

# Repository of intra- and inter-run variations of quantitative autoantibody assays: a European multicenter study

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# Repository of intra- and inter-run variations of quantitative autoantibody assays: a European multicenter study

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## Abstract

**Objectives:** No reference data are available on repositories to measure precision of autoantibody assays. The scope of this study was to document inter- and intra-run variations of quantitative autoantibody assays based on a real-world large international data set.

**Methods:** Members of the European Autoimmunity Standardisation Initiative (EASI) group collected the data of intra- and inter-run variability obtained with assays quantifying 15 different autoantibodies in voluntary

participating laboratories from their country. We analyzed the impact on the assay performances of the type of immunoassay, the number of measurements used to calculate the coefficient of variation (CVs), the nature and the autoantibody level of the internal quality control (IQC). **Results:** Data were obtained from 64 laboratories from 15 European countries between February and October 2021. We analyzed 686 and 1,331 values of intra- and inter-run CVs, respectively. Both CVs were significantly dependent on: the method of immunoassay, the level of IQC with higher imprecision observed when the antibody levels were lower than 2-fold the threshold for positivity, and the nature of the IQC with commercial IQCs having lower CVs than patients-derived IQCs. Our analyses also show that

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the type of autoantibody has low impact on the assay' performances and that 15 measurements are sufficient to establish reliable intra- and inter-run variations.

**Conclusions:** This study provides for the first time an international repository yielding values of intra- and inter-run variation for quantitative autoantibody assays. These data could be useful for ISO 15189 accreditation requirements and will allow clinical diagnostic laboratories to assure quality of patient results.

**Keywords:** immunoassay; inter-run variation; internal quality control; intra-run variation; laboratory accreditation; precision; quality assurance.

## Introduction

Specialized autoimmunity laboratories face the crucial problem of the lack of standardization mainly due to the variability of the nature of the antigens and of the immunoglobulin conjugate used in the different immunoassays available in the market. In addition, these latter assays, such as the classical ELISA (enzyme-linked immunosorbent assay) or the more recently developed automated methods FEIA (fluorescent-enzyme immunoassay), CLIA (chemiluminescent immunoassay) or ALBIA (addressable laser bead immunoassay), are using different methods of detection, different positive threshold and different arbitrary units. Moreover, other parameters may influence the precision' performances of the autoantibody assays, such as the autoantibody level and the nature of the samples used for the verification experiments (i.e. "in house" determination of the assays' precision).

The ISO 15189 norm advocates the assessment of several key parameters at the initiation of the accreditation process and regularly afterwards to maintain the label. Among these parameters included is the measurement precision, i.e. the extent to which repeated measurements of the same sample give similar results, often expressed as coefficient of variation (CV). This is extremely important for detecting errors during the analytical phase and helps to ensure day-to-day consistency of the process. Precision is measured for repeatability conditions of measurement (also called intra-run precision) and for reproducibility conditions of measurement (also called inter-run precision) that includes different locations, operators, measuring systems or procedures. These parameters need the use of internal quality controls (IQC) samples that are measured several times allowing the calculation of both intra- and inter-run precision. These samples may be derived from patient' sera or plasma, or can be from commercial origin containing a

defined quantity of a certain autoantibody in a defined physiological buffer. The commercial IQC samples may be included in the commercial kits to assess the interval ranges that should be used for the immediate validation of an experiment, or either may be bought from various independent companies and used for inter-laboratory comparisons.

Altogether, these parameters largely complicate the analysis of the assays' performances which have to be obtained in the context of ISO 15189 accreditation [1]. Until now no repository containing benchmark values for intra- and inter-run CVs exists to help the laboratory professionals considering the results of their autoantibody assays as acceptable or not [2].

Hence, the interpretation of precision data obtained in clinical diagnostic laboratories (CDL), remains challenging in the field of autoimmunity. Contrary to other specialties such as clinical chemistry [3, 4] or hematology [5], few reference data have been proposed for autoantibody assays and these values are not taking into account the different parameters described above [6, 7]. To our knowledge, there is only a one study that recently addressed the question of the precision in autoantibody assays [8].

Considering the complexity of the field, the European Autoimmunity Standardisation Initiative (EASI) has been formed to harmonize autoantibody diagnostics [9]. The objective of the present study was to establish an international repository of data in autoimmunity that could be used in routine practice and for the accreditation of CDL. A large number of European CDL, predominantly associated with members of the EASI community, were requested to participate in a survey aiming to collect and analyze the CVs of intra-run and inter-run precision routinely obtained by several analytical methods quantifying 15 different autoantibodies. The results, obtained from 66 European laboratories, allow the analysis of inter- and intra-run variations of quantitative autoantibody assays according to different parameters such as the type of immunoassay, the number of measurements used to calculate the CVs, the nature and the autoantibody level of the IQC.

## Materials and methods

### Selection criteria of autoantibody assays

Fifteen quantitative autoantibody assays for which the quantitation is of clinical relevance were included in the survey that comprised: IgG and IgM isotypes of anti-beta two glycoprotein 1 (B2GP1) and anti-cardiolipin (aCL) autoantibodies, IgG isotype of anti-myeloperoxidase (MPO), anti-proteinase 3 (PR3), anti-glomerular basement membrane (GBM), anti-double stranded-DNA (dsDNA), anti-thyroglobulin (TG),

anti-thyroidperoxidase (TPO), anti-thyroid stimulating hormone receptor (TSH-R), anti-cyclic citrullinated peptides (CCP), IgG anti-deaminated gliadin peptides (DGP), IgA anti-tissue transglutaminase (tTG) autoantibodies and IgM isotype for rheumatoid factor (RF); the latter, however, is also often measured by nephelometry/turbidimetry and these assays are not specific for IgM isotype only [10].

## Data collection

The survey was proposed to all EASI participant countries and the study was performed from February to October 2021 by completing an excel sheet using preformatted columns containing drop down menu. The collected data comprised of the name of the analyte; the type of immunoassay; the number of values used for the CV calculation (<5, 5–15, 15–30 or >30 values); the level of the sample used as IQC according to the positive threshold (low: <2-fold, medium: between two and 5-fold, high: >5-fold) and its nature (patient sample prepared in the CDL, assay manufacturer-related commercial control, or kit-independent commercial control); and the results of intra- and inter-run CVs completed in percentage. The survey table allowed entering the data for each IQC used for the respective analyte. No specific measurement was asked to be performed and only already obtained data was shared.

## Statistical analysis

Prior to analysis, CVs calculated from less than five measurements were excluded. In addition, reported CVs below 0.1% or above 50% were excluded.

Data were analyzed according to the studied parameters (name of the analyte, type of immunoassay, number of values used for the CV calculation, level of the IQC, nature of IQC) by different statistical analysis using Prism-GraphPad™ software. Descriptive data are given using median and interquartile ranges in brackets (IQR [25–75%]). Unpaired t-test, Mann–Whitney test and one-way ANOVA with multiple comparisons (Tukey's multiple comparison test) were used. A

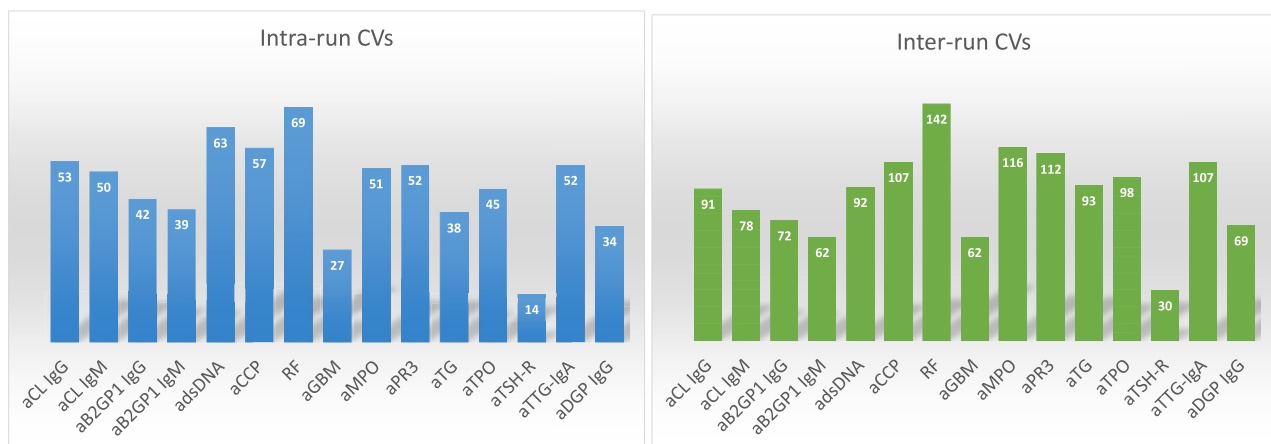
p-value less than 0.05 was considered significant. According to the model used by some societies of Biochemistry [6], we calculated two performance goals corresponding to the 90th percentile and the 50th percentile (median) from CVs distribution. The first target is the lowest CV value reached by 90% of laboratories and the second by 50% of laboratories. These performance goals were calculated for all studied parameters.

## Results

Data were collected from 64 laboratories from 15 European countries: Austria (1 laboratory), Belgium (6 laboratories), Croatia (6 laboratories), Estonia (4 laboratories), France (7 laboratories), Germany (2 laboratories), Greece (1 laboratory), Hungary (4 laboratories), Italy (16 laboratories), Netherlands (6 laboratories), Norway (4 laboratories), Spain (1 laboratory), Sweden (4 laboratories), Switzerland (5 laboratories) and United Kingdom (1 laboratory) (Supplementary Figure 1).

Altogether 2,354 values of CVs were collected, 686 values of intra-run CVs (4 values were excluded according to the predefined criteria) and 1,331 values of inter-run CVs (33 values excluded according to the predefined criteria). Assays accredited according to the EN ISO 1589 accounts for 60% of intra-run and 57% of inter-run CVs, respectively.

The number of CVs obtained for each analyte is depicted in Figure 1. It varied from 14 to 69 and from 30 to 142 values per analyte for the CVs of intra-run and inter-run precision, respectively. The highest numbers of collected data were for RF for both intra-run CVs as well inter run CVs. The lowest numbers of collected CVs were for anti-TSH-R autoantibody assays.



**Figure 1:** Number of CV values analyzed according to the type of autoantibody.

RF, rheumatoid factor; adsDNA, anti-double stranded-DNA; aTG, anti-thyroglobulin; aTPO, anti-thyroidperoxidase; aTSH-R, anti-TSH-receptor; aTTG, anti-tissue transglutaminase; aDGP IgG, IgG anti-deaminated peptide from gliadin; aB2GP1, anti-beta 2 glycoprotein 1 (IgG and IgM); aCL, anti-cardiolipin (IgG and IgM); aCCP, anti-cyclic citrullinated peptides; aMPO, anti-myeloperoxidase; aPR3, anti-proteinase 3; aGBM, anti-glomerular basement membrane.

## Impact of the type of immunoassay

The main types of immunoassays used by participants were fluorescent-enzyme immunoassays (FEIA: 525 values, 34%), chemiluminescence (CLIA: 466 values, 30%), enzyme linked immunosorbent assay (ELISA: 300 values, 19%), nephelometry/turbidimetry (101 values, 6%) and addressable laser bead immunoassay (ALBIA: 72 values, 5%). Ninety-seven values (6%) were obtained from other methods and represented each less than 1% of the reported methods (comprising immunodot (13 values), radio-immunoassays or immunocompetition). In total, the data were obtained from 19 different immunoassays' providers (Supplementary Figure 1A and B).

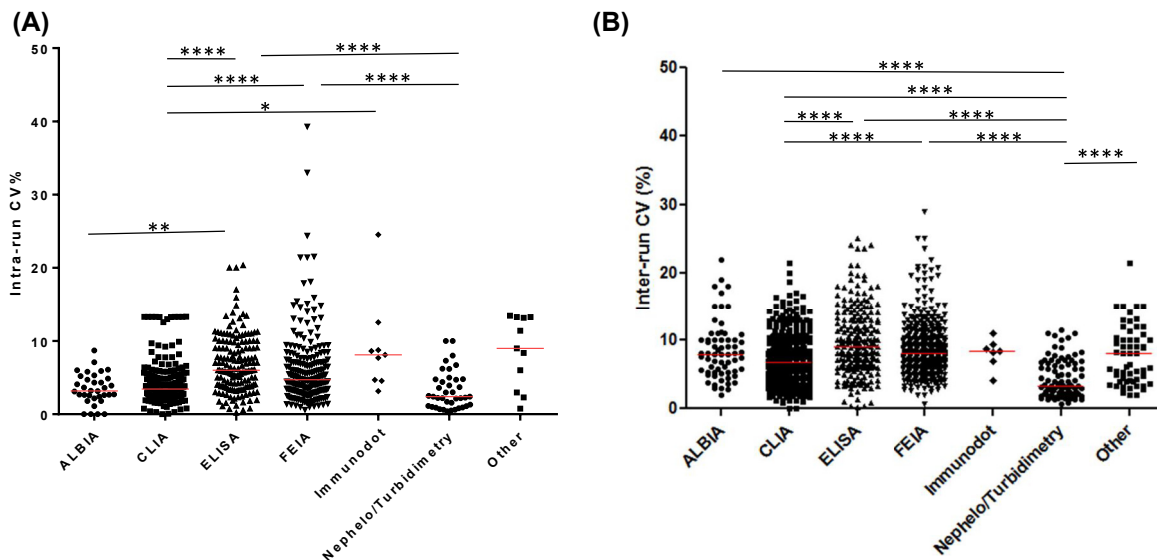
Overall, significantly lower CV values of intra-run (median and interquartiles: 2.4% [0.4–10]) and inter-run (median: 3.3% [0.6–11]) precision were obtained with immunoassays using nephelometry/turbidimetry as compared to all other methods (except for CLIA and ALBIA for intra-run CVs). Assays using CLIA had significantly lower intra-run and inter-run precision (median: 3.5% [0.3–13] and 6.8% [0.04–25]) than the other methods except for ALBIA methods and nephelometry/turbidimetry (Figure 2A,B, respectively and Table 1). As a consequence, we observed significant differences among the performances at 90th and 50th percentiles according to the type of immunoassay.

## Impact of the number of measurements

We first analyzed the results of CVs according to the number of measurements used for their determination (<5, 5–15, 15–30 or >30 values) (Figure 3). Surprisingly, higher CVs were observed when the number of measurements for intra-run precision was between 5 and 15 as compared to the other ranges (Figure 3A). However, no significant difference was observed between the four groups (one-way ANOVA) when considering inter-run CVs (Figure 3B). We then analyzed the same impact on inter-run according to the methods and observed significantly highest CVs when the number of measurements was >30 for ALBIA and FEIA, whereas no significant difference was observed for CLIA, ELISA and nephelometry/turbidimetry (Figure 3C).

## Impact of the levels of IQC

We next analyzed the impact of the analyte level of the IQC positivity in relation to the positive threshold (i.e., low: levels <2-fold the positive cut-off; medium: levels comprised between two and 5-fold the positive cut-off; or high: levels >5-fold the positive cut off) on the intra-run and inter-run precisions (Table 2). For both parameters we observed significantly lower CV values when the IQC was high as compared to the values observed when the IQC was



**Figure 2:** Levels of intra-run (A) and inter-run CVs (B) according to the method of the immunoassays.

FEIA, fluorescent-enzyme immunoassay; CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; ALBIA, addressable laser bead immunoassay. Only significant differences are shown. NS,  $p > 0.05$ ; \* $p < 0.05$ , \*\* $p = 0.01$ , \*\*\* $p = 0.001$ , \*\*\*\* $p < 0.0001$  (one way Anova). Red lines indicate the median.

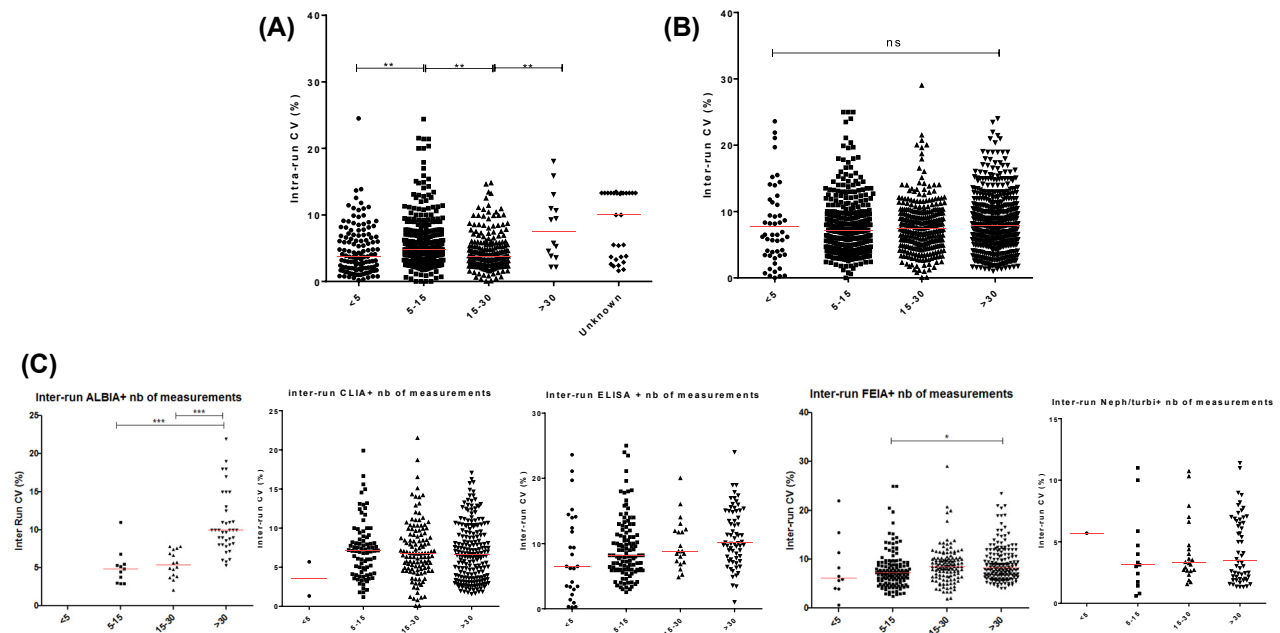
**Table 1:** Description of intra-run and inter-run CVs results according to the methods.

		ALBIA	CLIA	ELISA	FEIA	Immunodot	Nephelometry/ turbidimetry	Other
Intra-run CV	Number of values	30	203	164	224	9	39	11
	Minimum	1.1	0.3	0.2	0.6	3.2	0.4	0.8
	Maximum	8.7	13	20	39	25	10	13
	Median	<b>3.6</b>	<b>3.5</b>	<b>6</b>	<b>4.7</b>	<b>8.1</b>	<b>2.4</b>	<b>9</b>
	25% Percentile	2.6	2.5	3.9	3.2	4.6	1.1	3
	75% Percentile	5.2	5	9.7	7.4	11	4.8	13
90% Percentile	<b>6.1</b>	<b>7.8</b>	<b>11</b>	<b>12</b>	<b>25</b>	<b>7.3</b>	<b>13</b>	
Inter-run CV	Number of values	65	410	224	452	7	92	57
	Minimum	2	0.1	0.2	0.7	4.2	0.6	2
	Maximum	22	22	25	29	11	11	22
	Median	<b>7.8</b>	<b>6.8</b>	<b>9</b>	<b>8</b>	<b>8.3</b>	<b>3.3</b>	<b>8</b>
	25% Percentile	5.3	4.2	6.2	6.1	6.9	2.2	4.1
	75% Percentile	10	9.2	13	10	9.3	6.5	11
90% Percentile	<b>15</b>	<b>12</b>	<b>16</b>	<b>13</b>	<b>11</b>	<b>8.2</b>	<b>14</b>	

Bold values refer to the 50% (equivalent to the median) and 90% percentiles.

low (intra-run: median of CVs: 3.7% [2.5–5.8] vs. 4.9% [3.2–8.7],  $p=0.0002$ ; inter-run: median of CVs: 7% [4.5–9.5] vs. 8.1% [5.4–11.2],  $p=0.0001$  for high vs. low IQC, respectively) (Figure 4). A significant difference was also observed for the inter-run precision between low and medium levels of IQC ( $p=0.01$ ).

We then addressed the question whether the IQC levels have the same impact on inter-run precision, according to the method of immunoanalysis. For CLIA, ELISA and FEIA, we observed significantly higher imprecision for low IQC level in comparison to medium (ELISA and FEIA) and high IQC level (CLIA and FEIA) (Figure 4C).



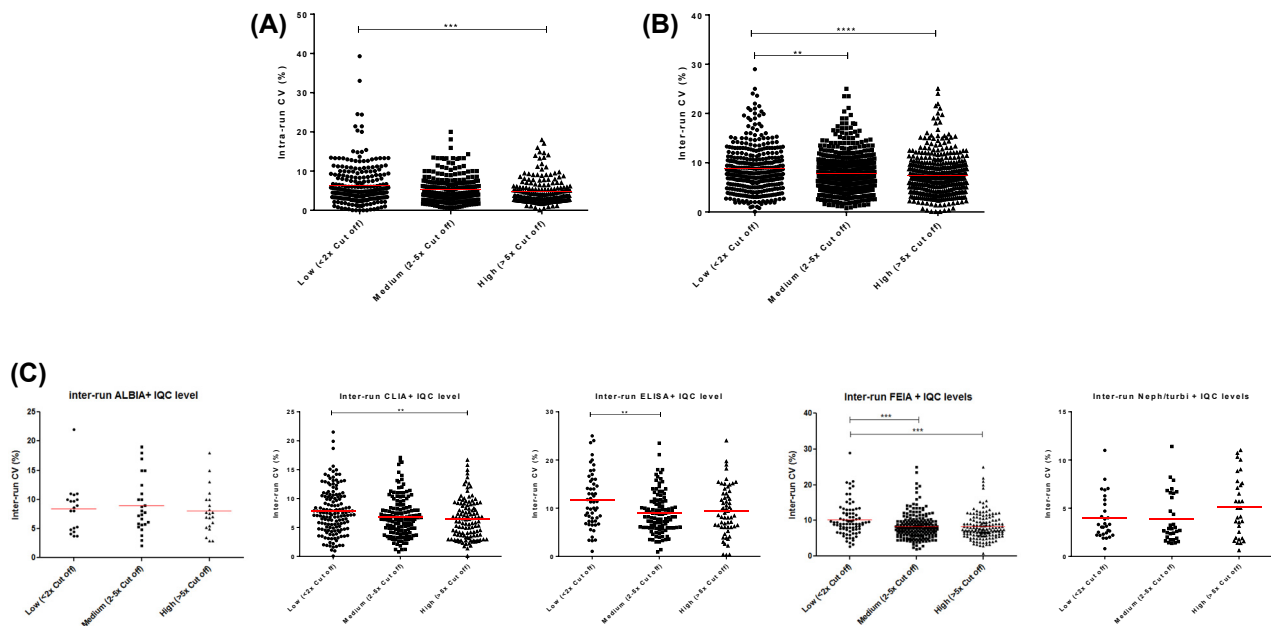
**Figure 3:** CVs according to the number of measurements used to calculate (A) the intra-run CVs and (B) the inter-run CVs. (C) The impact of the number of measurements for inter-run determination was also analyzed according to the methods (one way anova).

FEIA, fluorescent-enzyme immunoassay; CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; ALBIA, addressable laser bead immunoassay; nb, number. Only significant differences are shown.  $*p<0.05$ ,  $**p=0.01$ ,  $***p=0.001$ . Red lines indicate the median.

**Table 2:** Ranges of inter-run CV values according to the levels of IQC and the method of analysis.

IQC Levels	ALBIA			CLIA			ELISA			FEIA			Nephelometry/ turbidimetry		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Number of values	19	25	21	153	140	117	55	108	61	84	212	154	30	31	31
Median	<b>8.6</b>	<b>7.8</b>	<b>7.6</b>	<b>7.4</b>	<b>6.4</b>	<b>6</b>	<b>11</b>	<b>8.2</b>	<b>9</b>	<b>9.2</b>	<b>7.9</b>	<b>7.5</b>	<b>3.2</b>	<b>2.9</b>	<b>5</b>
25% Percentile	4.8	5.7	5.3	4.9	4.3	3.6	7.3	5.9	6	7.5	6	6	2.3	1.9	1.9
75% Percentile	10	12	10	10	8.5	8.9	16	11	13	12	9.7	10	5.5	6.6	7.6
90% Percentile	<b>11</b>	<b>17</b>	<b>15</b>	<b>13</b>	<b>11</b>	<b>11</b>	<b>20</b>	<b>15</b>	<b>15</b>	<b>17</b>	<b>12</b>	<b>12</b>	<b>7</b>	<b>7.7</b>	<b>10</b>

Low, IQC levels inferior to 2 fold the positive cut-off; medium, IQC levels comprised between 2 and 5 fold the positive cut-off; high, IQC levels superior to 5 fold the positive cut off. Bold values refer to the 50% (equivalent to the median) and 90% percentiles.



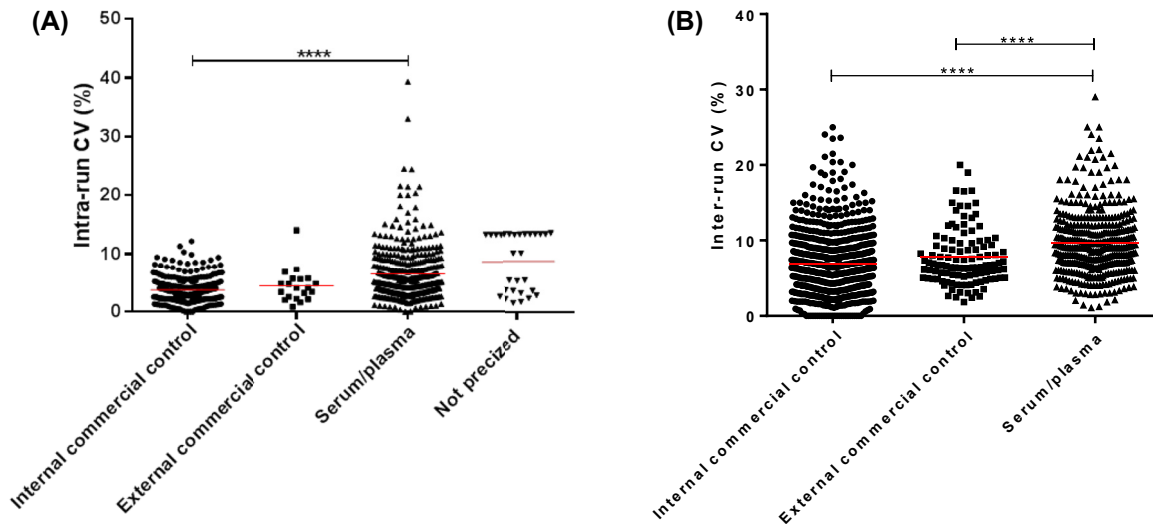
**Figure 4:** Intra- (A) and inter-run (B) CVs considering IQC levels. (C) The impact of IQC analyte levels for inter-run determination was also analyzed according to the methods (one way anova). FEIA, fluorescent-enzyme immunoassay; CLIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; ALBIA, addressable laser bead immunoassay Only significant differences are shown. \* $p < 0.05$ , \*\* $p = 0.01$ , \*\*\* $p = 0.001$ . Red lines indicate the median.

## Impact of the nature of IQC

We next analyzed the influence of the nature of the IQC on the results of the intra and inter-run CVs. Significantly higher values were observed if the IQC sample used for CV calculation originated from patient' sera or plasma, as compared to an internal commercial control (comprised in the reagent kit) for intra-run variation (median of CVs: 5.3% [3.3–8.5], vs. 3.3% [2.2–4.8]  $p < 0.0001$ ), or to both internal and external commercial (reagent-manufacturer-independent commercial control) controls for intra and inter-run variations (9% [6.8–12] vs. 6.8% [4.2–9] and 6.7% [5.1–9.6],  $p < 0.0001$ , respectively). No difference was observed between the two kinds of commercial IQC (Figure 5).

## Precision according to the analyte

We also analyzed the data for each analyte, i.e., the specific autoantibody quantified in the assays (Table 3). Overall, very few differences of intra-run CVs were observed between the different analytes (Supplementary Figure 3A, Supplementary Table 1). Intra-run CVs of RF assays (3.2% [1.6–5.6]) were significantly lower than those observed for anti-PR3 (6.1% [3.5–9.7]), aCL IgG (6% [3.5–7.8]), anti-TSH-R (7.4% [5.2–9.6]) and anti-tTG-IgA (4.7% [3.4–8.1]). Intra-run CVs for anti-TPO (2.9% [2.7–4.1]) were also lower than those obtained for anti-TSH-R and anti-tTG-IgA (Supplementary Figure 3A, Supplementary Table 1). Larger differences were observed for the inter-run CVs



**Figure 5:** Intra- and inter-run CVs according to the nature of the IQC.

Red lines indicates the median of CV. Only significant differences are shown. \*\*\*\* $p < 0.0001$ .

**Table 3:** Performance goals of intra- and inter-run CVs per analyte corresponding to the 90th percentile and the 50th percentile (median) from CVs distribution. The first target is the lowest CV value reached by 90% of laboratories and the second by 50% of laboratories.

	Intra-run CV, %		Inter-run CV, %	
	50th percentile	90th percentile	50th percentile	90th percentile
aCL IgG	6.5	10.0	8.4	15.1
aCL IgM	5.9	11.2	7.9	14.1
aB2GP1 IgG	5.7	11.3	8.5	14.1
aB2GP1 IgM	6.6	15.4	8.5	14.6
adsDNA	5.2	9.6	9.2	17.6
aCCP IgG	5.4	10.2	8.7	14.9
RF	3.9	8	5.5	10.9
aGBM	5.4	13.3	8.1	13.1
aMPO	5.4	11.2	8.5	12.9
aPR3	7.3	13.3	9.6	14.4
aTG	4.7	9.5	5	9
aTPO	3.8	7	6	10.7
aTSH-R	7.7	13.5	8.1	13.4
aTTG IgA	6.5	13.4	9	13.6
aDPG IgG	5.5	13.1	8	12.4

RF, rheumatoid factor; adsDNA, anti-double stranded-DNA; aTG, anti-thyroglobulin; aTPO, anti-thyroidperoxidase; aTSH-R, anti-TSH-receptor; aTTG, anti-tissue transglutaminase; aDPG IgG, IgG anti-deaminated peptide from gliadin; aB2GP1, anti-beta 2 glycoprotein 1 (IgG and IgM); aCL, anti-cardiolipin (IgG and IgM); aCCP, anti-cyclic citrullinated peptides; aMPO, anti-myeloperoxidase; aPR3, anti-proteinase 3; aGBM, anti-glomerular basement membrane; 50th per, 50th percentile; 90th per, 90th percentile.

(Supplementary Figure 3B, Supplementary Table 2). Overall, lower CVs were observed for RF assays (5.1% [2.5–7.8]) as compared to the majority of the other analytes (Supplementary Table 2). Similarly, anti-TG (4.5% [3.1–7]) and anti-TPO (5.3% [3.1–8]) assays exhibited lower intra-run CV than a majority of the other analytes. To further explore these differences, we analyzed the distribution of the methods used for detecting these autoantibodies. We noted that the most frequent methods were that of nephelometry/

turbidimetry for RF (61.6% of the laboratories) and CLIA for anti-TG and anti-TPO (50 and 54.8% of laboratories, respectively) (Supplementary Figure 4).

## Discussion

The present work is the result of an unprecedented collaboration between 64 Clinical Diagnostic Laboratories



(CDL) specialized in autoimmunity diagnostic from 15 European countries participating in the EASI group which aims at the improvement and harmonization of the diagnostic in the field of autoimmunity [1]. Our first objective was to establish a repository collecting performance parameters of the quantitative autoantibody immunoassays to which specialized laboratories may refer to interpret their verification results. Indeed, on the contrary to other specialties such as clinical chemistry (data available in some websites such as the Westgard, the European Federation of Laboratory Medicine (EFLM) or QUALAB websites), no benchmark data of intra and inter-run CVs have been established for the quantitative autoantibody assays. Only a recent national French study proposed the first data in this field [8]. Thus, our results, comprising more than 2,000 values collected from a large and international network of laboratories, represent a source of data for the precision of immunoassays detecting and quantifying 15 different autoantibodies. Every laboratory professional may now refer to these values to appreciate the performances of these assays and evaluate their robustness in their own laboratory. For this purpose, we propose two performance goals corresponding to the 90th percentile (lowest CV value reached by 90% of laboratories) and the 50th percentile (lowest CV value reached by 50% of laboratories) (median) from CVs distribution [4].

Interestingly, the detailed analyses of this large amount of collected data, was the impetus for the identification of crucial parameters which influence significantly the performances of these assays. First, our results show that the type of autoantibody has little or no impact on the assays' performances. Very few significant differences of performance were observed between the 15 different autoantibodies, and the differences that were found were merely correlated to the methods of immunoanalysis. Notably, we underline RF for which performances are influenced by the frequent use of nephelometry/turbidimetry (representing more than 61% of the methods used in our study). To our judgement, the second important finding of our results is highlighted by the importance of the method in use in the precision of the assays. Among the different methods assessed, significant lower intra- and inter-run CVs were observed for nephelometry/turbidimetry and CLIA as compared to the others. The high precision of these two methods is well known as they are routinely used for analytes measured in clinical chemistry [11, 12].

In addition, our analysis reveals the important influence of the IQC characteristics in the results of precision experiments. IQC samples can originate from different types of samples. They may be provided by the manufacturer of the respective immunoassay. These

reagents may be lyophilized or ready to use without dilution, and their reference values are usually provided by the manufacturer to be used for acceptance of the results. Alternatively, IQCs may be bought from providers other than the immunoassay manufacturer to ensure an independent assessment of performance as recommended [13–15], possibly requiring adapted dilutions on similar conditions than the patient's samples. Lastly, the CDL may use single or pooled anonymous patient samples. In the latter two situations the CDL has to determine the target value of the respective IQC. Information about the source of the respective controls in terms single patient derived or pooled material was, however, not investigated in this study.

Our results show that both nature and the level of the IQC significantly influence the results of the inter-run and intra-run CVs, a finding which is in accordance with that reported by the French study [8]. Indeed, significant lower imprecisions are observed with commercial IQC than patient' samples-derived IQC. This probably reflects the impact of the presence of preservatives used for the stabilization of commercial control samples, and also of the matrix proteins and of the immunoglobulins that are present in high concentrations in serum and may interfere with the interaction of the antigen-specific autoantibodies to their target. Moreover, controls included in commercial assays are frequently ready to use and are not treated in the same manner as patient samples that are diluted, thus introducing a significant analytical bias. As a consequence, they potentially provide a false measurement acceptance limit. For all these reasons, commercial IQCs do not provide the exact uncertainty of measurement that occurs in patient's samples, which is detrimental to the interpretation of patient results. Notably, this is important to interpret autoantibody level changes when they need to be monitored to adapt the therapeutics (for example anti-dsDNA in SLE, anti-PR3, anti-MPO in vasculitis or anti-GBM in Goodpature syndrome). If the uncertainty of measurement is calculated using a falsely low inter-run CV, the change in autoantibody level may be wrongfully interpreted as significant and may result incorrect clinical and therapeutic decisions. As previously recommended for antinuclear antibodies assays [16], our results confirm the need to use patients-derived IQC for quantitative autoantibody immunoassays. In addition to the nature of IQC, our large study confirms that the levels of positivity of the IQC affect the precision, lower precision is observed when values are below to 2-fold the positive threshold. This is probably due to the non-Gaussian distribution of autoantibody positivity in the population [17]. This borderline positivity may be observed in patients samples and its

clinical interpretation is challenging transforming a negative to a positive result and impacting the patient diagnosis and care. However, it is important to use an IQC closest to the levels of the clinical decision, so the positive threshold should take into account the calculated imprecision or a commentary to the physicians should be added to the results within this zone of high analytical imprecision, allowing appropriate interpretation of each patient result. The recent proposition of use of specific likelihood ratios for each assay may be a reliable way to circumvent this obstacle [18, 19].

Our large study confirms that the number of measurements used for the CV calculations had no significant influence on the intra- and inter-run CVs of autoantibody assays. Thus, this number may be moderate using only 10 to 15 measurements instead of 30 recommended by some accreditation technical guidances, which are sufficient to obtain reliable evaluation of the precision [8, 13]. These findings could substantially reduce the financial cost in achieving accreditation.

One limitation of our study is that most participating laboratories are highly specialized expertise in autoimmune diagnostics. This might induce some bias in the results, however, the collected data were obtained from the routine work up of the laboratories and no specific experiments were done for the study. In addition, Italian laboratories were somewhat over represented in the study (16/64, 2.5%). However, we did not observe any significant difference in terms of method distribution when we excluded their data from the analysis (data not shown).

In conclusion, this study provides a repository of data collected from a large number of different laboratories across Europe yielding benchmark values of intra- and inter-run variation for quantitative autoantibody assays, data that were lacking until now to help CDL in applying for ISO 15189 accreditation. Furthermore, our data reveals that the main parameters that influence autoantibody assays precision are the methods of detection/revelation, the nature and the level of the IQC used for their assessment. These data will allow CDL to assure quality of patient' results. Highly reproducible laboratory results are crucial to guarantee correct clinical interpretation of the results.

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## References

- Bizzaro N, Bossuyt X, Haapala AM, Shoenfeld Y, Sack U. Accreditation in autoimmune diagnostic laboratories. A position paper of the european autoimmunity standardisation initiative (EASI). *Autoimmun Rev* 2016;16:81–6.
- Mulder L, van der Molen R, Koelman C, van Leeuwen E, Roos A, Damoiseaux J. Validation conform ISO-15189 of assays in the field of autoimmunity: joint efforts in The Netherlands. *Autoimmun Rev* 2018;17:513–7.
- Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, et al. Current databases on biological variation: pros, cons and progress. *Scand J Clin Lab Invest* 1999;59:491–500.
- Vassault A, Grafmeyer D, de Graeve J, Cohen R, Beaudonnet A, Bienvenu J. [Quality specifications and allowable standards for validation of methods used in clinical biochemistry]. *Ann Biol Clin Paris* 1999;57:685–95.
- Vis JY, Huisman A. Verification and quality control of routine hematology analyzers. *Int J Lab Hematol* 2016;38:100–9.
- Westgard J. Westgard QC 2019. Available from: <https://www.westgard.com>.
- Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988 (clia) proficiency testing regulations related to analytes and acceptable performance. *Fed Regist* 2019;84:1536–67.
- Senant M, Musset L, Chyderiotis G, Guis-Cabanne L, Damoiseaux J, Fabien N, et al. Precision of autoantibody assays in clinical diagnostic laboratories: what is the reality? *Clin Biochem* 2020;83:57–64.
- Damoiseaux J, Olschowka N, Shoenfeld Y. EASI - european autoimmunity standardisation initiative: facing the challenges of diagnostics in autoimmunity. *Clin Chem Lab Med* 2018;56:1620–3.
- Sieghart D, Platzer A, Studenic P, Alasti F, Grundhuber M, Swiniarski S, et al. Determination of autoantibody isotypes increases the sensitivity of serodiagnostics in rheumatoid arthritis. *Front Immunol* 2018;9:876.
- Wang M, Li J, Huang Y, Chen T, Dong S, Zhang R, et al. Analytical validation of the LiCA<sup>®</sup> high-sensitivity human thyroid stimulating hormone assay. *Clin Biochem* 2022;101:42–9.
- Benard C, Chauzeix J, Blancher A, Puissant-Lubrano B. Analytical performances of SPAPLUS<sup>®</sup> turbidimeter for the quantification of albumin and IgG in serum and cerebrospinal fluid. *Clin Biochem* 2016;49:828–31.
- SH GTA 06. Guide technique d'accréditation: contrôle de qualité en biologie médicale. COFRAC; 2012.
- Kinns H, Pitkin S, Housley D, Freedman DB. Internal quality control: best practice. *J Clin Pathol* 2013;66:1027–32.
- CLSI. Statistical quality control for quantitative measurement procedures: principles and definitions. Clinical and Laboratory Standards Institute; 2016, C24-A4.
- Bogaert L, Van den Brems S, Schouwers S, Bossuyt X, Van Hoovels L. Harmonizing by reducing inter-run variability: performance evaluation of a quality assurance program for antinuclear antibody detection by indirect immunofluorescence. *Clin Chem Lab Med* 2019;57:990–8.
- Sepe V, Eldridge S, Loviselli A, Cirillo R, Bottazzo GF. Definition of cut-off points for autoantibody assays in cohorts of healthy individuals. The sardinian school children-IDDM (SSI) & newborn-IDDM (SNI) study groups. *Lancet* 1996;347:693.
- Sack U, Bossuyt X, Andreeva H, Antal-Szalmás P, Bizzaro N, Bogdanos D, et al. Quality and best practice in medical laboratories: specific requests for autoimmunity testing. *Auto - Immun Highlights* 2020;11:12.
- Bossuyt X, Damoiseaux J, Rasmussen N, van Paassen P, Hellmich B, Baslund B, et al. Harmonization of antineutrophil cytoplasmic antibodies (ANCA) testing by reporting test result-specific likelihood ratios: position paper. *Clin Chem Lab Med* 2020;59:e35–9.

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