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Running head: global ring trial NDV and IBDV antibodies

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Summary

The results of global proficiency testing schemes (PTS) for serological tests to detect antibodies against Infectious Bursal Disease Virus (IBDV) and Newcastle Disease Virus (NDV) in chicken serum, in which 125 and 120 laboratories respectively participated from Africa, Asia, Europe, Central and South America were used to analyse the performances of different antibody test systems such as virus neutralization tests (VNT) haemagglutination inhibition (HI) tests, enzyme-linked immunosorbent assays (ELISA) and agar gel precipitation tests (AGPT). All laboratories were asked to carry out their routine diagnostic tests for the detection of IBDV and NDV antibodies as usual. This global ring trial provided a large amount of data on variation within and between laboratories and test systems used worldwide.

The data showed that the variation between the quantitative test results of different laboratories ($R_{between}$) using the IBDV VNT and the NDV HI test was higher (about double) compared to the variation within commercial ELISA systems. Although both tests are often referred to and used as the "gold standard" in experimental and scientific studies, official procedures and for the validation of tests, this study shows that there is an urgent need for a global implementation of recommended test procedures and/or the inclusion of international reference sera in these studies.

Introduction

Serology for IBDV and NDV is widely used as a monitoring tool, a diagnostic tool and for estimation of the optimal time for IBD vaccination(s). Serology is also used in many field trials, efficacy testing of live and inactivated vaccines and to control the import and export of hatching eggs and animals. For these purposes several kinds of tests are used, such as the virus neutralization tests (VNT), haemagglutination inhibition (HI) test and enzyme-linked immunosorbant assays (ELISAs). VNT and HI are usually "in-house" test systems, using different antigens and protocols. For some of these test systems, such as the HI test for NDV, there are recommendations on their performance (OIE Manual, 2004a; 2004b), but these protocols do not guarantee the same test results from different laboratories. Compared to “in-house tests”, commercially available test systems have the advantages of uniform test protocols and test reagents. Therefore, when such a test system has proved to be robust, and the variation between different batches is low, it might be expected to find smaller differences between the test results from different laboratories, compared to the test results of “in-house tests” such as HI and VNT.

Proficiency testing schemes (PTSs) are an important tool for laboratories and their customers to be able to compare the results with those from other laboratories. PTSs are also important subjects within laboratory quality systems, and participation is strongly advised for ISO 17025 accredited laboratories (International Standard ISO/IEC 17025 2005). PTSs can also provide information about the variation in test results of different test systems. Detection of a wide variation in results of test systems that are used on an international scale might stimulate laboratories to use more standardized protocols in order to be able to produce more comparable test results. The Dutch Animal Health Service (GD /AHS) organized proficiency testing schemes for serological tests for IBDV and NDV antibodies in 2005, in which 125 and 120 laboratories respectively participated from Africa, Asia, Europe, Central and South America. The results are presented and discussed in this paper.

Materials and Methods

Sera. Both IBDV and NDV antibody PTSs consisted of 8 freeze-dried chicken serum samples, that were sent to each of the participants, with the request to test the samples for antibodies against IBDV or NDV, respectively. It was requested to test the samples twice in different test runs. Results were expected to include AGPT, VNT, HI and/or ELISAs (commercially available or "in-house" test systems). In each PTSs, the sera (Table 1) were identified by sample numbers only.

Serum 1 was a pooled serum from 2 groups of vaccinated broilers (D78 or 228E) that had been challenged with the very virulent IBDV strain, D6948 at 21 days after vaccination. The birds were bled at 2 weeks after challenge.
Sera 2 – 7 were produced in a vaccination experiment in which 16-week-old specified pathogen free (SPF) White Leghorn chickens kept in Horsefall-Bauer isolators were individually vaccinated with a single dose of live IBDV vaccine according to the manufacturer's instructions. At 7, 10 or 14 days post vaccination (dpv), birds were removed for blood sampling. The sera were pooled (according to sampling day) before freeze-drying.
Serum 8 was a pooled serum from 52 week-old SPF layers and was used in both PTSs as the negative sample.
Serum 9 was a pooled serum from SPF chickens that were inoculated twice intratracheally with a high dose of NDV, LaSota strain. The birds were bled 4 weeks after the second inoculation.
Sera 10 - 15 were produced in a vaccination experiment in which 3-week-old SPF White Leghorn chickenss kept in Horsefall-Bauer isolators were individually vaccinated with a single dose of live NDV vaccine according to the manufacturer's instructions. At 7, 10 or 14 dpv, birds were removed for bleeding. The sera were pooled before freeze-drying.

In a pilot study, the homogeneity, stability, possible damage in transport and effects of ambient conditions on the samples had been checked and approved (ISO 43-1).

Participants. Each of the participating laboratories was given a unique ID-code which was only disclosed to the laboratory itself. In total, 125 laboratories from 39

countries from Africa (7), Asia (11), Europe (86) and Central and South America (21) participated in the IBDV antibody trial. One hundred and twenty laboratories participated from 37 countries from Africa (7), Asia (10), Europe (81) and Central and South America (22) in the NDV antibody trial. All laboratories reported their test results, except for one (for both the IBDV and the NDV PTS).

Tests. In the ring trial for IBDV antibodies, the participating laboratories reported the results (qualitative and quantitative) from 18 AGPTs, 13 VNTs and 127 ELISAs. The results of 11 ELISA systems (9 commercial and 2 "in-house" systems) were reported, in which 4 commercial ELISAs were used by 7 to 66 participants. The data of these 4 ELISAs and the VNT were used for statistical analyses.

The reported NDV results included data from VNT (1), HI test (52) and ELISA (97). There were 8 ELISA systems used in this PTS, of which 3 were commercial ELISAs, used by 6 to 57 participants. The data of these 3 ELISA systems and the HI test were used for further statistical analyses. The laboratories were also asked to include the cut-off level they used for the VNT and HI test (to distinguish between negative and positive test results). For the commercially available test systems, the cut-off level was used as indicated by the producer.

Statistical analyses. Laboratories were asked to test the serum panel twice on two different days. The (absolute) difference of the test results (expressed as $\log_2$ titres) of the duplicates was used for calculating the within laboratory reproducibility (R_{within}) of a test system. The R_{within} of a test system (HI test, VNT or ELISAs) was defined as the mean of all (absolute) differences between the duplicates of all sera of all laboratories performing that test system.

The variation in reported titres by all laboratories using the same test system was used for calculating the between laboratory reproducibility ($R_{between}$). The standard deviation (SD) of all reported titres was calculated for each of the 7 sera from vaccinated and/or challenged birds. The $R_{between}$ of a test system was defined as the mean of the SDs of these 7 sera.

The range of titres for a serum within a test system was determined by calculating the difference between the highest and the lowest reported titre (expressed as $\log_2$ titres) by the laboratories using that test system. The mean range of titres of a

test system was defined as the mean of all ranges of the 7 sera from vaccinated and/or challenged birds. No outliner testing was done.

For each laboratory and test system, the total mean reported titre (log_2) of all sera was calculated. The accuracy within the same test system was expressed as the percentage of laboratories with one or more log_2 steps difference in their total mean titre compared to the total mean titre of all laboratories together using the same system (ISO 5725-2 (1994).

Results

IBDV AGPT. Table 2 shows the qualitative results for the AGPT system performed by the 18 laboratories. Despite the use of 14 different IBDV strains as antigen, all AGPT results from these 18 laboratories were positive for the vaccination/challenge serum and the sera collected 10 and 14 dpv. Samples 2 and 4 (both collected 7 dpv) were negative in most AGPT systems. No false positive results were reported.

IBDV VNT. The quantitative and qualitative results for the VNT as performed by 13 laboratories are also presented in Table 2. Using IBDV serotype 1 antigens, all laboratories scored the SPF serum sample negative. VNTs of all laboratories scored positive results on 6 out of 7 sera that originated from vaccinated and/or challenged chickens. This interpretation of the reported VNT titres was based on the cut-off titre as indicated by the laboratory itself. The reported cut-off titres varied between 4 (lowest log_2 titre reported for a positive sample) and <6 (highest titre reported for a negative sample), and from 6 to 8 for "suspect" results. Forty-six percent of the laboratories reported a total mean titre that differed by more than 1 log_2 step from the total mean titre of all laboratories' results taken together (Table 3). Eight percent of the laboratories reported a total mean titre that differed by more than 2 log_2 steps from the total mean titre of all the results from the different laboratories.

The mean range between the lowest and highest result of the laboratories on each of the 7 individual sera that originated from vaccinated and/or challenged chickens was 7.0 log_2 steps. The total mean R_{within} and $R_{between}$ were 0.5 and 1.7 log_2 steps.

IBDV ELISAs. The qualitative and quantitative results obtained using 4 commercially available ELISA systems used by 7 to 66 participants are presented in Table 2. The qualitative test results were based on the cut off values/ thresholds as indicated by the respective manufacturers. According to the ELISA used, between 72% and 96% of the laboratories scored the SPF sample negative, and between 84% and 100% of the laboratories scored a positive result on 6 out of 7 of the sera that originated from vaccinated and/or challenged chickens.

Between 0 and 3% of the laboratories reported a total mean titre that differed more than 1 $\log_2$ step from the total mean titres of all laboratories taken together for the different ELISA systems (Table 3). In one of the ELISA systems a laboratory reported a total mean titre that differed more than 2 $\log_2$ steps from the total mean titre of all laboratories. The total mean R_{within} and $R_{between}$ of the ELISA systems were 0.3 and 0.7 ($\log_2$) steps.

The total mean titre of ELISA B on the 7 sera from vaccinated and/or challenged chickens were, on average, 3.9, 2.7, and 0.9 $\log_2$ steps higher than the total mean titres of ELISAs D, A and C, respectively.

NDV VNT. The reported VNT (performed by one laboratory only) was negative on the SPF serum and positive on all other sera. Further statistical analysis was not possible.

HI test for NDV. The quantitative and qualitative results for the HI tests, as performed by 52 laboratories, are presented in Table 4. Many different NDV antigens were used. In total, 98% of all laboratories scored the SPF sample negative, and between 69% and 98% of the laboratories scored a positive result on the 7 sera that originated from the vaccinated chickens. Most false negative results were observed with the 2 sera that were collected 7 dpv. The interpretation on the reported HI titres was based on the cut-off titre as indicated by the laboratory itself. The cut-offs of the HI tests varied between 2 (lowest $\log_2$ titre reported for a positive sample) and 4 (highest titre reported for a negative sample), and between 3 and 7 for "suspect" results.

Twenty percent of the laboratories reported a total mean titre that differed more than 1 $\log_2$ step from the total mean titre of the results from all laboratories

together (Table 3). Four percent of the laboratories reported a total mean titre that differed by more than 2 $\log_2$ steps from the total mean titre of all laboratories.

The mean range between the lowest and highest result of the laboratories for each of the 7 individual sera that originated from vaccinated and/or challenged chickens was 6.3 $\log_2$ steps. The total mean R_{within} and $R_{between}$ were 0.4 and 1.3 $\log_2$ steps.

NDV ELISA. The qualitative and quantitative results, obtained from the 3 commercially available ELISA systems that were used by 6 to 57 participants, are presented in Table 4. Results were based on the cut off values/ thresholds as indicated by the respective manufacturers. Depending on the ELISA used, between 43% and 96% of the laboratories scored the SPF sample negative and 54% to100% of the laboratories reported a positive ELISA result on the 7 sera that originated from vaccinated chickens. Most false negative results were observed with serum sample 11 that was collected 7 dpv.

Between 0 and 6 % of the laboratories reported a total mean titre that differed by more than 1 $\log_2$ step from the total mean titre of all laboratories together for the different ELISA systems (Table 3). In one of the ELISAs, a laboratory reported a total mean titre that differed more than 2 $\log_2$ steps from the total mean titre of all laboratories.

The mean R_{within} and $R_{between}$ of the ELISA systems were 0.3 and 0.7 $\log_2$ steps. The total mean of all titres of ELISA B on the 7 sera from vaccinated and/or challenged chickens were on average 1.1, and 0.8 $\log_2$ steps higher than the titres of ELISA C and A respectively. The mean of the ranges of the 3 ELISAs between the lowest and highest result of the 7 sera that originated from vaccinated chickens was 3.3 $\log_2$ steps.

Discussion

The data from a global proficiency testing scheme using different serological tests for NDV and IBDV antibodies were used to analyse the variation in test results within and between laboratories and test systems used worldwide. These results cannot be used to determine the quality of individual test systems, as the results could have been influenced by mistakes in test performance, sample treatment or logistics (exchange of sera) caused by some of the participating laboratories. When the number of participants using a certain test increases, there is an increasing risk that one or more laboratories report "odd" results, resulting in a wider range of reported titres for that serum or test system. Also, the high percentage of false positive results on the SPF serum in several ELISAs does not justify the conclusion that these tests have low specificities. Only one SPF serum sample (from 52 week-old SPF chickens) was used, and many more known negative sera from all ages of bird are required to determine the quality of a specific test system (ISO 17025), since it is known that older birds can show more non specific factors in their serum than young birds (Roberts *et al.,* 1992).

The quantitative results (antibody titres) obtained using the IBDV and NDV ELISAs, showed that the variation in reported titres between laboratories using the same ELISA was about half that of the VNT for IBDV or the HI test for NDV. However, differences in mean total titres of more than 2 $\log_2$ steps were recorded between different ELISAs. This confirms that it is essential for the interpretation of an ELISA titre to know which ELISA system was used.

The data show that the variation between quantitative test results of the different laboratories ($R_{between}$) using the IBDV VNT and the NDV HI test was higher (about double) compared to the variation within commercial ELISA systems. The remarkable differences in reported cut-off levels, the number of different antigens, the amounts of antigen, incubation times and temperatures used by the laboratories using the IBDV VNT or NDV HI test, showed that there are major differences in test procedures. Although both tests are often referred to and used as the so called "gold standard" in experimental and scientific studies (Alexander & Gough, 2003; Lukert & Saif, 2003) and in official procedures, such as vaccine efficacy testing, import, export and validation of tests (Thayer *et al*, 1987; De Wit *et al,* 1992, 2001; De Wit & Van Loon, 1998), this study shows that there is an urgent need for a global implementation

of recommended test procedures (for example, according to the World Organisation for Animal Health (OIE) Manual) and for the inclusion of international reference sera in these studies.

As global standardization of test procedures, for example VNTs, HI tests and ELISA systems, is difficult to achieve, the inclusion of international reference sera could be a relatively fast and easy step forward. When international reference sera are included in these studies, the quantitative results of such sera would provide very useful information on the comparability of the test results of this study to those of other studies. This study shows that when a laboratory reports a correlation between an antibody titre for IBDV or NDV and protection (Thayer *et al*, 1987; Van den Berg & Meulemans, 1991; Maas *et al*, 2001, 2003; Rahman *et al.,* 2004), or vaccine efficacy (Maas *et al*, 2001, 2003; Mebatsion *et al*, 2002; Claassen *et al*, 2004), or breakthrough titres for vaccination, and so on, without including results for any international reference sera, these titres cannot be interpreted correctly by other laboratories or authorities. Without the use of reference sera it is not possible to know the variation between laboratories or the comparability with other laboratories. Inclusion of reference sera will also increase the visibility of the need of standardization of test systems.

The Office International des Epizooties (OIE) has recommended use of reference sera for standardization of several test systems, for example in bovine herpesvirus type 1 serological tests. A negative, a weak positive and a positive serum have been adopted as OIE international standards for bovine herpesvirus type 1 tests (OIE manual, 2004c). Based on the results from these global PTSs for IBDV and NDV antibody tests, we propose that a SPF serum sample, as used in this study, could be used as a negative international reference serum sample; while a serum sample collected 7 days after inoculation of SPF chickens with either IBDV or NDV could be used as a weak positive international reference sample. A serum sample from vaccinated and boosted SPF chickens could be used as a strong positive control serum.

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Table 1. *Description of serum samples provided to each participating laboratory*

Proficiency testing scheme	Serum No.	Number in proficiency testing scheme	Age at inoculation (weeks)	Virus strain (1 dose)	Sampling (days post vaccination)
IBDV	1	5	3	D78/228E, D6948	14 (days post challenge)
IBDV	2	8	16	D78	7
IBDV	3	1	16	D78	14
IBDV	4	6	16	Bursine 2	7
IBDV	5	7	16	Bursine 2	14
IBDV	6	3	16	Gallivac	10
IBDV	7	4	16	Gallivac	14
IBDV and NDV	8	2 and 4	52	No inoculation	
NDV	9	7	3	LaSota x2	28 (after booster)
NDV	10	8	3	Avipro ND HB1	10
NDV	11	5	3	Avinew VG/GA	7
NDV	12	6	3	Avinew VG/GA	10
NDV	13	3	3	NDW	10
NDV	14	1	3	Clone 30	7
NDV	15	2	3	Clone 30	14

Table 2. *Overview of the results of the AGPT, VNT and 4 ELISAs in the 2005 global ring test for IBDV antibody detection*

Test	Test result	IBDV serum sample identification, vaccines used and number of days between vaccination and blood sampling							
		8	1	2	3	4	5	6	7
		SPF	D78-228E/D6948	D78	D78	Bursine 2	Bursine 2	Gallivac	Gallivac
		-	14	7	14	7	14	10	14
AGPT	pos (%)	-	100	11	100	6	100	100	100
	sus[a] (%)	-	-	6	-	6	-	-	-
	neg (%)	100	-	83	-	88	-	-	-
VNT	pos (%)	-	100	100	100	85	100	100	100
	sus (%)	-	-	-	-	15	-	-	-
	neg (%)	100	-	-	-	-	-	-	-
	Range[b]	<1 - <6	8 - 15	4 - 13	8 – 13	5 - 11	6 - 11	7 - >12	8 – 12
	mean[b]		11.4	8.7	11.3	7.2	8.5	10.2	10.5
	$R_{between}$[c]		2.0	2.3	1.5	1.8	1.4	1.7	1.2
	R_{within}[d]	0.1	0.5	0.7	0.7	0.4	0.6	0.3	0.3
ELISA A	pos (%)	3	100	84	99	1	99	100	100
	sus (%)	1	-	6	1	3	1	-	-
	neg (%)	96	-	10	-	96	-	-	-
	Range	0 – 9.8	11.0 – 12.9	6.4 - 11.1	8.6 – 12.6	0 – 10.2	0 – 11.4	10.8 – 11.9	11.1 – 12.7
	Mean	6.5	11.8	9.1	11.8	5.9	10.4	11.4	11.8
	$R_{between}$	2.1	0.3	1.0	0.4	2.1	0.9	0.3	0.3
	R_{within}	1.0	0.1	0.5	0.2	1.0	0.4	0.1	0.2
ELISA B	pos (%)	20	100	100	100	88	100	100	97
	sus (%)	6	-	-	-	6	-	-	-
	neg (%)	74	-	-	-	6	-	-	3
	Range	5.9 – 9.9	12.8 – 14.1	11.2 – 14.3	13.0 – 14.4	6.1 - 11.0	12.7 – 14.0	13.1 – 14.5	0 – 14.5
	Mean	8.0	13.5	12.9	13.8	9.3	13.4	13.8	14.0
	$R_{between}$	0.9	0.3	0.6	0.3	0.8	0.3	0.3	0.3
	R_{within}	0.3	0.1	0.2	0.1	0.3	0.1	0.1	0.1
ELISA C	pos (%)	-	100	100	100	22	100	100	100

	sus (%)	22	-	-	-	22	-	-	-
	neg (%)	78	-	-	-	56	-	-	-
	range	6.4 – 8.8	11.8 – 13.2	10.7 – 12.7	12.6 – 14.0	5.0 – 9.6	11.8 – 13.2	12.7 – 13.8	12.7 – 14.0
	mean	7.7	12.6	11.6	13.5	7.9	12.6	13.3	13.5
	$R_{between}$	0.6	0.5	0.6	0.3	1.0	0.4	0.3	0.3
	R_{within}	0.3	0.1	0.2	0.1	0.6	0.2	0.1	0.1
ELISA D	pos (%)	28	100	100	100	-	100	100	100
	sus (%)	-	-	-	-	-	-	-	-
	neg (%)	72	-	-	-	100	-	-	-
	range	0 – 6.6	9.6 – 12.2	6.6 – 8.6	10.1 – 11.6	0 – 5.6	8.6 – 10.4	6.3 – 11.1	10.4 – 12.6
	mean	5.1	11.1	7.7	11.2	2.4	9.7	10.4	11.5
	$R_{between}$	2.3	0.9	0.6	0.5	2.9	0.5	1.1	0.9
	R_{within}	0.0	0.3	0.4	0.3	1.6	0.4	0.8	0.5

[a] : a result is considered to be “suspect” when both duplicate test results are not the same.
[b] : presented as $\log_2$ titre.
[c] : between laboratory reproducibility.
[d] : within laboratory reproducibility.

Table 3. *Differences between the total mean titre obtained in different laboratories and the total mean titre of a test system*

Test	Difference (log_2) between the total mean titre of laboratories (expressed as the percentage of participating laboratories) and the total mean titre of a test system							
	- 3.999 – 3.0[a]	- 2.999 – 2.0	- 1.999 – 1.0	- 0.999 - 0	0 – 0.999	1.0 – 1.999	2.0 – 2.999	3.0 – 3.999
IBDV								
VNT			23[b]	39	15	15	8	
ELISA A				50	49	1		
ELISA B		3		46	51			
ELISA C				55	45			
ELISA D				43	57			
NDV								
HI		2	8	40	40	8	2	
ELISA A	2	2		30	64	2		
ELISA B				42	58			
ELISA C				43	57			

[a]: difference in log_2 steps between the total mean titre of a laboratory for a test system, and the total mean titres of all laboratories in the same test system.
[b]: percentage of the laboratories using that test system.

Table 4. *Overview of the results of the VNT, HI test and 3 ELISAs in the 2005 global ring test for NDV antibody detection*

Test	Test result	NDV serum used, vaccines and number of days between vaccination and blood sampling							
		8	9	10	11	12	13	14	15
		SPF	LaSota (x2)	HB1	Avinew	Avinew	NDW	Clone 30	Clone 30
		-	28	10	7	10	10	7	14
VNT	pos (%)	-	100	100	100	100	100	100	100
	neg (%)	100	-	-	-	-	-	-	-
	mean[b]	<1	10.3	5.5	4.0	4.3	5.3	2.1	8.0
HI	pos (%)	2	98	96	69	96	94	81	98
	Sus[a] (%)	-	-	-	4	2	2	4	-
	neg (%)	98	2	4	27	2	4	15	2
	range[b]	1 - 3	8 - 13	4 - 9	<1 - 7	5 - 9	3 - 8	1 - 7	7 - 12
	mean[b]	1.1	10.3	6.2	4.1	6.9	5.9	4.2	9.0
	$R_{between}$[c]	1.0	1.4	1.2	1.5	1.2	1.1	1.4	1.3
	R_{within}[d]	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.4
ELISA A	pos (%)	2	100	98	55	98	100	98	98
	Sus (%)	2	-	2	11	-	-	-	-
	neg (%)	96	-	-	34	2	-	2	2
	range	0 – 11.4	9.8 – 15.5	8.5 – 13.8	0 – 11.2	5.5 – 13.5	8.6 – 12.3	7.5 – 12.1	6.8 – 14.4
	mean	5.7	14.0	11.7	8.6	12.1	10.4	10.1	13.6
	$R_{between}$	2.5	0.9	0.7	1.4	1.0	0.6	0.7	1.0
	R_{within}	1.4	0.4	0.4	0.4	0.4	0.5	0.4	0.4
ELISA B	pos (%)	4	100	100	54	100	92	88	100
	sus (%)	4	-	-	15	-	8	8	-
	neg (%)	92	-	-	31	-	-	4	-
	range	5.5 – 10.9	14.1 – 15.2	11.2 – 12.8	9.2 – 11.5	11.5 – 13.6	9.9 – 11.9	10.0 – 11.8	13.6 – 14.9
	mean	8.0	14.7	12.1	10.3	12.6	11.1	11.0	14.1
	$R_{between}$	1.3	0.3	0.4	0.7	0.4	0.4	0.5	0.3
	R_{within}	0,7	0.1	0.2	0.3	0.3	0.2	0.3	0.1
ELISA C	pos (%)	43	100	100	58	100	100	100	100
	sus (%)	14	-	-	28	-	-	-	-

neg (%)	43	-	-	14	-	-	-	-
range	0 – 8.4	13.2 – 15.6	10.4 – 11.6	7.6 – 8.4	11.2 – 12.6	8.1 – 9.4	8.6 – 9.4	11.5 – 15.6
mean	6.8	14.8	11.2	8.0	12.1	8.8	9.0	14.5
$R_{between}$	3.0	1.3	0.3	0.3	0.5	0.4	0.4	1.4
R_{within}	0.1	0.9	0.3	0.1	0.4	0.2	0.2	0.9

[a] : a result is considered to be "suspect" when both duplicate test results are not the same.
[b]: expressed as $\log_2$ titres.
[c]: between laboratory reproducibility.
[d]: within laboratory reproducibility.