targets¹ you accept to include in the study²

¹ Species or group of species

e.g. Pig
Ruminant

² The participant agrees to deliver the protocol of the targets used
in the study

**Name(s), detailed shipping address, phone
number and e-mail of person(s) to whom the
material should be directed**

e.g. Olivier Fumière
Department Quality of Agricultural Products
Chaussée de Namur 21
B-5030 Gembloux
Belgium
+32 (0)81 62.03.51
fumiere@cra.wallonie.be

Name and signature of the NRL responsible



Please read carefully this information before fill in the form		
Instructions		PCR interlaboratory study 2011
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	Summarized report page (to print, sign and fax)	
2. Instructions		
Informations to help you how fill in the reporting sheet		
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 your data. It is filled automatically while encoding the report form.		
Start filling the "Report form" worksheet		
2.2. Except for the sample numbers, data entry on the form is limited to pick lists.		
2.3. <u>Laboratory identification</u> The first data to enter is your unique laboratory code (cell B5). <i>This code is to find in the sample shipment</i> . 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 11 (use the scroll-bar on the right if needed to visualise other values), click on the correct value (this closes the pick-list). The chosen lab code appears upon every column of the different samples analysis (cells D9 to AJ9). The second data to enter is the agreement on responsibility (cell B6). 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. <i>Please note that your data will never be deleted by doing so.</i>		
2.4. <u>Report</u> Data related to one sample are organised in columns. Each column must contain the sample number "Sample N" (cells D11 to AJ11). This number is an entire referring to the one indicated on the vial containing the DNA to analyse. <i>This data is mandatory. Please fill the column according the increasing order of the sample numbers.</i> Please indicate your results according to the targets you have tested. If there are the species for which you don't have a target, please indicate "Not tested". ! More than 1 animal species can be present in the samples simultaneously.		
"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. <i>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.</i>		
3. Sending of the results The deadline for sending the results is 2 weeks (10 working days) after reception of the samples . 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@crl.cra.wallonie.be with as mail subject: EURL-AP PCR ILS RESULTS 2011 The Report summary has to be sent by fax to +32(0)81 62 03 88		

Annex VI: sheet for the recording of the results sent to the participants

Interlaboratory Study 2011 

Evaluation of NRLs PCR methods

Laboratory identification

Laboratory code:

Responsibility agreement:

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

Report

Lab code	Lab code?																
Sample rank	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th
Sample N°	<input type="text"/>																
PCR target tested																	
Animal	<input type="text"/>																
Cattle	<input type="text"/>																
Sheep	<input type="text"/>																
Goat	<input type="text"/>																
Ruminant	<input type="text"/>																
Pig	<input type="text"/>																
Chicken	<input type="text"/>																
Turkey	<input type="text"/>																
Goose	<input type="text"/>																
Duck	<input type="text"/>																
Poultry	<input type="text"/>																
Avian	<input type="text"/>																
Horse	<input type="text"/>																
Rabbit	<input type="text"/>																
Fish	<input type="text"/>																

Annex V: automatically generated sheet generated for the reporting

**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 e-mail**



Report summary

PCR interlaboratory study 2011

Laboratory identification code Lab code?

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Remark(s):

Date:

Name:

Signature:

First name:

Annex VI: List of material from which the DNA extracts originate

Description	
1	Blank (soyabean free from PAP)
2	Blank + 0.1 % in weight of cattle MBM
3	Blank + 0.1 % in weight of sheep MBM
4	Blank + 0.1 % in weight of pig MBM
5	Blank + 0.1 % in weight of chicken MBM
6	Blank + 0.1 % in weight of fishmeal
7	Blank + 0.1 % in weight of cattle MBM + 0.5 % in weight of fishmeal
8	Blank + 0.1 % in weight of sheep MBM + 0.5 % in weight of fishmeal
9	Blank + 0.1 % in weight of pig MBM + 0.5 % in weight of fishmeal
10	Blank + 0.1 % in weight of chicken MBM + 0.5 % in weight of fishmeal
11	Blank + 0.1 % in weight of cattle MBM + 0.5 % in weight of pig MBM
12	Blank + 0.1 % in weight of sheep MBM + 0.5 % in weight of pig MBM
13	Blank + 0.1 % in weight of pig MBM + 0.5 % in weight of chicken MBM
14	Blank + 0.1 % in weight of chicken MBM + 0.5 % in weight of chicken MBM
15	Blank + 0.1 % in weight of pig MBM + 0.5 % in weight of chicken MBM
16	Blank + 0.1 % in weight of pig MBM + 0.5 % in weight of chicken MBM
17	Fresh turkey meat

Annex VII: Results of the participants

Legend:

-  Correct result
-  False result
-  No conclusion on the result
-  Coding error

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 e-mail



Report summary

PCR interlaboratory study 2011

Laboratory identification code 1

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish		
1	1	Positive	Not tested	Negative	Not tested	Negative	Negative	Positive	Positive	Not tested	Negative						
2	12	Positive	Not tested	Positive	Not tested	Positive	Negative	Negative	Negative	Not tested	Positive						
3	19	Positive	Not tested	Negative	Not tested	Negative	Negative	Negative	Negative	Not tested	Negative						
4	30	Positive	Not tested	Negative	Not tested	Positive	Negative	Negative	Negative	Not tested	Negative						
5	37	Positive	Not tested	Negative	Not tested	Positive	Negative	Negative	Negative	Not tested	Positive						
6	48	Positive	Not tested	Positive	Not tested	Positive	Positive	Negative	Negative	Not tested	Negative						
7	55	Positive	Not tested	Negative	Not tested	Positive	Positive	Negative	Negative	Not tested	Negative						
8	66	Positive	Not tested	Negative	Not tested	Negative	Negative	Negative	Negative	Not tested	Negative	Not tested	Positive				
9	73	Positive	Not tested	Positive	Not tested	Positive	Negative	Positive	Positive	Not tested	Negative						
10	84	Positive	Not tested	Negative	Not tested	Negative	Positive	Negative	Negative	Not tested	Negative	Positive					
11	91	Positive	Not tested	Negative	Not tested	Negative	Positive	Negative	Negative	Not tested	Negative	Negative					
12	102	Positive	Not tested	Negative	Not tested	Negative	Positive	Positive	Positive	Not tested	Negative						
13	109	Positive	Not tested	Negative	Negative	Negative	Negative	Positive	Positive	Not tested	Positive						
14	127	Positive	Not tested	Negative	Not tested	Negative	Negative	Negative	Positive	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Negative
15	145	Positive	Not tested	Positive	Not tested	Positive	Negative	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Negative
16	163	Positive	Not tested	Negative	Not tested	Negative	Positive	Positive	Positive	Not tested	Negative	Negative					
17	181	Positive	Not tested	Negative	Not tested	Positive	Negative	Positive	Positive	Not tested	Negative						

Remark(s): Animal PCR assay is based on 18S eukariotic rRNA gene, common region for mammals, reptiles, birds, amphibians, arthropods, shelfish, greenplants and fungi

Date: 25/02/2011

Name:

Signature:

First name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 2

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish
1	2	Negative	Negative	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
2	13	Negative	Positive	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
3	20	Negative	Positive	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
4	31	Positive	Negative	Negative	Negative	Not tested	Positive	Negative	Negative	Not tested					
5	38	Positive	Positive	Negative	Negative	Not tested	Positive	Negative	Negative	Not tested					
6	49	Negative	Positive	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
7	56	Positive	Negative	Negative	Negative	Not tested	Negative	Positive	Negative	Not tested					
8	67	Positive	Negative	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
9	74	Negative	Negative	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
10	85	Positive	Positive	Negative	Negative	Not tested	Positive	Positive	Negative	Not tested					
11	92	Positive	Negative	Negative	Negative	Not tested	Negative	Positive	Negative	Not tested					
12	110	Positive	Negative	Negative	Negative	Not tested	Negative	Negative	Positive	Not tested					
13	128	Negative	Negative	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
14	146	Positive	Negative	Negative	Negative	Not tested	Negative	Positive	Negative	Not tested					
15	164	Positive	Positive	Negative	Negative	Not tested	Negative	Positive	Positive	Not tested					
16	171	Positive	Negative	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					
17	182	Negative	Negative	Negative	Negative	Not tested	Negative	Negative	Negative	Not tested					

Remark(s):

Date: 29/03/2011

Name:

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First name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 3

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish	
1	10	Not tested	Negative	Negative	Not tested	Not tested	Negative	Positive	Negative	Negative	Negative	Not tested				
2	28	Not tested	Negative	Negative	Not tested	Not tested	Negative	Positive	Negative	Negative	Negative	Not tested				
3	35	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
4	46	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
5	53	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
6	64	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
7	71	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
8	82	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Not tested					
9	89	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
10	100	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
11	107	Not tested	Negative	Negative	Not tested	Not tested	Negative	Positive	Negative	Negative	Negative	Not tested				
12	118	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
13	125	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
14	136	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
15	143	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				
16	161	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Positive	Negative	Negative	Not tested				
17	179	Not tested	Negative	Negative	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative	Not tested				

Remark(s): Result of sample 82 (duck assay) does not correspond with the entered result in the report form ; it should be "negative" insted of "not tested"

Date: 01/03/2011

Name:

Signature:

First name:

Laboratory identification code 4

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish	
1	9	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
2	27	Not tested	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
3	45	Not tested	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Negative
4	52	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
5	63	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
6	70	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
7	81	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Negative
8	88	Not tested	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Not tested	Not tested	Not tested
9	99	Not tested	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
10	106	Not tested	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
11	117	Not tested	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
12	124	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
13	135	Not tested	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
14	142	Not tested	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
15	153	Not tested	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
16	160	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
17	178	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested

Remark(s): Result of sample 88 is "not tested" for duc. We can't change this cell (R31) in Report form. Our sample set was defrosted.

Date: 08/03/2011

Name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 5

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish	
1	8	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Positive	Not tested	Not tested	Not tested				
2	26	Negative	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
3	44	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Positive	Not tested	Not tested	Not tested				
4	62	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Positive	Not tested	Not tested	Not tested				
5	69	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Positive	Not tested	Not tested	Not tested				
6	80	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
7	87	Negative	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
8	98	Negative	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
9	105	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
10	116	Negative	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
11	123	Negative	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
12	134	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
13	141	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Positive	Not tested	Not tested	Not tested				
14	152	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
15	159	Negative	Negative	Negative	Not tested	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested				
16	170	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Positive	Not tested	Not tested	Not tested				
17	177	Positive	Negative	Negative	Not tested	Not tested	Negative	Not tested	Positive	Not tested	Not tested	Not tested				

Remark(s):

Date: 08/03/2011

Name:

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First name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 8

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish
1	5	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
2	16	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
3	23	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
4	34	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
5	41	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
6	59	Positive	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested						
7	77	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
8	95	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
9	113	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
10	120	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
11	131	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
12	138	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
13	149	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
14	156	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
15	167	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
16	174	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						
17	185	Negative	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested						

Remark(s):

Date: 07/03/2011

Name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 9

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish
1	4	Not tested	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
2	15	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested						
3	22	Not tested	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
4	33	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested						
5	40	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested						
6	51	Not tested	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
7	58	Not tested	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
8	76	Not tested	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
9	94	Not tested	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
10	112	Not tested	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
11	130	Not tested	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
12	137	Not tested	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
13	148	Not tested	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
14	155	Not tested	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested						
15	166	Not tested	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
16	173	Not tested	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
17	184	Not tested	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Not tested	Negative	Not tested	Not tested	Not tested	Not tested

Remark(s):

Date: 03/03/2011

Name:

Signature:

First name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 10

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish
1	3	Not tested	Positive	Not tested	Not tested	Positive	Negative	Negative	Not tested						
2	14	Not tested	Negative	Not tested	Not tested	Positive	Positive	Negative	Not tested						
3	21	Not tested	Positive	Not tested	Not tested	Negative	Positive	Negative	Not tested						
4	32	Not tested	Negative	Not tested	Not tested	Positive	Negative	Negative	Not tested						
5	39	Not tested	Negative	Not tested	Not tested	Positive	Negative	Positive	Not tested						
6	50	Not tested	Negative	Not tested	Not tested	Negative	Positive	Negative	Not tested						
7	57	Not tested	Negative	Not tested	Not tested	Negative	Negative	Negative	Not tested						
8	68	Not tested	Negative	Not tested	Not tested	Negative	Positive	Positive	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
9	75	Not tested	Negative	Not tested	Not tested	Negative	Negative	Positive	Not tested						
10	93	Not tested	Negative	Not tested	Not tested	Negative	Negative	Positive	Not tested						
11	111	Not tested	Negative	Not tested	Not tested	Positive	Negative	Negative	Not tested						
12	129	Not tested	Negative	Not tested	Not tested	Negative	Positive	Positive	Not tested						
13	147	Not tested	Positive	Not tested	Not tested	Positive	Negative	Positive	Not tested						
14	154	Not tested	Negative	Not tested	Not tested	Negative	Negative	Positive	Not tested						
15	165	Not tested	Negative	Not tested	Not tested	Positive	Negative	Negative	Not tested						
16	172	Not tested	Negative	Not tested	Not tested	Negative	Negative	Negative	Not tested						
17	183	Not tested	Positive	Not tested	Not tested	Positive	Negative	Negative	Not tested						

Remark(s): Examinations with own methods

Date: 08/03/2011

Name:

Signature:

First name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 10

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish	
1	3	Not tested	Negative	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
2	14	Not tested	Positive	Positive	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
3	21	Not tested	Negative	Negative	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
4	32	Not tested	Positive	Positive	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
5	39	Not tested	Negative	Positive	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
6	50	Not tested	Negative	Negative	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
7	57	Not tested	Negative	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
8	68	Not tested	Negative	Negative	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
9	75	Not tested	Negative	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
10	93	Not tested	Positive	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
11	111	Not tested	Negative	Positive	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
12	129	Not tested	Negative	Negative	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
13	147	Not tested	Negative	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
14	154	Not tested	Negative	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Positive	Not tested	Not tested	Not tested	Not tested
15	165	Not tested	Negative	Positive	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
16	172	Not tested	Negative	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested
17	183	Not tested	Positive	Negative	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested	Negative	Not tested	Not tested	Not tested	Not tested

Remark(s): Examinations with kit

Date: 08/03/2011

Name:

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First name:

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Report summary

PCR interlaboratory study 2011

Laboratory identification code 11

Sample N°	Animal	Cattle	Sheep	Goat	Ruminant	Pig	Chicken	Turkey	Goose	Duck	Poultry	Avian	Horse	Rabbit	Fish
1	11	Not tested	Positive	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Negative					
2	18	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Negative					
3	29	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Positive					
4	36	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Negative					
5	47	Not tested	Positive	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Negative					
6	54	Not tested	Positive	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Positive					
7	65	Not tested	Negative	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Negative					
8	72	Not tested	Positive	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Negative					
9	83	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Positive					
10	90	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Negative					
11	101	Not tested	Negative	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Positive					
12	108	Not tested	Negative	Not tested	Not tested	Not tested	Positive	Negative	Not tested	Negative					
13	119	Not tested	Negative	Not tested	Not tested	Not tested	Positive	Positive	Not tested	Negative					
14	126	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Positive					
15	144	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Positive					
16	162	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Negative	Not tested	Negative					
17	180	Not tested	Negative	Not tested	Not tested	Not tested	Negative	Positive	Not tested	Negative					

Remark(s):

Date: 11/03/2011

Name:

Signature:

First name: