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172-PT

## **PROFICIENCY TESTING 2013**

***AUJESZKY'S DISEASE VIRUS (ADV)***

***Detection of ADV gB- and gE-specific antibodies in serum by  
Enzyme Linked Immunosorbent Assay (ELISA)***

**OPERATIONAL UNIT  
COORDINATION OF VETERINARY DIAGNOSIS  
EPIDEMIOLOGY AND RISK ASSESSMENT  
(CVD-ERA)**

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## I. Introduction

Details relevant to the proficiency test (PT) are available in the Procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle', which is summarized in the 'Manual for the participant'.

## II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of ADV gB- and/or ADV gE-specific antibodies in porcine serum by ELISA.

## III. Materials and methods

### III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be analyzed by means of an ELISA test. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

### III.2. Reference samples

#### III.2.1. ADV gB reference samples

Replicates of 6 reference serum samples of porcine origin, either free from detectable ADV gB-specific antibodies ( $n = 2$ ; coded 'PT2013AUJgBSERNS1' and 'PT2013AUJgBSERNS2') or containing detectable ADV gB-specific antibodies ( $n = 4$ ; coded 'PT2013AUJgBSERPS1', 'PT2013AUJgBSERPS2', 'PT2013AUJgBSERPS3' and 'PT2013AUJgBSERPS4'), were used. In total, 160 aliquots were distributed to 8 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2013AUJgBSERNS2, PT2013AUJgBSERPS1, PT2013AUJgBSERPS2 and PT2013AUJgBSERPS3, and 4 aliquots of the reference serum samples PT2013AUJgBSERNS1 and PT2013AUJgBSERPS4. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 4).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification using the PrioCheck PRV gB antibody ELISA test from Prionics and a seroneutralisation assay (SN). The reference serum samples PT2013AUJgBSERNS1 and PT2013AUJgBSERNS2 were obtained from both uninfected and non-vaccinated animals. The reference serum samples PT2013AUJgBSERPS1 and PT2013AUJgBSERPS2 were obtained from 2 uninfected animals that were vaccinated twice with the live attenuated marker vaccine Suvaxyn Aujeszky 783+0/W (ADV attenuated NIA3-783 strain in mineral oil adjuvant). Hereby, reference serum sample PT2013AUJgBSERPS2 was a 1/16 dilution of the original serum. The reference serum sample PT2013AUJgBSERPS3 was a 1/4 dilution of a serum obtained from a naturally ADV-infected animal, whereas the reference serum sample PT2013AUJgBSERPS4 was a 1/64 dilution of a serum obtained from an experimentally ADV-infected animal (with an ADV strain isolated from a wolf in 2011 in Belgium). For each reference serum sample, the same qualitative result was obtained with both test methods used. Taken together, the reference serum samples PT2013AUJgBSERNS1 and PT2013AUJgBSERNS2 were considered as negative sera, and the reference serum samples PT2013AUJgBSERPS1, PT2013AUJgBSERPS2, PT2013AUJgBSERPS3 and PT2013AUJgBSERPS4 as variably positive sera in SN but (strong) positive sera in ADV gB ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the PrioCheck PRV gB antibody ELISA test from Prionics, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of ADV gB-specific antibodies in porcine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the PrioCheck PRV gB antibody ELISA test from Prionics.

**Remark:** For the PT ADV gB, an additional panel consisting of 9 serum samples with variable qualitative results in ELISA but negative in SN, the reference test (golden standard) for the detection of ADV gB-specific antibodies in serum, was sent to the 8 participating laboratories along with the regular ADV gB PT samples. Since this was out of scope of this PT according to ISO 17043, a description of these additional serum samples as well as the qualitative and quantitative data analysis is summarized in Annex 3.

### III.2.2. ADV gE reference samples

Replicates of 6 reference serum samples of porcine origin, either free from detectable ADV gE-specific antibodies ( $n = 3$ ; coded 'PT2013AUJgESERNS1', 'PT2013AUJgESERNS2' and 'PT2013AUJgESERNS3') or containing detectable ADV gE-specific antibodies ( $n = 3$ ; coded 'PT2013AUJgESERPS1', 'PT2013AUJgESERPS2' and 'PT2013AUJgESERPS3'), were used. In total, 180 aliquots were distributed to 9 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2013AUJgESERNS1, PT2013AUJgESERNS2, PT2013AUJgESERNS3 and PT2013AUJgESERPS1, and 4 aliquots of the reference serum samples PT2013AUJgESERPS2 and PT2013AUJgESERPS3. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 5).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained during pre-verification using the HerdChek PRV gpl antibody ELISA test from IDEXX. The reference serum samples PT2013AUJgESERNS1 (=PT2013AUJgBSERNS1) and PT2013AUJgESERNS2 (=PT2013AUJgBSERNS2) were obtained from both uninfected and non-vaccinated animals, whereas the reference serum sample PT2013AUJgESERNS3 (=PT2013AUJgBSERPS1) was obtained from an uninfected animal that was vaccinated twice with the live attenuated marker vaccine Suvaxyn Aujeszky 783+0/W (ADV attenuated NIA3-783 strain in mineral oil adjuvant). The reference serum sample PT2013AUJgESERPS1 was a 1/8 dilution of a serum obtained from a naturally ADV-infected animal, whereas the reference serum samples PT2013AUJgESERPS2 and PT2013AUJgESERPS3 were a 1/2 and a 1/16 dilution of a serum obtained from an experimentally ADV-infected animal (with an ADV strain isolated from a wolf in 2011 in Belgium). Taken together, the reference serum samples PT2013AUJgESERNS1, PT2013AUJgESERNS2 and PT2013AUJgESERNS3 were considered as negative sera, whereas the reference serum samples PT2013AUJgESERPS1, PT2013AUJgESERPS2 and PT2013AUJgESERPS3 as variably positive sera in ADV gE ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the HerdChek PRV gpl antibody ELISA test from IDEXX, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of ADV gE-specific antibodies in porcine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the HerdChek PRV gpl antibody ELISA test from IDEXX.

## III.3. Classification of results, level of agreement and threshold for qualification

### III.3.1. Classification of results

Results provided by the participating laboratories are categorized as *success* when the reported result matches with the assigned status or *failure* when the reported result does not match with the assigned status.

### III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 20 aliquots of reference samples used for either the PT ADV gB or the PT ADV gE.

### III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 aliquots of reference samples is at least 95% for the PT ADV gB and 90% for the PT ADV gE.

## IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the operational unit CVD-ERA of CODA-CERVA.

### IV.1. Transfer and start of the analyses of the reference samples

LAB1 until LAB8 participated in both the PT ADV gB and the PT ADV gE and hence received 40 aliquots of reference serum samples (20 for the PT ADV gB and 20 for the PT ADV gE). In contrast, LAB9 only participated in the PT ADV gE and thus received 20 aliquots of reference serum samples. The reference serum samples were sent frozen (dry ice) to each of the participating laboratories by national or international courier on 26<sup>th</sup> of August 2013 (340 aliquots in total). LAB6, LAB7, LAB8 and LAB9 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 27<sup>th</sup> (LAB2 and LAB5) or 28<sup>th</sup> (LAB1, LAB3 and LAB4) of August 2013. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between 27<sup>th</sup> of August and 13<sup>th</sup> of September 2013. LAB5 did not communicate the date of analysis for the PT ADV gB for 1 out of the 2 ELISA kits used (Table 1).

### IV.2. Dates at which results were returned to the operational unit CVD-ERA

Results from the participating laboratories were submitted to the operational unit CVD-ERA between 28<sup>th</sup> of August and 13<sup>th</sup> of September 2013. All participants hereby respected the deadline of 13<sup>th</sup> of September 2013 for submission of the results (Table 1).

**Table 1.** Overview of the dates on which (i) the reference serum samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the operational unit CVD-ERA of CODA-CERVA.

| Laboratory | Reference samples received | Start of analysis gB                  | Start of analysis gE | Submission of the results (Excel file) |
|------------|----------------------------|---------------------------------------|----------------------|--|
| LAB1       | 28/08/2013                 | 28/08/2013                            | 28/08/2013           | 03/09/2013                             |
| LAB2       | 27/08/2013                 | 28/08/2013                            | 28/08/2013           | 13/09/2013 ( )                         |
| LAB3       | 28/08/2013                 | 05/09/2013                            | 05/09/2013           | 09/09/2013                             |
| LAB4       | 28/08/2013                 | 11/09/2013                            | 13/09/2013           | 13/09/2013                             |
| LAB5       | 27/08/2013                 | 04/09/2013 & <b>NOT MENTIONED</b> (#) | 05/09/2013           | 09/09/2013                             |
| LAB6       | 26/08/2013                 | 30/08/2013                            | 28/08/2013           | 02/09/2013                             |
| LAB7       | 26/08/2013                 | 02/09/2013                            | 28/08/2013           | 03/09/2013                             |
| LAB8       | 26/08/2013                 | 27/08/2013                            | 27/08/2013           | 28/08/2013 (*)                         |
| LAB9       | 26/08/2013                 | NA                                    | 27/08/2013           | 28/08/2013                             |

**Legend:** NA = not applicable; ( ) = LAB2 was asked on 21/10/2013 to resubmit their results because the reporting of the qualitative results was not in compliance with the instructions of the PT provider (a corrected version was received on 24/10/2013); (#) = this laboratory tested ELISA kits from different producers; (\*) = LAB8 was asked on 27/01/2014 to resubmit their results because of a transcription error for sample 18 in the PT ADV gB: the qualitative result did not correspond with the quantitative result (a corrected version was received on 27/01/2014)

### IV.3. Compliance with the procedure

Except LAB2 and LAB5, all participating laboratories have provided a duly dated and signed copy of the results.

#### IV.4. Qualitative data analysis

LAB5 analysed the 20 aliquots of reference serum samples for the PT ADV gB using ELISA kits from 2 different producers and hence submitted 2 sets of results. In order to analyse the provided data, this participating laboratory has been divided into different sublaboratories, namely LAB5.1 (kit 1) and LAB5.2 (kit 2).

##### IV.4.1. Level of agreement

Qualitative data analysis showed that:

- (i) For the detection of **ADV gB-specific antibodies**, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence obtained 100% of agreement. Hereby, LAB5 used 2 different ELISA kits (Table 2).
- (ii) For the detection of **ADV gE-specific antibodies**, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence obtained 100% of agreement (Table 3).

**Table 2.** Agreement between results obtained by the participating laboratories (LABNR) and the status of the **ADV gB** reference serum samples assigned by CODA-CERVA. All participating laboratories received 20 aliquots of ADV gB reference serum samples. Results are presented as absolute values and percentages (in parentheses).

|                | LABNR      |            |            |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
|                | 1          | 2          | 3          | 4          | 5.1        | 5.2        | 6          | 7          | 8          |
| <b>failure</b> | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    |
| <b>success</b> | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) |

**Table 3.** Agreement between results obtained by the participating laboratories (LABNR) and the status of the **ADV gE** reference serum samples assigned by CODA-CERVA. All participating laboratories received 20 aliquots of ADV gE reference serum samples. Results are presented as absolute values and percentages (in parentheses).

|                | LABNR      |            |            |            |            |            |            |            |            |
|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
|                | 1          | 2          | 3          | 4          | 5          | 6          | 7          | 8          | 9          |
| <b>failure</b> | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    | 0 (0.0)    |
| <b>success</b> | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) | 20 (100.0) |

A quantitative data analysis (including box plots) is shown for educational purposes in Annex 1 and Annex 2.

##### IV.4.2. Variability among participating laboratories

Since all participating laboratories reached 100% of agreement for the detection of both ADV gB- and ADV gE-specific antibodies in reference serum samples, no variability between qualitative laboratory results could be observed.

For each participating laboratory, the obtained results and the assigned statuses for the reference serum samples are shown in Table 4 for the PT ADV gB and in Table 5 for the PT ADV gE.

**Table 4.** The responses (RESULT) of the participating laboratories (LABNR) with the identification of the **ADV gB** reference serum samples (SAMPLE), the positions of the ADV gB reference serum samples as placed in the block (LABPOSIT), and the status assigned by CODA-CERVA (STATUS). NEG: negative; POS: positive.

|    | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|----|-------|----------|-------------------|--------|--------|---------|
| 1  | 1     | 1        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 2  | 1     | 2        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 3  | 1     | 3        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 4  | 1     | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 5  | 1     | 5        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 6  | 1     | 6        | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 7  | 1     | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 8  | 1     | 8        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 9  | 1     | 9        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 10 | 1     | 10       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 11 | 1     | 11       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 12 | 1     | 12       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 13 | 1     | 13       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 14 | 1     | 14       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 15 | 1     | 15       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 16 | 1     | 16       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 17 | 1     | 17       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 18 | 1     | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 19 | 1     | 19       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 20 | 1     | 20       | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 21 | 2     | 1        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 22 | 2     | 2        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 23 | 2     | 3        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 24 | 2     | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 25 | 2     | 5        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 26 | 2     | 6        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 27 | 2     | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 28 | 2     | 8        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 29 | 2     | 9        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 30 | 2     | 10       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 31 | 2     | 11       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 32 | 2     | 12       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 33 | 2     | 13       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 34 | 2     | 14       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 35 | 2     | 15       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 36 | 2     | 16       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 37 | 2     | 17       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 38 | 2     | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 39 | 2     | 19       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 40 | 2     | 20       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |



(Table 4 - CONTINUED)

|    | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|----|-------|----------|-------------------|--------|--------|---------|
| 41 | 3     | 1        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 42 | 3     | 2        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 43 | 3     | 3        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 44 | 3     | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 45 | 3     | 5        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 46 | 3     | 6        | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 47 | 3     | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 48 | 3     | 8        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 49 | 3     | 9        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 50 | 3     | 10       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 51 | 3     | 11       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 52 | 3     | 12       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 53 | 3     | 13       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 54 | 3     | 14       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 55 | 3     | 15       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 56 | 3     | 16       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 57 | 3     | 17       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 58 | 3     | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 59 | 3     | 19       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 60 | 3     | 20       | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 61 | 4     | 1        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 62 | 4     | 2        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 63 | 4     | 3        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 64 | 4     | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 65 | 4     | 5        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 66 | 4     | 6        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 67 | 4     | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 68 | 4     | 8        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 69 | 4     | 9        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 70 | 4     | 10       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 71 | 4     | 11       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 72 | 4     | 12       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 73 | 4     | 13       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 74 | 4     | 14       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 75 | 4     | 15       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 76 | 4     | 16       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 77 | 4     | 17       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 78 | 4     | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 79 | 4     | 19       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 80 | 4     | 20       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |



(Table 4 - CONTINUED)

|     | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|-----|-------|----------|-------------------|--------|--------|---------|
| 81  | 5.1   | 1        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 82  | 5.1   | 2        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 83  | 5.1   | 3        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 84  | 5.1   | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 85  | 5.1   | 5        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 86  | 5.1   | 6        | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 87  | 5.1   | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 88  | 5.1   | 8        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 89  | 5.1   | 9        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 90  | 5.1   | 10       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 91  | 5.1   | 11       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 92  | 5.1   | 12       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 93  | 5.1   | 13       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 94  | 5.1   | 14       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 95  | 5.1   | 15       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 96  | 5.1   | 16       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 97  | 5.1   | 17       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 98  | 5.1   | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 99  | 5.1   | 19       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 100 | 5.1   | 20       | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 101 | 5.2   | 1        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 102 | 5.2   | 2        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 103 | 5.2   | 3        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 104 | 5.2   | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 105 | 5.2   | 5        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 106 | 5.2   | 6        | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 107 | 5.2   | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 108 | 5.2   | 8        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 109 | 5.2   | 9        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 110 | 5.2   | 10       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 111 | 5.2   | 11       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 112 | 5.2   | 12       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 113 | 5.2   | 13       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 114 | 5.2   | 14       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 115 | 5.2   | 15       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 116 | 5.2   | 16       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 117 | 5.2   | 17       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 118 | 5.2   | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 119 | 5.2   | 19       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 120 | 5.2   | 20       | PT2013AUJgBSERPS1 | POS    | POS    | 1       |





(Table 4 - CONTINUED)

|     | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|-----|-------|----------|-------------------|--------|--------|---------|
| 121 | 6     | 1        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 122 | 6     | 2        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 123 | 6     | 3        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 124 | 6     | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 125 | 6     | 5        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 126 | 6     | 6        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 127 | 6     | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 128 | 6     | 8        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 129 | 6     | 9        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 130 | 6     | 10       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 131 | 6     | 11       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 132 | 6     | 12       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 133 | 6     | 13       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 134 | 6     | 14       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 135 | 6     | 15       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 136 | 6     | 16       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 137 | 6     | 17       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 138 | 6     | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 139 | 6     | 19       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 140 | 6     | 20       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 141 | 7     | 1        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 142 | 7     | 2        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 143 | 7     | 3        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 144 | 7     | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 145 | 7     | 5        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 146 | 7     | 6        | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 147 | 7     | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 148 | 7     | 8        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 149 | 7     | 9        | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 150 | 7     | 10       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 151 | 7     | 11       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 152 | 7     | 12       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 153 | 7     | 13       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 154 | 7     | 14       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 155 | 7     | 15       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 156 | 7     | 16       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 157 | 7     | 17       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 158 | 7     | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 159 | 7     | 19       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 160 | 7     | 20       | PT2013AUJgBSERPS1 | POS    | POS    | 1       |



(Table 4 - CONTINUED)

|     | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|-----|-------|----------|-------------------|--------|--------|---------|
| 161 | 8     | 1        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 162 | 8     | 2        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 163 | 8     | 3        | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 164 | 8     | 4        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 165 | 8     | 5        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 166 | 8     | 6        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 167 | 8     | 7        | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 168 | 8     | 8        | PT2013AUJgBSERPS1 | POS    | POS    | 1       |
| 169 | 8     | 9        | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 170 | 8     | 10       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 171 | 8     | 11       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 172 | 8     | 12       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 173 | 8     | 13       | PT2013AUJgBSERNS1 | NEG    | NEG    | 1       |
| 174 | 8     | 14       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 175 | 8     | 15       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 176 | 8     | 16       | PT2013AUJgBSERPS2 | POS    | POS    | 1       |
| 177 | 8     | 17       | PT2013AUJgBSERNS2 | NEG    | NEG    | 1       |
| 178 | 8     | 18       | PT2013AUJgBSERPS4 | POS    | POS    | 1       |
| 179 | 8     | 19       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |
| 180 | 8     | 20       | PT2013AUJgBSERPS3 | POS    | POS    | 1       |



**Table 5.** The responses (RESULT) of the participating laboratories (LABNR) with the identification of the **ADV gE** reference serum samples (SAMPLE), the positions of the ADV gE reference serum samples as placed in the block (LABPOSIT), and the status assigned by CODA-CERVA (STATUS). NEG: negative; POS: positive.

|    | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|----|-------|----------|-------------------|--------|--------|---------|
| 1  | 1     | 1        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 2  | 1     | 2        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 3  | 1     | 3        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 4  | 1     | 4        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 5  | 1     | 5        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 6  | 1     | 6        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 7  | 1     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 8  | 1     | 8        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 9  | 1     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 10 | 1     | 10       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 11 | 1     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 12 | 1     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 13 | 1     | 13       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 14 | 1     | 14       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 15 | 1     | 15       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 16 | 1     | 16       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 17 | 1     | 17       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 18 | 1     | 18       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 19 | 1     | 19       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 20 | 1     | 20       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 21 | 2     | 1        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 22 | 2     | 2        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 23 | 2     | 3        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 24 | 2     | 4        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 25 | 2     | 5        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 26 | 2     | 6        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 27 | 2     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 28 | 2     | 8        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 29 | 2     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 30 | 2     | 10       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 31 | 2     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 32 | 2     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 33 | 2     | 13       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 34 | 2     | 14       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 35 | 2     | 15       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 36 | 2     | 16       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 37 | 2     | 17       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 38 | 2     | 18       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 39 | 2     | 19       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 40 | 2     | 20       | PT2013AUJgESERPS1 | POS    | POS    | 1       |



(Table 5 - CONTINUED)

|    | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|----|-------|----------|-------------------|--------|--------|---------|
| 41 | 3     | 1        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 42 | 3     | 2        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 43 | 3     | 3        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 44 | 3     | 4        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 45 | 3     | 5        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 46 | 3     | 6        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 47 | 3     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 48 | 3     | 8        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 49 | 3     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 50 | 3     | 10       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 51 | 3     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 52 | 3     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 53 | 3     | 13       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 54 | 3     | 14       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 55 | 3     | 15       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 56 | 3     | 16       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 57 | 3     | 17       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 58 | 3     | 18       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 59 | 3     | 19       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 60 | 3     | 20       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 61 | 4     | 1        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 62 | 4     | 2        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 63 | 4     | 3        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 64 | 4     | 4        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 65 | 4     | 5        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 66 | 4     | 6        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 67 | 4     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 68 | 4     | 8        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 69 | 4     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 70 | 4     | 10       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 71 | 4     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 72 | 4     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 73 | 4     | 13       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 74 | 4     | 14       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 75 | 4     | 15       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 76 | 4     | 16       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 77 | 4     | 17       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 78 | 4     | 18       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 79 | 4     | 19       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 80 | 4     | 20       | PT2013AUJgESERPS1 | POS    | POS    | 1       |



(Table 5 - CONTINUED)

|     | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|-----|-------|----------|-------------------|--------|--------|---------|
| 81  | 5     | 1        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 82  | 5     | 2        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 83  | 5     | 3        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 84  | 5     | 4        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 85  | 5     | 5        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 86  | 5     | 6        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 87  | 5     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 88  | 5     | 8        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 89  | 5     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 90  | 5     | 10       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 91  | 5     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 92  | 5     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 93  | 5     | 13       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 94  | 5     | 14       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 95  | 5     | 15       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 96  | 5     | 16       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 97  | 5     | 17       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 98  | 5     | 18       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 99  | 5     | 19       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 100 | 5     | 20       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 101 | 6     | 1        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 102 | 6     | 2        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 103 | 6     | 3        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 104 | 6     | 4        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 105 | 6     | 5        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 106 | 6     | 6        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 107 | 6     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 108 | 6     | 8        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 109 | 6     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 110 | 6     | 10       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 111 | 6     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 112 | 6     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 113 | 6     | 13       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 114 | 6     | 14       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 115 | 6     | 15       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 116 | 6     | 16       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 117 | 6     | 17       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 118 | 6     | 18       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 119 | 6     | 19       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 120 | 6     | 20       | PT2013AUJgESERPS1 | POS    | POS    | 1       |



(Table 5 - CONTINUED)

|     | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|-----|-------|----------|-------------------|--------|--------|---------|
| 121 | 7     | 1        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 122 | 7     | 2        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 123 | 7     | 3        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 124 | 7     | 4        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 125 | 7     | 5        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 126 | 7     | 6        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 127 | 7     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 128 | 7     | 8        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 129 | 7     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 130 | 7     | 10       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 131 | 7     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 132 | 7     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 133 | 7     | 13       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 134 | 7     | 14       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 135 | 7     | 15       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 136 | 7     | 16       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 137 | 7     | 17       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 138 | 7     | 18       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 139 | 7     | 19       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 140 | 7     | 20       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 141 | 8     | 1        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 142 | 8     | 2        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 143 | 8     | 3        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 144 | 8     | 4        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 145 | 8     | 5        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 146 | 8     | 6        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 147 | 8     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 148 | 8     | 8        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 149 | 8     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 150 | 8     | 10       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 151 | 8     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 152 | 8     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 153 | 8     | 13       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 154 | 8     | 14       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 155 | 8     | 15       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 156 | 8     | 16       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 157 | 8     | 17       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 158 | 8     | 18       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 159 | 8     | 19       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 160 | 8     | 20       | PT2013AUJgESERPS1 | POS    | POS    | 1       |



(Table 5 - CONTINUED)

|     | LABNR | LABPOSIT | SAMPLE            | STATUS | RESULT | SUCCESS |
|-----|-------|----------|-------------------|--------|--------|---------|
| 161 | 9     | 1        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 162 | 9     | 2        | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 163 | 9     | 3        | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 164 | 9     | 4        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 165 | 9     | 5        | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 166 | 9     | 6        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 167 | 9     | 7        | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 168 | 9     | 8        | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |
| 169 | 9     | 9        | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 170 | 9     | 10       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 171 | 9     | 11       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 172 | 9     | 12       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 173 | 9     | 13       | PT2013AUJgESERNS1 | NEG    | NEG    | 1       |
| 174 | 9     | 14       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 175 | 9     | 15       | PT2013AUJgESERPS1 | POS    | POS    | 1       |
| 176 | 9     | 16       | PT2013AUJgESERPS3 | POS    | POS    | 1       |
| 177 | 9     | 17       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 178 | 9     | 18       | PT2013AUJgESERNS2 | NEG    | NEG    | 1       |
| 179 | 9     | 19       | PT2013AUJgESERPS2 | POS    | POS    | 1       |
| 180 | 9     | 20       | PT2013AUJgESERNS3 | NEG    | NEG    | 1       |

## V. Discussion

The purpose of this PT was to assess performances of the participating laboratories when analyzing reference serum samples of porcine origin for the detection of ADV gB- and/or ADV gE-specific antibodies by ELISA.

For the detection of ADV gB-specific antibodies in reference serum samples, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). Hereby, LAB5 used 2 ELISA kits from different producers (Table 2 and Table 4).

One participating laboratory used an in-house developed ADV gB antibody ELISA kit, whereas the other participants used ADV gB antibody ELISA kits from 3 different commercial kit producers. Hereby, different batches from the same ELISA kit were used: IDEXX (3 batches: KH557, HJ055, BJ055), Prionics (2 batches: Z130301L, Z120701L) and LSI (1 batch: 2-PRVGB-005). LAB1, LAB2 and LAB5.2 on the one hand, and LAB5.1, LAB6, LAB7 and LAB8 on the other hand, used the same ADV gB ELISA kit. Hereby, LAB5.1, LAB6 and LAB8 used the same batch. In addition, LAB1 and LAB2 performed the long incubation protocol, whereas LAB5.2 performed the short incubation protocol of the same ELISA kit (different batches).

For the detection of ADV gE-specific antibodies in reference serum samples, all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 3 and Table 5).

One participating laboratory used an in-house developed ADV gE antibody ELISA kit, whereas the other participants used ADV gE antibody ELISA kits from 2 different commercial kit producers. Hereby, different batches from the same ELISA kit were used: IDEXX (5 batches: KH539, LH074, LH618, LH047, DJ333) and LSI (1 batch: 2-PRVGE-003). LAB1, LAB2, LAB5, LAB6, LAB7, LAB8 and LAB9 used the same ADV gE ELISA kit. Hereby, LAB5 and LAB9 on the one hand, and LAB7 and LAB8 on the other hand, used the same batch. Furthermore, LAB1 performed the long incubation protocol, whereas LAB6, LAB7, LAB8 and LAB9 performed the short incubation protocol (LAB2 and LAB5 did not provide information about the used incubation protocol).

## VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 95% (PT ADV gB) or at least 90% (PT ADV gE) of the results provided by this laboratory is in agreement with the status of the reference serum samples assigned by CODA-CERVA (see III.3.3.). Consequently, all participants to the PT ADV gB achieved a satisfactory performance for the detection of ADV gB-specific antibodies in porcine serum samples with all kits used, and all participants to the PT ADV gE achieved a satisfactory performance for the detection of ADV gE-specific antibodies in porcine serum samples.

Head CVD-ERA  
Yves Van der Stede





## Appendix

### Names of the participating laboratories

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES)

(Ploufragan, France)

Association Régionale de Santé et d'Identification Animales (ARSIA) (Loncin, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

IDEXX Switzerland AG (Bern-Liebefeld, Switzerland)

Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)

Laboratoire Service International (LSI) (Lissieu, France)

State Veterinary Institute Zvolen (Zvolen, Slovakia)

Veterinair Laboratorium Gelderland (VLG) (Epe, The Netherlands)

Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, Belgium)



## Annex 1: Quantitative data analysis

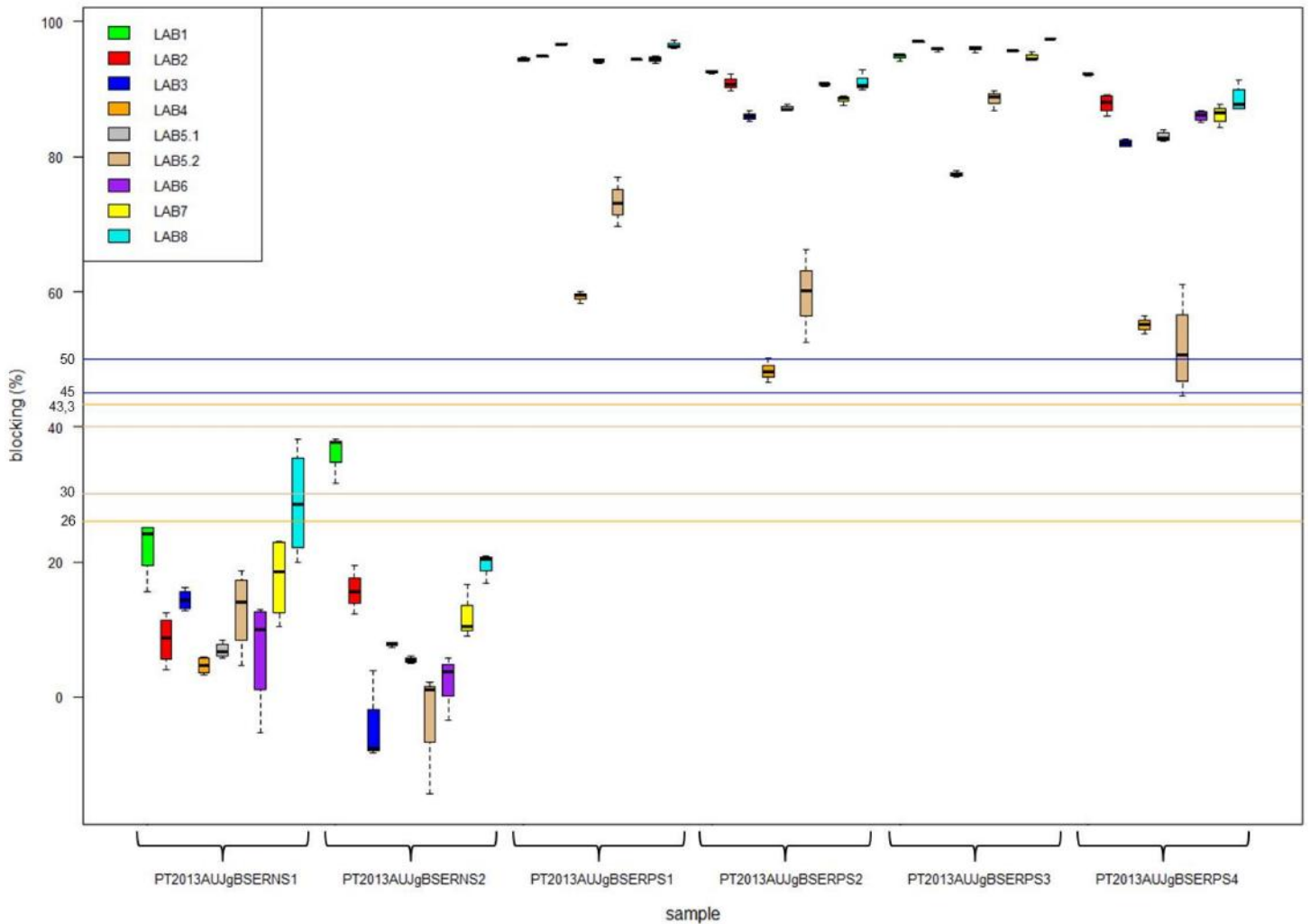
Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software programs R (box plots) and SAS 9.2. (summary statistics). All quantitative data analyses were performed on the normalized data, namely the percentages blocking calculated according to the instructions for this PT:  $[1 - (OD_{\text{Sample}} / \text{mean } OD_{\text{Negative Kit Controls}})] \times 100$ .

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

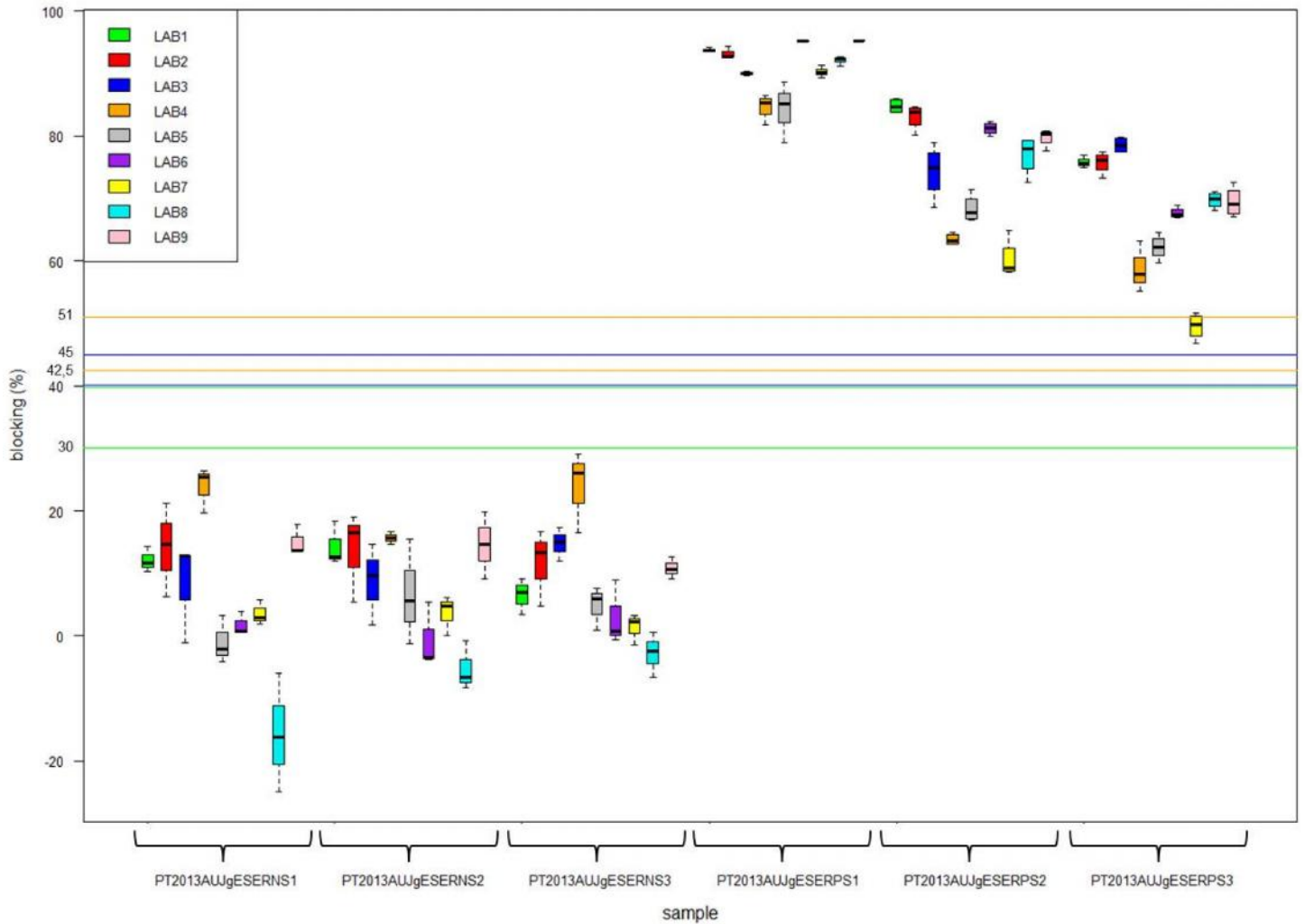
### I. Box plots

Box plots of the percentages blocking per reference serum sample and per participating (sub)laboratory were made using the statistical software R. Box plots for the (sub)laboratories participating in the PT ADV gB and the PT ADV gE are shown in Figure 1 and Figure 2, respectively.

**Remark:** To calculate the percentages blocking, the PT provider used the formula  $[1 - (OD_{\text{Sample}} / \text{mean } OD_{\text{Negative Kit Controls}})] \times 100$  for both the PT ADV gB and the PT ADV gE. Because LAB4 calculated the percentages blocking for both PTs using the formula  $[(\text{mean } OD_{\text{Negative Kit Controls}} - OD_{\text{Sample}}) / (\text{mean } OD_{\text{Negative Kit Controls}} - \text{mean } OD_{\text{Positive Kit Controls}})] \times 100$ , the cut-offs for the ADV gB and ADV gE antibody ELISA kits used by LAB4 were adapted accordingly: 26,0-43,3% instead of 30-50% for the ADV gB antibody ELISA kit and 42,5-51,0% instead of 50-60% for the ADV gE antibody ELISA kit.



**Figure 1. Box plots showing the percentage blocking per ADV gB reference serum sample and per participating (sub)laboratory.** Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. (Adapted) cut-off values applied by the participating laboratories are: 26-43,3% (orange; LAB4), 30-40% (light brown; LAB5.2), 45-50% (blue; LAB3), 40-50% (upper light brown and upper blue line; LAB1, LAB2, LAB5.1) and 50% (upper blue line; LAB6, LAB7, LAB8). LAB1, LAB2 and LAB5.2 on the one hand, and LAB5.1, LAB6, LAB7 and LAB8 on the other hand, used the same ADV gB ELISA kit (LAB5.1 applied alternative cut-off values compared to LAB6, LAB7 and LAB8). Hereby, LAB5.1, LAB6 and LAB8 used the same batch. In addition, LAB1 and LAB2 performed the long incubation protocol, whereas LAB5.2 performed the short incubation protocol of the same ELISA kit (different batches; different cut-off values for short and long incubation protocol).



**Figure 2. Box plots showing the percentage blocking per ADV gE reference serum sample and per participating laboratory.** Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. (Adapted) cut-off values applied by the participating laboratories are shown in green (30-40%; LAB1, LAB2, LAB5, LAB6, LAB7, LAB8 and LAB9), blue (40-45%; LAB3) and orange (42,5-51%; LAB4), respectively. LAB1, LAB2, LAB5, LAB6, LAB7, LAB8 and LAB9 used the same ADV gE ELISA kit. Hereby, LAB5 and LAB9 used the same batch. Furthermore, LAB1 performed the long incubation protocol, whereas LAB6, LAB7, LAB8 and LAB9 performed the short incubation protocol (LAB2 and LAB5 did not provide information about the used incubation protocol).

## II. Mandel's h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-lab variability (reproducibility) and within-lab variability (repeatability) were estimated through Mandel's h- and k-statistics, respectively, using the statistical software SAS 9.2. Mandel's h- and k-statistics were calculated per reference serum sample and per participating laboratory.

The h-statistic depends on the number of participants, whereas the k-statistic depends on both the number of participants and the number of repeats per sample. When 30 participants or more are involved in a PT, a satisfactory between-lab and within-lab consistency is obtained when the (absolute) value for the h- and k-statistic is smaller than 2. An unsatisfactory result (a corrective action is required) is reached when the (absolute) value is larger than 3. (Absolute) values between 2 and 3 indicate a questionable consistency. Importantly, in case of a smaller number of participants (which is the case in this PT), other indicator values apply for Mandel's h- and k-statistics (Table 1).

**Table 1.** Indicators for Mandel's h- and k-statistics at the 5% significance level in function of the number of participating laboratories (p) and the number of repeats per sample (n) as described in ISO 5725-2.

| p (# labs) | h    | k             |      |      |      |      |      |      |      |      |
|------------|------|---------------|------|------|------|------|------|------|------|------|
|            |      | n (# repeats) |      |      |      |      |      |      |      |      |
|            |      | 2             | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |
| 3          | 1,15 | 1,65          | 1,53 | 1,45 | 1,40 | 1,37 | 1,34 | 1,32 | 1,30 | 1,29 |
| 4          | 1,42 | 1,76          | 1,59 | 1,50 | 1,44 | 1,40 | 1,37 | 1,35 | 1,33 | 1,31 |
| 5          | 1,57 | 1,81          | 1,62 | 1,53 | 1,46 | 1,42 | 1,39 | 1,36 | 1,34 | 1,32 |
| 6          | 1,66 | 1,85          | 1,64 | 1,54 | 1,48 | 1,43 | 1,40 | 1,37 | 1,35 | 1,33 |
| 7          | 1,71 | 1,87          | 1,66 | 1,55 | 1,49 | 1,44 | 1,41 | 1,38 | 1,36 | 1,34 |
| 8          | 1,75 | 1,88          | 1,67 | 1,56 | 1,50 | 1,45 | 1,41 | 1,38 | 1,36 | 1,34 |
| 9          | 1,78 | 1,90          | 1,68 | 1,57 | 1,50 | 1,45 | 1,42 | 1,39 | 1,36 | 1,35 |
| 10         | 1,80 | 1,90          | 1,68 | 1,57 | 1,50 | 1,46 | 1,42 | 1,39 | 1,37 | 1,35 |
| 11         | 1,82 | 1,91          | 1,69 | 1,58 | 1,51 | 1,46 | 1,42 | 1,39 | 1,37 | 1,35 |
| 12         | 1,83 | 1,92          | 1,69 | 1,58 | 1,51 | 1,46 | 1,42 | 1,40 | 1,37 | 1,35 |
| 13         | 1,84 | 1,92          | 1,69 | 1,58 | 1,51 | 1,46 | 1,43 | 1,40 | 1,37 | 1,35 |
| 14         | 1,85 | 1,92          | 1,70 | 1,59 | 1,52 | 1,47 | 1,43 | 1,40 | 1,37 | 1,35 |
| 15         | 1,86 | 1,93          | 1,70 | 1,59 | 1,52 | 1,47 | 1,43 | 1,40 | 1,38 | 1,36 |

Based on Table 1, the maximum absolute value for Mandel's h-statistic is 1,78 for the PT ADV gB (p=9) and the PT ADV gE (p=9). For the PT ADV gB, the maximum value for Mandel's k-statistic is 1,68 for the reference serum samples PT2013AUJgBSERNS2, PT2013AUJgBSERPS1, PT2013AUJgBSERPS2 and PT2013AUJgBSERPS3 (p=9 and n=3), and 1,57 for the reference serum samples PT2013AUJgBSERNS1 and PT2013AUJgBSERPS4 (p=9 and n=4). For the PT ADV gE, the maximum value for Mandel's k-statistic is 1,68 for the reference serum samples PT2013AUJgESERNS1, PT2013AUJgESERNS2, PT2013AUJgESERNS3 and PT2013AUJgESERPS1 (p=9 and n=3) and 1,57 for the reference serum samples PT2013AUJgESERPS2 and PT2013AUJgESERPS3 (p=9 and n=4).

For the detection of ADV gB-specific antibodies, 5 out of 9 participating (sub)laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. This was not the case for LAB1, LAB4, LAB5.2 and LAB8, which showed an increased value for Mandel's h-statistic for at least 1 reference serum sample: LAB1 for the negative reference serum sample PT2013AUJgBSERNS2 (h=2,05), LAB4 for the positive reference serum samples PT2013AUJgBSERPS1 (h=-2,23), PT2013AUJgBSERPS2 (h=-2,07) and PT2013AUJgBSERPS3 (h=-2,44), LAB5.2 for the positive reference serum sample PT2013AUJgBSERPS4 (h=-1,84), and LAB8 for the negative reference serum sample PT2013AUJgBSERNS1 (h=1,88). LAB1, LAB2 and LAB5.2 on the one hand, and LAB5.1, LAB6, LAB7 and LAB8 on the other hand, used the same ADV gB ELISA kit. Hereby, LAB5.1, LAB6 and LAB8 used the same batch. In addition, LAB1 and LAB2 performed the long incubation protocol, whereas LAB5.2 performed the short incubation protocol of the same ELISA kit (different batches).

Furthermore, 7 out of 9 participating (sub)laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples. This was not the case for LAB5.2 and LAB6 which showed an increased value for Mandel's k-statistic for at least 1 reference serum sample: LAB5.2 for the negative reference serum sample PT2013AUJgBSERNS2 (k=1,93) and for the positive reference serum samples PT2013AUJgBSERPS1 (k=2,82), PT2013AUJgBSERPS2

( $k=2,76$ ), PT2013AUJgBSERPS3 ( $k=2,38$ ) and PT2013AUJgBSERPS4 ( $k=2,72$ ), and LAB6 for the negative reference serum sample PT2013AUJgBSERNS1 ( $k=1,60$ ).

For the detection of ADV gE-specific antibodies, 6 out of 9 participating laboratories obtained a satisfactory between-laboratory consistency for all reference serum samples. This was not the case for LAB4, LAB7 and LAB8, which showed an increased value for Mandel's h-statistic for 1 reference serum sample: LAB4 for the negative reference serum sample PT2013AUJgESERNS3 ( $h=1,95$ ), LAB7 for the positive reference serum sample PT2013AUJgESERPS3 ( $h=-1,92$ ) and LAB8 for the negative reference serum sample PT2013AUJgESERNS1 ( $h=-1,98$ ). LAB1, LAB2, LAB5, LAB6, LAB7, LAB8 and LAB9 used the same ADV gE ELISA kit. Hereby, LAB5 and LAB9 on the one hand, and LAB7 and LAB8 on the other hand, used the same batch. Furthermore, LAB1 performed the long incubation protocol, whereas LAB6, LAB7, LAB8 and LAB9 performed the short incubation protocol (LAB2 and LAB5 did not provide information about the used incubation protocol).

Furthermore, 5 out of 9 participating laboratories obtained a satisfactory within-laboratory consistency for all reference serum samples. This was not the case for LAB3, LAB4, LAB5 and LAB8 which showed an increased value for Mandel's k-statistic for 1 reference serum sample: LAB3 for the positive reference serum sample PT2013AUJgESERPS2 ( $k=1,80$ ), LAB4 for the positive reference serum sample PT2013AUJgESERPS3 ( $k=1,74$ ), LAB5 for the positive reference serum sample PT2013AUJgESERPS1 ( $k=2,56$ ) and LAB8 for the negative reference serum sample PT2013AUJgESERNS1 ( $k=1,77$ ).

All data used for the calculations of Mandel's h- and k-statistics can be found in Annex 2.

### III. ANOVA

Using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the normalized OD values as a dependent variable, it was investigated whether statistically significant differences exist ( $\alpha=0,05$ ) between participating laboratories. Comparisons were made at the global level (all reference serum samples were analysed together), status level (all reference serum samples with the same status were analysed together) and sample level (all reference serum samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits, batches or incubation protocols) is most relevant at the status level (less variation than at a global level), we focused on the latter.

#### III.1. ADV gB

For the PT ADV gB, no statistically significant differences were observed between laboratories or between different kits at a global level. However, statistically significant differences existed at both sample and status level.

At the status level, significant differences were observed for both the negative and positive reference serum samples. For the negative reference serum samples, LAB1 and LAB8 reported percentages blocking that were significantly higher than those reported by LAB3, LAB5.1, LAB4, LAB5.2 and LAB6. For the positive reference serum samples, LAB4 and LAB5.2 reported percentages blocking that were significantly lower than those reported by the other participants. For the 2 ELISA kits used by LAB5, a significant difference in percentages blocking was observed for the positive but not for the negative reference serum samples. Hereby, the percentages blocking obtained by LAB5.1 were significantly higher than those obtained by LAB5.2.

#### III.2. ADV gE

For the PT ADV gE, no statistically significant differences were observed between laboratories at a global level. However, statistically significant differences existed at both sample and status level.

At the status level, significant differences were observed for both the negative and positive reference serum samples. For the negative reference serum samples, LAB4 reported percentages blocking that were significantly higher than those reported by LAB1, LAB3, LAB5, LAB7, LAB6 and LAB8. Furthermore, LAB8 reported percentages blocking that were significantly lower than those reported by the other participants. For the positive reference serum samples, LAB4 and LAB7 reported percentages blocking that were significantly lower than those reported by LAB1 and LAB2. In addition, the percentages blocking reported by LAB7 were significantly lower compared to LAB1, LAB2, LAB9, LAB3 and LAB6.

## Annex 2: Calculations of Mandel's h- and k-statistics (based on % blocking)

### A. ADV qB

| Sample                   | Labnr      | n_i | v_i   | x_i_m | x_g_m | between_<br>lab_coef | STDEV<br>_repeat | STDEV<br>_repro | STDEV<br>_betweenlab | h            | k           | cv      |
|--------------------------|------------|-----|-------|-------|-------|----------------------|------------------|-----------------|----------------------|--------------|-------------|---------|
| PT2013AUJgBSERNS1        | 1          | 4   | 20,79 | 22,25 | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | 1,07         | 0,86        | 20,50   |
| PT2013AUJgBSERNS1        | 2          | 4   | 13,35 | 8,45  | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | -0,64        | 0,69        | 43,23   |
| PT2013AUJgBSERNS1        | 3          | 4   | 2,42  | 14,36 | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | 0,09         | 0,29        | 10,84   |
| PT2013AUJgBSERNS1        | 4          | 4   | 1,55  | 4,64  | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | -1,11        | 0,23        | 26,82   |
| PT2013AUJgBSERNS1        | 5.1        | 4   | 1,33  | 6,87  | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | -0,84        | 0,22        | 16,81   |
| PT2013AUJgBSERNS1        | 5.2        | 4   | 37,09 | 12,79 | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | -0,10        | 1,15        | 47,63   |
| <b>PT2013AUJgBSERNS1</b> | <b>6</b>   | 4   | 71,85 | 6,84  | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | -0,84        | <b>1,60</b> | 123,94  |
| PT2013AUJgBSERNS1        | 7          | 4   | 38,84 | 17,64 | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | 0,50         | 1,17        | 35,34   |
| <b>PT2013AUJgBSERNS1</b> | <b>8</b>   | 4   | 66,71 | 28,78 | 13,62 | 0,20                 | 5,31             | 5,96            | 2,69                 | <b>1,88</b>  | 1,54        | 28,38   |
| <b>PT2013AUJgBSERNS2</b> | <b>1</b>   | 3   | 13,39 | 35,82 | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | <b>2,05</b>  | 0,77        | 10,22   |
| PT2013AUJgBSERNS2        | 2          | 3   | 13,13 | 15,76 | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | 0,45         | 0,76        | 22,99   |
| PT2013AUJgBSERNS2        | 3          | 3   | 46,21 | -4,04 | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | -1,12        | 1,43        | -168,38 |
| PT2013AUJgBSERNS2        | 4          | 3   | 0,20  | 7,77  | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | -0,18        | 0,09        | 5,78    |
| PT2013AUJgBSERNS2        | 5.1        | 3   | 0,28  | 5,46  | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | -0,36        | 0,11        | 9,74    |
| <b>PT2013AUJgBSERNS2</b> | <b>5.2</b> | 3   | 84,60 | -3,76 | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | -1,10        | <b>1,93</b> | -244,65 |
| PT2013AUJgBSERNS2        | 6          | 3   | 23,61 | 2,01  | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | -0,64        | 1,02        | 241,60  |
| PT2013AUJgBSERNS2        | 7          | 3   | 16,96 | 12,05 | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | 0,16         | 0,87        | 34,18   |
| PT2013AUJgBSERNS2        | 8          | 3   | 4,98  | 19,35 | 10,05 | 0,45                 | 4,75             | 6,44            | 4,34                 | 0,74         | 0,47        | 11,53   |
| PT2013AUJgBSERPS1        | 1          | 3   | 0,10  | 94,44 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | 0,43         | 0,24        | 0,33    |
| PT2013AUJgBSERPS1        | 2          | 3   | 0,01  | 94,86 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | 0,47         | 0,07        | 0,09    |
| PT2013AUJgBSERPS1        | 3          | 3   | 0,01  | 96,72 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | 0,61         | 0,06        | 0,08    |
| <b>PT2013AUJgBSERPS1</b> | <b>4</b>   | 3   | 0,79  | 59,25 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | <b>-2,23</b> | 0,68        | 1,50    |
| PT2013AUJgBSERPS1        | 5.1        | 3   | 0,11  | 94,21 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | 0,42         | 0,26        | 0,36    |
| <b>PT2013AUJgBSERPS1</b> | <b>5.2</b> | 3   | 13,74 | 73,27 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | -1,17        | <b>2,82</b> | 5,06    |
| PT2013AUJgBSERPS1        | 6          | 3   | 0,02  | 94,44 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | 0,43         | 0,10        | 0,13    |
| PT2013AUJgBSERPS1        | 7          | 3   | 0,29  | 94,46 | 88,69 | 0,93                 | 1,31             | 4,85            | 4,67                 | 0,44         | 0,41        | 0,57    |



| Sample                   | Labnr      | n_i | v_i   | x_i_m | x_g_m | between_lab_coef | STDEV_repeat | STDEV_repro | STDEV_betweenlab | h            | k           | cv    |
|--------------------------|------------|-----|-------|-------|-------|------------------|--------------|-------------|------------------|--------------|-------------|-------|
| PT2013AUJgBSERPS1        | 8          | 3   | 0,46  | 96,57 | 88,69 | 0,93             | 1,31         | 4,85        | 4,67             | 0,60         | 0,51        | 0,70  |
| PT2013AUJgBSERPS2        | 1          | 3   | 0,06  | 92,58 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | 0,68         | 0,10        | 0,27  |
| PT2013AUJgBSERPS2        | 2          | 3   | 1,57  | 90,91 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | 0,58         | 0,50        | 1,38  |
| PT2013AUJgBSERPS2        | 3          | 3   | 0,60  | 86,04 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | 0,27         | 0,31        | 0,90  |
| <b>PT2013AUJgBSERPS2</b> | <b>4</b>   | 3   | 3,19  | 48,28 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | <b>-2,07</b> | 0,72        | 3,70  |
| PT2013AUJgBSERPS2        | 5.1        | 3   | 0,25  | 87,21 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | 0,35         | 0,20        | 0,57  |
| <b>PT2013AUJgBSERPS2</b> | <b>5.2</b> | 3   | 46,75 | 59,63 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | -1,37        | <b>2,76</b> | 11,47 |
| PT2013AUJgBSERPS2        | 6          | 3   | 0,10  | 90,69 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | 0,56         | 0,13        | 0,34  |
| PT2013AUJgBSERPS2        | 7          | 3   | 0,46  | 88,42 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | 0,42         | 0,27        | 0,77  |
| PT2013AUJgBSERPS2        | 8          | 3   | 2,41  | 91,11 | 81,65 | 0,84             | 2,48         | 6,18        | 5,67             | 0,59         | 0,63        | 1,70  |
| PT2013AUJgBSERPS3        | 1          | 3   | 0,34  | 94,75 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | 0,26         | 0,92        | 0,62  |
| PT2013AUJgBSERPS3        | 2          | 3   | 0,00  | 97,00 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | 0,61         | 0,11        | 0,07  |
| PT2013AUJgBSERPS3        | 3          | 3   | 0,05  | 95,89 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | 0,44         | 0,37        | 0,24  |
| <b>PT2013AUJgBSERPS3</b> | <b>4</b>   | 3   | 0,26  | 77,43 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | <b>-2,44</b> | 0,81        | 0,66  |
| PT2013AUJgBSERPS3        | 5.1        | 3   | 0,25  | 95,98 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | 0,45         | 0,79        | 0,52  |
| <b>PT2013AUJgBSERPS3</b> | <b>5.2</b> | 3   | 2,28  | 88,55 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | -0,71        | <b>2,38</b> | 1,71  |
| PT2013AUJgBSERPS3        | 6          | 3   | 0,01  | 95,70 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | 0,41         | 0,13        | 0,09  |
| PT2013AUJgBSERPS3        | 7          | 3   | 0,41  | 94,84 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | 0,28         | 1,01        | 0,67  |
| PT2013AUJgBSERPS3        | 8          | 3   | 0,01  | 97,50 | 93,07 | 0,93             | 0,63         | 2,35        | 2,26             | 0,69         | 0,13        | 0,08  |
| PT2013AUJgBSERPS4        | 1          | 4   | 0,03  | 92,19 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | 0,87         | 0,07        | 0,18  |
| PT2013AUJgBSERPS4        | 2          | 4   | 1,91  | 87,87 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | 0,58         | 0,54        | 1,57  |
| PT2013AUJgBSERPS4        | 3          | 4   | 0,26  | 81,97 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | 0,19         | 0,20        | 0,62  |
| PT2013AUJgBSERPS4        | 4          | 4   | 1,18  | 55,12 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | -1,61        | 0,42        | 1,97  |
| PT2013AUJgBSERPS4        | 5.1        | 4   | 0,58  | 83,00 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | 0,26         | 0,30        | 0,92  |
| <b>PT2013AUJgBSERPS4</b> | <b>5.2</b> | 4   | 48,57 | 51,69 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | <b>-1,84</b> | <b>2,72</b> | 13,48 |
| PT2013AUJgBSERPS4        | 6          | 4   | 0,64  | 86,05 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | 0,46         | 0,31        | 0,93  |
| PT2013AUJgBSERPS4        | 7          | 4   | 1,96  | 86,27 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | 0,47         | 0,55        | 1,62  |
| PT2013AUJgBSERPS4        | 8          | 4   | 3,98  | 88,55 | 79,19 | 0,81             | 2,56         | 5,86        | 5,26             | 0,63         | 0,78        | 2,25  |



**Legend:** **Labnr** = number attributed to a laboratory during the PT; **n<sub>i</sub>** = number of replicates; **v<sub>i</sub>** = total variability (variance) in the normalized data (% blocking); **x<sub>i,m</sub>** = mean of normalized data (% blocking); **x<sub>g,m</sub>** = mean of normalized data (% blocking) obtained by all laboratories; **between\_lab\_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV\_repeat** = repeatability standard deviation over all laboratories; **STDEV\_repro** = reproducibility standard deviation over all laboratories; **STDEV\_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).

## B. ADV qE

| Sample                   | Labnr    | n <sub>i</sub> | v <sub>i</sub> | x <sub>i,m</sub> | x <sub>g,m</sub> | between_lab_coeff | STDEV_repeat | STDEV_repro | STDEV_betweenlab | h            | k           | cv      |
|--------------------------|----------|----------------|----------------|------------------|------------------|-------------------|--------------|-------------|------------------|--------------|-------------|---------|
| PT2013AUJgESERNS1        | 1        | 3              | 3,95           | 12,08            | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | 0,46         | 0,37        | 16,45   |
| PT2013AUJgESERNS1        | 2        | 3              | 56,31          | 14,03            | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | 0,63         | 1,41        | 53,50   |
| PT2013AUJgESERNS1        | 3        | 3              | 64,89          | 8,17             | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | 0,12         | 1,51        | 98,57   |
| PT2013AUJgESERNS1        | 4        | 3              | 13,05          | 23,75            | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | 1,48         | 0,68        | 15,21   |
| PT2013AUJgESERNS1        | 5        | 3              | 14,70          | -1,02            | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | -0,69        | 0,72        | -374,39 |
| PT2013AUJgESERNS1        | 6        | 3              | 3,74           | 1,75             | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | -0,45        | 0,36        | 110,58  |
| PT2013AUJgESERNS1        | 7        | 3              | 4,02           | 3,57             | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | -0,29        | 0,38        | 56,20   |
| <b>PT2013AUJgESERNS1</b> | <b>8</b> | 3              | 88,98          | -15,67           | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | <b>-1,98</b> | <b>1,77</b> | -60,21  |
| PT2013AUJgESERNS1        | 9        | 3              | 6,06           | 14,98            | 6,85             | 0,35              | 5,33         | 6,59        | 3,88             | 0,71         | 0,46        | 16,43   |
| PT2013AUJgESERNS2        | 1        | 3              | 12,50          | 14,33            | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | 0,86         | 0,66        | 24,66   |
| PT2013AUJgESERNS2        | 2        | 3              | 51,58          | 13,61            | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | 0,76         | 1,34        | 52,79   |
| PT2013AUJgESERNS2        | 3        | 3              | 41,41          | 8,68             | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | 0,10         | 1,20        | 74,17   |
| PT2013AUJgESERNS2        | 4        | 3              | 0,94           | 15,63            | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | 1,04         | 0,18        | 6,20    |
| PT2013AUJgESERNS2        | 5        | 3              | 70,62          | 6,60             | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | -0,18        | 1,57        | 127,36  |
| PT2013AUJgESERNS2        | 6        | 3              | 27,34          | -0,54            | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | -1,14        | 0,97        | -975,60 |
| PT2013AUJgESERNS2        | 7        | 3              | 9,92           | 3,59             | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | -0,58        | 0,59        | 87,68   |
| PT2013AUJgESERNS2        | 8        | 3              | 15,78          | -5,24            | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | -1,77        | 0,74        | -75,88  |
| PT2013AUJgESERNS2        | 9        | 3              | 28,89          | 14,57            | 7,91             | 0,17              | 5,36         | 5,87        | 2,39             | 0,90         | 1,00        | 36,88   |
| PT2013AUJgESERNS3        | 1        | 3              | 8,67           | 6,48             | 8,20             | 0,29              | 4,19         | 4,99        | 2,71             | -0,21        | 0,70        | 45,46   |
| PT2013AUJgESERNS3        | 2        | 3              | 37,93          | 11,60            | 8,20             | 0,29              | 4,19         | 4,99        | 2,71             | 0,42         | 1,47        | 53,11   |
| PT2013AUJgESERNS3        | 3        | 3              | 7,10           | 14,75            | 8,20             | 0,29              | 4,19         | 4,99        | 2,71             | 0,82         | 0,64        | 18,07   |
| <b>PT2013AUJgESERNS3</b> | <b>4</b> | 3              | 43,44          | 23,83            | 8,20             | 0,29              | 4,19         | 4,99        | 2,71             | <b>1,95</b>  | 1,57        | 27,65   |



| Sample                   | Labnr    | n_i | v_i   | x_i_m | x_g_m | between_lab_coeff | STDEV_repeat | STDEV_repro | STDEV_betweenlab | h     | k           | cv      |
|--------------------------|----------|-----|-------|-------|-------|-------------------|--------------|-------------|------------------|-------|-------------|---------|
| PT2013AUJgESERNS3        | 5        | 3   | 12,38 | 4,81  | 8,20  | 0,29              | 4,19         | 4,99        | 2,71             | -0,42 | 0,84        | 73,20   |
| PT2013AUJgESERNS3        | 6        | 3   | 26,26 | 3,02  | 8,20  | 0,29              | 4,19         | 4,99        | 2,71             | -0,65 | 1,22        | 169,84  |
| PT2013AUJgESERNS3        | 7        | 3   | 6,06  | 1,33  | 8,20  | 0,29              | 4,19         | 4,99        | 2,71             | -0,86 | 0,59        | 185,27  |
| PT2013AUJgESERNS3        | 8        | 3   | 13,03 | -2,80 | 8,20  | 0,29              | 4,19         | 4,99        | 2,71             | -1,37 | 0,86        | -129,00 |
| PT2013AUJgESERNS3        | 9        | 3   | 3,03  | 10,80 | 8,20  | 0,29              | 4,19         | 4,99        | 2,71             | 0,32  | 0,42        | 16,11   |
| PT2013AUJgESERPS1        | 1        | 3   | 0,11  | 93,77 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | 0,68  | 0,18        | 0,36    |
| PT2013AUJgESERPS1        | 2        | 3   | 0,92  | 93,16 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | 0,54  | 0,51        | 1,03    |
| PT2013AUJgESERPS1        | 3        | 3   | 0,11  | 89,98 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | -0,23 | 0,18        | 0,37    |
| PT2013AUJgESERPS1        | 4        | 3   | 6,01  | 84,52 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | -1,54 | 1,30        | 2,90    |
| <b>PT2013AUJgESERPS1</b> | <b>5</b> | 3   | 23,46 | 84,24 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | -1,61 | <b>2,56</b> | 5,75    |
| PT2013AUJgESERPS1        | 6        | 3   | 0,02  | 95,18 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | 1,02  | 0,08        | 0,15    |
| PT2013AUJgESERPS1        | 7        | 3   | 0,91  | 90,24 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | -0,17 | 0,50        | 1,05    |
| PT2013AUJgESERPS1        | 8        | 3   | 0,60  | 92,08 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | 0,28  | 0,41        | 0,84    |
| PT2013AUJgESERPS1        | 9        | 3   | 0,02  | 95,19 | 90,93 | 0,36              | 1,89         | 2,36        | 1,42             | 1,03  | 0,08        | 0,16    |
| PT2013AUJgESERPS2        | 1        | 4   | 1,20  | 84,79 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | 1,14  | 0,45        | 1,29    |
| PT2013AUJgESERPS2        | 2        | 4   | 4,39  | 83,11 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | 0,96  | 0,86        | 2,52    |
| <b>PT2013AUJgESERPS2</b> | <b>3</b> | 4   | 19,21 | 74,32 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | -0,04 | <b>1,80</b> | 5,90    |
| PT2013AUJgESERPS2        | 4        | 4   | 0,83  | 63,39 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | -1,28 | 0,37        | 1,44    |
| PT2013AUJgESERPS2        | 5        | 4   | 4,94  | 68,32 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | -0,72 | 0,91        | 3,25    |
| PT2013AUJgESERPS2        | 6        | 4   | 1,08  | 81,22 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | 0,74  | 0,43        | 1,28    |
| PT2013AUJgESERPS2        | 7        | 4   | 9,98  | 60,19 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | -1,64 | 1,30        | 5,25    |
| PT2013AUJgESERPS2        | 8        | 4   | 9,77  | 76,96 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | 0,26  | 1,28        | 4,06    |
| PT2013AUJgESERPS2        | 9        | 4   | 2,07  | 79,74 | 74,67 | 0,62              | 2,44         | 3,94        | 3,10             | 0,57  | 0,59        | 1,80    |
| PT2013AUJgESERPS3        | 1        | 4   | 0,69  | 75,73 | 67,45 | 0,74              | 1,93         | 3,81        | 3,28             | 0,89  | 0,43        | 1,10    |
| PT2013AUJgESERPS3        | 2        | 4   | 3,19  | 75,75 | 67,45 | 0,74              | 1,93         | 3,81        | 3,28             | 0,89  | 0,93        | 2,36    |
| PT2013AUJgESERPS3        | 3        | 4   | 1,68  | 78,51 | 67,45 | 0,74              | 1,93         | 3,81        | 3,28             | 1,18  | 0,67        | 1,65    |
| <b>PT2013AUJgESERPS3</b> | <b>4</b> | 4   | 11,19 | 58,53 | 67,45 | 0,74              | 1,93         | 3,81        | 3,28             | -0,95 | <b>1,74</b> | 5,72    |
| PT2013AUJgESERPS3        | 5        | 4   | 4,10  | 62,16 | 67,45 | 0,74              | 1,93         | 3,81        | 3,28             | -0,57 | 1,05        | 3,26    |
| PT2013AUJgESERPS3        | 6        | 4   | 0,79  | 67,68 | 67,45 | 0,74              | 1,93         | 3,81        | 3,28             | 0,03  | 0,46        | 1,32    |



| Sample                   | Labnr           | n <sub>i</sub> | v <sub>i</sub> | x <sub>i_m</sub> | x <sub>g_m</sub> | between_<br>lab_coeff | STDEV<br>_repeat | STDEV<br>_repro | STDEV<br>_betweenlab | h                   | k    | cv   |
|--------------------------|-----------------|----------------|----------------|------------------|------------------|-----------------------|------------------|-----------------|----------------------|---------------------|------|------|
| <b>PT2013AUJgESERPS3</b> | <b><u>7</u></b> | 4              | 4,35           | 49,53            | 67,45            | 0,74                  | 1,93             | 3,81            | 3,28                 | <b><u>-1,92</u></b> | 1,08 | 4,21 |
| PT2013AUJgESERPS3        | 8               | 4              | 1,80           | 69,73            | 67,45            | 0,74                  | 1,93             | 3,81            | 3,28                 | 0,24                | 0,70 | 1,92 |
| PT2013AUJgESERPS3        | 9               | 4              | 5,63           | 69,41            | 67,45            | 0,74                  | 1,93             | 3,81            | 3,28                 | 0,21                | 1,23 | 3,42 |

**Legend:** **Labnr** = number attributed to a laboratory during the PT; **n<sub>i</sub>** = number of replicates; **v<sub>i</sub>** = total variability (variance) in the normalized data (% blocking); **x<sub>i\_m</sub>** = mean of normalized data (% blocking); **x<sub>g\_m</sub>** = mean of normalized data (% blocking) obtained by all laboratories; **between\_lab\_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV\_repeat** = repeatability standard deviation over all laboratories; **STDEV\_repro** = reproducibility standard deviation over all laboratories; **STDEV\_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).



## Annex 3: Qualitative and quantitative data analysis of the additional panel ADV gB samples

For the PT ADV gB, an additional panel consisting of serum samples with variable qualitative results in ELISA but all negative in SN, the reference test (golden standard) for the detection of ADV gB-specific antibodies in serum, was sent to the participating laboratories along with the regular ADV gB PT samples. The aim was to include serum samples that score weak positive and cut-off in ELISA in order to achieve more information about the analytical sensitivity of the used ADV gB ELISA kits by the participating laboratories. This part was out of scope of this PT according to ISO 17043, and should only be considered as educational information for the participants. The data analysis of these additional serum samples was thus not used to evaluate the participants in this PT.

### I. Information about the additional IBRgB serum samples

In total, 72 aliquots of these additional serum samples were distributed to the 8 laboratories participating in the PT ADV gB. All participants received 3 aliquots of 3 different serum samples of porcine origin (PT2013AUJgBSER-S1A, PT2013AUJgBSER-S1B, PT2013AUJgBSER-S1C), i.e. 9 aliquots in total. The positions of the serum samples in the sent blocks were randomized for each participant.

The serum samples PT2013AUJgBSER-S1A, PT2013AUJgBSER-S1B and PT2013AUJgBSER-S1C were a 1/1024, a 1/2048 and a 1/4096 dilution, respectively, of a serum obtained from an animal that was experimentally infected with an ADV strain isolated from a wolf in 2011 in Belgium (different animal than the one reference serum sample PT2013AUJgBSERPS4 was derived from (see III.2.1)). During pre-verification, all 3 additional serum samples were negative using SN, whereas variable results were obtained using the PrioCheck PRV gB antibody ELISA test from Prionics (POS - POS/NEG - NEG, respectively).

After aliquoting the different serum samples, a homogeneity check was performed on 10 aliquots of each serum sample using the PrioCheck PRV gB antibody ELISA test from Prionics. In addition, 3 aliquots of each serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the PrioCheck PRV gB antibody ELISA test from Prionics.

### II. Qualitative data analysis

The qualitative results reported by the participating laboratories are summarized in Table 1.

**Table 1.** Overview of the qualitative results reported by the participating laboratories for the 12 aliquots of additional IBRgB serum samples.

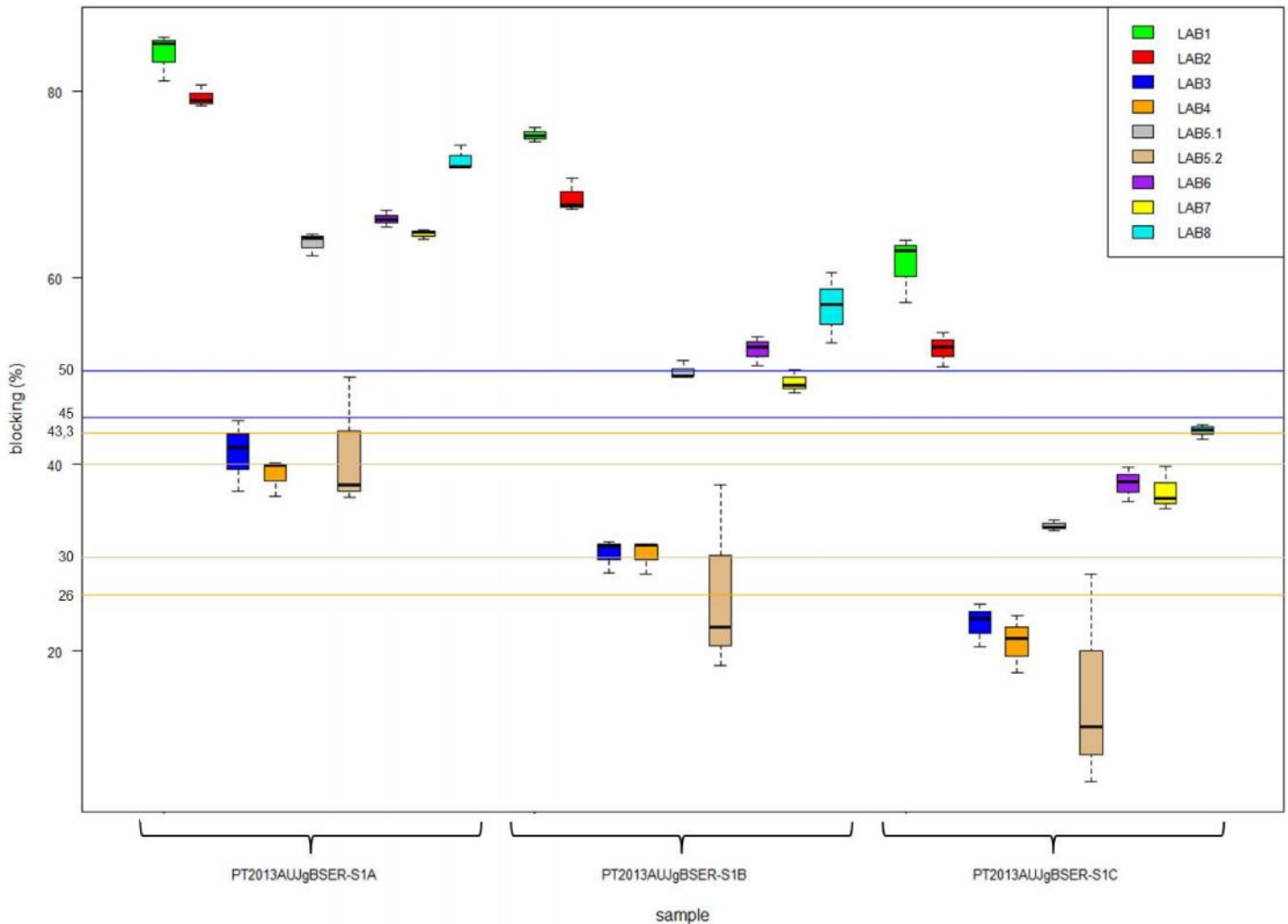
|                           | LAB1 | LAB2 | LAB3 | LAB4 | LAB5.1 | LAB5.2 | LAB6 | LAB7 | LAB8 | TOTAL | GRAND TOTAL |
|---------------------------|------|------|------|------|--------|--------|------|------|------|-------|-------------|
| <b>PT2013AUJgBSER-S1A</b> |      |      |      |      |        |        |      |      |      |       |             |
| POS                       | 3    | 3    | 0    | 0    | 3      | 1      | 3    | 3    | 3    | 19    | 27          |
| NI                        | 0    | 0    | 0    | 3    | 0      | 2      | 0    | 0    | 0    | 5     |             |
| NEG                       | 0    | 0    | 3    | 0    | 0      | 0      | 0    | 0    | 0    | 3     |             |
| <b>PT2013AUJgBSER-S1B</b> |      |      |      |      |        |        |      |      |      |       |             |
| POS                       | 3    | 3    | 0    | 0    | 1      | 0      | 3    | 1    | 3    | 14    | 27          |
| NI                        | 0    | 0    | 0    | 3    | 2      | 1      | 0    | 0    | 0    | 6     |             |
| NEG                       | 0    | 0    | 3    | 0    | 0      | 2      | 0    | 2    | 0    | 7     |             |
| <b>PT2013AUJgBSER-S1C</b> |      |      |      |      |        |        |      |      |      |       |             |
| POS                       | 3    | 3    | 0    | 0    | 0      | 0      | 0    | 0    | 0    | 6     | 27          |
| NI                        | 0    | 0    | 0    | 0    | 0      | 0      | 0    | 0    | 0    | 0     |             |
| NEG                       | 0    | 0    | 3    | 3    | 3      | 3      | 3    | 3    | 3    | 21    |             |
| <b>TOTAL</b>              | 9    | 9    | 9    | 9    | 9      | 9      | 9    | 9    | 9    |       |             |
| POS                       | 9    | 9    | 0    | 0    | 4      | 1      | 6    | 4    | 6    | 39    | 81          |
| NI                        | 0    | 0    | 0    | 6    | 2      | 3      | 0    | 0    | 0    | 11    |             |
| NEG                       | 0    | 0    | 9    | 3    | 3      | 5      | 3    | 5    | 3    | 31    |             |

### III. Quantitative data analysis

The quantitative data analysis was performed using the statistical software programs R (box plots) and SAS 9.2. (summary statistics), all as described in Annex 1.

#### III.1 Box plots

Box plots of the percentages blocking per additional ADV gB serum sample and per participating (sub)laboratory were made using the statistical software R and are shown in Figure 1.



**Figure 1. Box plots showing the percentage blocking per additional ADV gB reference serum sample and per participating (sub)laboratory.** Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. (Adapted) cut-off values applied by the participating laboratories are: 26-43,3% (orange; LAB4), 30-40% (light brown; LAB5.2), 45-50% (blue; LAB3), 40-50% (upper light brown and upper blue line; LAB1, LAB2, LAB5.1) and 50% (upper blue line; LAB6, LAB7, LAB8). LAB1, LAB2 and LAB5.2 on the one hand, and LAB5.1, LAB6, LAB7 and LAB8 on the other hand, used the same ADV gB ELISA kit (LAB5.1 applied alternative cut-off values compared to LAB6, LAB7 and LAB8). Hereby, LAB5.1, LAB6 and LAB8 used the same batch. In addition, LAB1 and LAB2 performed the long incubation protocol, whereas LAB5.2 performed the short incubation protocol of the same ELISA kit (different batches; different cut-off values for short and long incubation protocol).



### III.2 Mandel's h- and k-statistics

Based on Table 1 in Annex 1, the maximum absolute value for Mandel's h-statistic is 1,78 (p=9) and the maximum value for Mandel's k-statistic is 1,68 for all serum samples (p=9 and n=3).

For the detection of ADV gB-specific antibodies in the additional serum samples, all participating (sub)laboratories obtained a satisfactory between-laboratory consistency for all serum samples. Furthermore, 8 out of 9 participating (sub)laboratories obtained a satisfactory within-laboratory consistency for all serum samples. This was not the case for LAB5.2 which showed an increased value for Mandel's k-statistic for the 3 serum samples: PT2013ADVgBSER-S1A (k=2,36), PT2013ADVgBSER-S1B (k=2,64) and PT2013ADVgBSER-S1C (k=2,62).

All data used for the calculations of Mandel's h- and k-statistics for the additional ADV gB serum samples can be found in Table 2 of this Annex.

### III.3 ANOVA

For the additional ADV gB serum samples, there was a significant difference between the participating (sub)laboratories at a global level. LAB1 reported percentages blocking that were significantly higher than those reported by LAB6, LAB7, LAB5.1, LAB3, LAB4 and LAB5.2. Furthermore, LAB1, LAB2, LAB8, LAB6 and LAB7 reported percentages blocking that were significantly higher than those reported by LAB3, LAB4 and LAB5.2. For the 2 ELISA kits used by LAB5, LAB5.1 obtained percentages blocking that were significantly higher compared to LAB5.2.



**Table 2.** Calculations of Mandel's h- and k-statistics (based on % blocking) for the panel additional ADV gB serum samples.

| Sample                    | Labnr      | n_i | v_i    | x_i_m | x_g_m | between_lab_coef | STDEV_repeat | STDEV_repr | STDEV_betweenla | h     | k           | cv    |
|---------------------------|------------|-----|--------|-------|-------|------------------|--------------|------------|-----------------|-------|-------------|-------|
| PT2013AUJgBSER-S1A        | 1          | 3   | 6,19   | 84,02 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | 1,33  | 0,83        | 2,96  |
| PT2013AUJgBSER-S1A        | 2          | 3   | 1,39   | 79,36 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | 1,06  | 0,40        | 1,49  |
| PT2013AUJgBSER-S1A        | 3          | 3   | 14,38  | 41,19 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | -1,18 | 1,27        | 9,21  |
| PT2013AUJgBSER-S1A        | 4          | 3   | 4,10   | 38,86 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | -1,32 | 0,68        | 5,21  |
| PT2013AUJgBSER-S1A        | 5.1        | 3   | 1,65   | 63,75 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | 0,14  | 0,43        | 2,01  |
| <b>PT2013AUJgBSER-S1A</b> | <b>5.2</b> | 3   | 49,79  | 41,20 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | -1,18 | <b>2,36</b> | 17,13 |
| PT2013AUJgBSER-S1A        | 6          | 3   | 0,75   | 66,31 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | 0,29  | 0,29        | 1,31  |
| PT2013AUJgBSER-S1A        | 7          | 3   | 0,30   | 64,72 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | 0,20  | 0,18        | 0,85  |
| PT2013AUJgBSER-S1A        | 8          | 3   | 1,75   | 72,69 | 61,34 | 0,80             | 2,99         | 6,70       | 6,00            | 0,67  | 0,44        | 1,82  |
| PT2013AUJgBSER-S1B        | 1          | 3   | 0,59   | 75,31 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | 1,55  | 0,20        | 1,02  |
| PT2013AUJgBSER-S1B        | 2          | 3   | 3,33   | 68,59 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | 1,15  | 0,47        | 2,66  |
| PT2013AUJgBSER-S1B        | 3          | 3   | 3,37   | 30,46 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | -1,07 | 0,48        | 6,03  |
| PT2013AUJgBSER-S1B        | 4          | 3   | 3,22   | 30,34 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | -1,07 | 0,47        | 5,91  |
| PT2013AUJgBSER-S1B        | 5.1        | 3   | 0,97   | 49,99 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | 0,07  | 0,26        | 1,97  |
| <b>PT2013AUJgBSER-S1B</b> | <b>5.2</b> | 3   | 103,54 | 26,27 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | -1,31 | <b>2,64</b> | 38,73 |
| PT2013AUJgBSER-S1B        | 6          | 3   | 2,41   | 52,26 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | 0,20  | 0,40        | 2,97  |
| PT2013AUJgBSER-S1B        | 7          | 3   | 1,58   | 48,77 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | 0,00  | 0,33        | 2,58  |
| PT2013AUJgBSER-S1B        | 8          | 3   | 14,29  | 56,91 | 48,77 | 0,71             | 3,85         | 7,14       | 6,02            | 0,47  | 0,98        | 6,64  |
| PT2013AUJgBSER-S1C        | 1          | 3   | 12,64  | 61,46 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | 1,69  | 0,81        | 5,79  |
| PT2013AUJgBSER-S1C        | 2          | 3   | 3,25   | 52,39 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | 1,08  | 0,41        | 3,44  |
| PT2013AUJgBSER-S1C        | 3          | 3   | 5,35   | 22,95 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | -0,88 | 0,53        | 10,08 |
| PT2013AUJgBSER-S1C        | 4          | 3   | 9,75   | 20,94 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | -1,01 | 0,71        | 14,92 |
| PT2013AUJgBSER-S1C        | 5.1        | 3   | 0,32   | 33,39 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | -0,18 | 0,13        | 1,69  |
| <b>PT2013AUJgBSER-S1C</b> | <b>5.2</b> | 3   | 131,98 | 15,37 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | -1,38 | <b>2,62</b> | 74,76 |
| PT2013AUJgBSER-S1C        | 6          | 3   | 3,33   | 37,93 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | 0,12  | 0,42        | 4,81  |
| PT2013AUJgBSER-S1C        | 7          | 3   | 5,72   | 37,12 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | 0,07  | 0,55        | 6,44  |
| PT2013AUJgBSER-S1C        | 8          | 3   | 0,59   | 43,55 | 36,12 | 0,59             | 4,38         | 6,83       | 5,24            | 0,49  | 0,18        | 1,76  |

**Legend:** Labnr = number attributed to a laboratory during the PT; n\_i = number of replicates; v\_i = total variability (variance) in the normalized data (% blocking); x\_i\_m = mean of normalized data (% blocking); x\_g\_m = mean of normalized data (% blocking) obtained by all laboratories; between\_lab\_coef = fraction of total variability due to differences between labs for each sample; STDEV\_repeat = repeatability standard deviation over all laboratories; STDEV\_repro = reproducibility standard deviation over all laboratories; STDEV\_betweenlab = between-lab standard deviation over all laboratories; h-

**statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %. Values for Mandel's h- and k-statistics shown in red/underlined/bold exceed the corresponding limit value as determined in Annex 1 (Table 1).