



HAL
open science

Precision of autoantibody assays in clinical diagnostic laboratories: What is the reality?

Marie Senant, Lucile Musset, Georges Chyderiotis, Laurence Guis-Cabanne,
Jan Damoiseaux, Nicole Fabien, Marie-Agnès Dragon-Durey

► To cite this version:

Marie Senant, Lucile Musset, Georges Chyderiotis, Laurence Guis-Cabanne, Jan Damoiseaux, et al.. Precision of autoantibody assays in clinical diagnostic laboratories: What is the reality?. *Clinical Biochemistry*, 2020, 83, pp.57 - 64. 10.1016/j.clinbiochem.2020.05.019 . hal-03491299

HAL Id: hal-03491299

<https://hal.science/hal-03491299v1>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Precision of autoantibody assays in Clinical Diagnostic

Laboratories: what is the reality?

Running title : **Precision of autoantibody assays**

Marie Senant^{1,2}, Lucile Musset³, Georges Chyderiotis⁴, Laurence Guis-Cabanne⁵, Jan Damoiseaux⁶, Nicole Fabien⁷, Marie-Agnès Dragon-Durey^{1,8}

1: Laboratoire d'Immunologie, Hôpital Européen Georges Pompidou, APHP, Paris, France.

2: Cerballiance, 41 rue du bois chaland, 91090 Lisses, France.

3: Département d'Immunologie, UF immunochimie & autoimmunité, CHU Pitié Salpêtrière-Ch Foix, APHP, Paris, France.

4: Immunologie, Laboratoire Eurofins Biomnis, Lyon, France.

5: Immunologie, Laboratoire Eurofins Biomnis, Ivry-sur-Seine, France.

6: Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, The Netherlands.

7: Service d'Immunologie, UF Autoimmunité, Hospices Civils de Lyon, CHLS, Pierre-Bénite, France.

8: Université de Paris, Paris, France.

Corresponding author: Marie-Agnès Dragon-Durey, MD, PhD for EASI France group, Laboratoire d'Immunologie, Hôpital Européen Georges Pompidou, APHP, Université de Paris, 20 rue Leblanc, 75015 Paris, France
Tel + 33 1 56 09 59 99
Fax : + 33 1 56 09 20 80
Email : marie-agnes.durey@aphp.fr

Number of words: 3058, **Number of Figures:** 5, **Number of tables:** 2, **Number of references:** 28.

ABSTRACT

Background: ISO 15189 accreditation remains a challenge for specialized laboratories. In the field of autoimmunity, beside the crucial problem of absence of standardization, laboratories have to manage the analytical performances of the large panel of assays in terms of sensitivity and specificity, but also on their measurement precision for which no reference values are available on biorepositories.

Methods: As an initiative of the French EASI (European Autoimmunity Standardization Initiative) group, French clinical diagnostic laboratories were requested to participate in a survey aiming to analyze the coefficients of variation (CVs) of intra-run and inter-run variability obtained with assays quantifying 14 different autoantibodies. Two performance goals corresponding to the 90th percentile and the 50th percentile (lowest CV values reached by 90 % and 50% of laboratories respectively) defined for three levels of concentration were calculated. The impact on the assay performances of the number of measurements, of the nature of the internal quality control (IQC) and the type of immunoassay, was also analyzed.

Results: 414 and 616 values of intra-run and inter-run CVs were collected, respectively. The 50th percentile performance goals were comprised between 1.0% and 8.9% for the intra-run CVs, and between 1.8% and 14.6% for the inter-run CVs. At 90th percentile, the performance goals were comprised between 3.2% and 13.5% for the intra-run CVs, and between 7.3% and 30.8% for the inter-run CVs. CVs calculated from 10 values were similar to those obtained from more values. Higher imprecision was observed when the antibody levels of the IQC was lower than 2 fold the positive threshold. Commercial IQCs gave lower CVs than IQCs derived from patient samples.

Conclusion: Our results allow proposing some acceptability limits for the precision performances of the autoantibody assays, compatible with the reality of life in diagnostic laboratories and clinical care.

Key words: Autoantibody, laboratory accreditation, coefficient of variation, analytic performances, Internal Quality Control.

ABBREVIATIONS

ALBIA: addressable laser bead immunoassay

B2GP1: β 2 glycoprotein 1

CCP: cyclic citrullinated peptides

CDL: clinical diagnostic laboratories

CL: cardiolipin

CLIA: chemiluminescent immunoassay

CV: coefficient of variation

dsDNA: double stranded-DNA

ELISA: enzyme-linked immunosorbent assay

FEIA: fluorescent-enzyme immunoassay

GBM: glomerular basement membrane

IQC: internal quality control

MPO: myeloperoxidase

PR3: proteinase 3

RF: rheumatoid factor

TG: thyroglobulin

TPO: thyroperoxidase

TSH-R: thyroid stimulating hormone (TSH)-receptor

TTG: tissue transglutaminase

1. INTRODUCTION

Specialized laboratories have to take up the challenge of applying for and maintaining ISO 15189 accreditation, with specific difficulties relative to the laboratory equipment, the pre-examination phase and examination procedures [1]. In the field of autoimmunity, the laboratories have to face the crucial issue of the absence of assay standardization. This is linked to the nature of the antigens, the immunoglobulin conjugate, the methods of detection and even the units of the results [2]. Consequently, laboratories have to manage the analytical performance of the large panel of assays available on the market [3]. The evaluation of such performances involves not only the clinical relevance of the assays in terms of sensitivity and specificity, but also their measurement precision. Precision of measurement is the extent to which repeated measurements of the same sample give similar results. It is expressed as standard deviation, variance or, more often, coefficient of variation (CV). Precision is measured for repeatability conditions of measurement (also called intra-serial, within-run or intra-run precision) that includes the same measurement procedure, operator, measuring system, operating conditions, time, and location. Precision is also measured for intermediate conditions of measurement (or inter-serial, between run, within-lab or inter-run precision) that includes the same measurement procedure and location, and replicate measurements on the same or similar objects over an extended period of time, and, as such, may include other conditions involving changes that include other lots of reagents, calibrations, calibrators, operators, and measuring systems. Reproducibility conditions of measurement also include different locations, operators, measuring systems or procedures [4]. While external quality control is used to determine reproducibility, the use of internal quality controls (IQC) allow the calculation of precision, both intra- and inter-run precision which are essential for detecting errors during the analytical phase. This helps to ensure day-to-day consistency of the process and determine whether patient results are reliable enough to be communicated.

The interpretation of precision data obtained in clinical diagnostic laboratories (CDL), *i.e.*, the decision to consider them as acceptable or not, remains challenging in the field of autoimmunity. To date very few reference values have been defined for autoantibody assays and no reference is available on-line or published. Such reference books exist for different specialties, mainly for clinical chemistry [5, 6] or hematology [7]. Only some reference CVs may be found for anti-thyroperoxidase (TPO), anti-thyroglobulin (TG) antibodies and rheumatoid factor (RF) assays [8, 9]. The mere absence of reference values for autoantibody diagnostics is partly due to the absence of reference materials in autoimmunity [10]. The Federal Drug Administration (FDA) published guidelines for companies developing diagnostics assays [11], giving global rules and analytical objectives to reach. However, these recommendations are not specific to the autoantibody measured neither to the method of detection used by each assay.

Considering the complexity of the field, the European Autoimmunity Standardization Initiative (EASI) has been formed to harmonize autoantibody diagnostics [12]. The objective of this study was to elaborate a reference support in autoimmunity that could be used in routine practice and for the accreditation of CDL. As an initiative of the French EASI group, a large number of French CDL were requested to participate in a survey aiming to analyze the CVs of intra-run and inter-run precision obtained for assays quantifying 14 different autoantibodies. The results, obtained from 32 specialist laboratories, allow the proposal of acceptability limits for the performances of the autoantibody assays, allowing accurate clinical interpretation of the test and being compatible with the diagnostic laboratories' reality.

2. MATERIAL AND METHODS

2.1. Selection criteria of autoantibody assays

The assays included in this study were selected according to the following criteria: quantitative autoantibody assays for which the quantitation is of clinical relevance and performed in a large number of laboratories. So the assays studied were: IgG and IgM isotypes for anti- beta 2 glycoprotein 1 (B2GP1) and anti-cardiolipin (CL) autoantibodies, IgG isotype for anti-myeloperoxidase (MPO-ANCA), anti-proteinase 3 (PR3-ANCA), anti-glomerular basement membrane (GBM), anti-double stranded-DNA (dsDNA), anti-thyroglobulin (TG), anti-thyroperoxidase (TPO), anti-TSH-receptor (TSH-R), and anti-cyclic citrullinated peptides (CCP), IgA isotype for 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 [13].

2.2. Data collection

A participation invitation for this study was sent by email in March 2018 to 88 French CDL, 68 being public hospital laboratories and 20 private CDL. To ensure uniformity of the responses, this e-mail was accompanied by an Excel sheet, ready to be completed for predefined items by fixed drop down menus. Figure 1A depicts the data collected. In addition to the analyte, for both intra- and inter-run CVs, information was collected on the type of immunoassay, the type of automation (manual, semi- or full automation), the number of values used for the CV calculation, the level of IQC according to the positive threshold (low: <2 fold, medium: between 2 and 5 fold, high: superior to 5 fold) and the nature of the sample used as IQC (patient sample prepared in the CDL, kit-related commercial control, or kit-independent commercial control). Each line was to be completed for each analyte and level of IQC. It was possible to complete as many lines as there were controls for the respective analyte. A 5 weeks deadline was given to complete the file and a reminder email was sent 10

days before the end of the study. The questionnaire did not ask for information about the time frame when measurements for intra- and inter-run CV's were done.

2.3. Data analysis

Prior to analysis, CV calculated from less than 5 measurements were excluded. In addition, reported CVs below 0.1% or above 80% were excluded.

Criteria for acceptable performance were defined for each autoantibody assay from data of CVs sent by participating laboratories. According to the model used by the French Society of Biochemistry [6] (SFBC), we fixed 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 defined for the 3 levels of autoantibodies.

In addition, we analyzed the impact of the number of measurements, the nature of the IQC used, as well as the method (type of immunoassay and automation), on the assay performance.

2.4. Statistical analysis

Prism-GraphPad™ software was used to perform different statistical analysis. Unpaired t-test, Mann-Whitney test and one-way ANOVA with multiple comparisons (Turkey's multiple comparison test) were used. .

3. RESULTS

The overall response rate of the invited laboratories was 36% (32/88). 20% of private laboratories (4/20) and 41% of hospital laboratories (28/68) participated, allowing collection of 440 values of intra-run CV (25 values were to be excluded, leaving 414 for analysis) and 654 values of inter-run CV (38 values were excluded leaving 616 for analysis). A mean of 34 values of CVs was provided by the participating laboratories. 41% of the assays were declared accredited by laboratories at the time of the data collection, according to the ISO 15189 guidelines.

The number of CVs obtained for each analyte is depicted in figure 1B. It varied from 7 to 53 and from 14 to 68 values per analyte for the CVs of intra-run and inter-run precision, respectively. The highest numbers of collected data were for anti-CCP and RF. The lowest numbers of collected CVs were for anti-TSH-R autoantibody assays.

3.1. Impact of the number of measurements on CV results

We first analyzed the results of CVs according to the number of measurements used for their determination (5-10, 11-20, 21-30 or >30 values) (Figure 2). No significant difference was observed between the 4 groups (one-way ANOVA) when considering all data (intra- and inter-run CVs).

3.2. Impact of the source of the quality controls on CV results

Different types of samples may be used as IQC to perform the measurements allowing the determination of the intra- and inter-run CVs. They may be provided by the manufacturer of the respective immunoassay. Reference values are usually provided in the technical data sheet and are to be used for acceptance of the data obtained. These reagents are frequently ready to use without dilution and may also be lyophilized. Alternatively, IQCs may be obtained from

providers independent of the immunoassay manufacturer, possibly requiring adapted dilutions. Lastly, the CDL may use single or pooled patient samples. In the latter two situations the CDL has to determine the target value of the respective IQC. We analyzed if the CVs of the IQCs are different depending on the source of the IQC sample (Figure 3). We observed that lower CVs were obtained with commercial controls when compared to IQCs derived from in-house samples, especially for the intra-run CVs ($p < 0.0001$).

3.3. Determination of acceptable precision performance

Acceptable precision performance was calculated for each autoantibody according to the levels of the IQC. The highest CV values were obtained with low level IQCs (<2 fold the positive threshold). CV values also depend on the type of autoantibody measured. In particular, assays for anti-CL or anti-B2GP1 autoantibodies have higher CVs. Irrespective of the analyte, the method and the level of the IQC, the 90th percentile precision performance goals (lowest CV values reached by 90 % of laboratories) were between 3.3% and 16.2% for the intra-run CVs, and between 6.7% and 23.2 for the inter-run CVs. At the 50th percentile precision performance goal values (lowest CV values reached by 50 % of laboratories) were between 2.9% and 10.7% for the intra-run CVs, and between 2.8% and 13.2% for the inter-run CVs (Table 1).

3.4. Impact of the type of immunoassay on CV results

The main types of immunoassays used by participants were fluorescent-enzyme immunoassays (FEIA; 39%), chemiluminescence (CLIA; 26%), enzyme linked immunosorbent assay (ELISA; 23%), addressable laser bead immunoassay (ALBIA; 7%), and nephelometry/turbidimetry (5%). Other methods (radio-immunoassays or immunocompetition) represented less than 1% of the methods reported. In total twenty

suppliers of in vitro diagnostic reagents were represented in the study (supplemental Figure 1).

We analyzed the results of intra-run and inter-run CVs and calculated the performances at 90th and 50th percentiles according to the type of immunoassay used (Figures 4 and 5).

This analysis revealed larger distribution of the intra-run CVs (Figure 4A) and higher CV values with ELISA compared to the other immunoassays (Figure 4B, range of CVs at 90th percentile: 11.4-13.5% for ELISA vs 3.2-12.2% for the other immunoassays), and especially for the low level IQCs.

Table 2 depicts the 616 results of inter-run CVs collected according to the type of immunoassay and the levels of IQCs. Irrespective of the IQC levels, larger distribution was observed for the inter-run CVs obtained with ELISA (median: 11.06%, IQRs (7.15-15.82) as compared to the other methods (FEIA: median: 8%, IQRs (6.07-10.71; CLIA: median: 6.78%, IQRs (4.1-9.67); ALBIA: median: 7.8%, IQRs (6.1-9.19); nephelometry/turbidimetry: median: 2.55%, IQRs (1.97-5.37) (Figure 5A).

Moreover, for the 90th percentile values, higher CVs of intra- and inter-run CVs were observed for low level IQCs (mean 10.9% and 16.2%, respectively) as compared to high level IQCs (8.2% and 13.8%, respectively, p=0.008 and 0.09, unpaired t test), irrespective of immunoassay type.

We then analyzed the impact of the type of immunoassay for specified autoantibodies on precision performance. We observed significant differences in inter-run CVs for IgG anti-CL assays, with higher values observed with ELISA as compared to FEIA, CLIA and ALBIA. For RF, significantly lower values were observed with nephelometry/turbidimetry as compared to FEIA and ELISA. For the other autoantibodies low or no differences were observed (Mann and Whitney, supplemental data, Figure 2).

4. DISCUSSION

This article presents the results of a study proposed to French CDL, aiming to collect and analyze the CVs of intra-run and inter-run variations of assays used for the detection of 14 autoantibodies, obtained in real life conditions. Despite the low rate of responses (36% of the invited laboratories), the data collected allowed the analysis of 414 values of intra-run CV and 616 values of inter-run CV. The difference in the number of data for intra- and inter-run variations is linked to the fact that inter-run variation is a parameter followed by all laboratories but the determination of intra-run variation is usually performed only for the initial verification of the methods.

The number of measurements used for the CV calculations had no significant influence on the intra- and inter-run CVs of autoantibody assays. This information is very important because the accreditation rules were defined for biochemistry analytes, such as blood electrolyte concentration, for which low levels of variation may have important clinical significance. Most of the accreditation guidelines have applied these rules to all laboratory specialties without any adaptation according to the clinical significance, the cost-effectiveness, or even feasibility. Indeed the cost for measuring an autoantibody is considerably higher than the measurement of blood electrolytes [11]. In France, the accreditation technical guidance for the use of IQC, recommends calculating the inter-run variability during at least 15 days of measurements, or at least 30 values [14]. Based on our results we conclude that more than 10 measurements for either intra- or inter-run CVs will not improve the estimation of the assay precision, whatever the type of immunoassay used. These findings could substantially reduce cost of achieving accreditation if the criteria was reduced from a minimum of 30 observations to 10, if our findings are validated by other studies.

Our study also revealed an impact of the source of the IQC on intra- and inter-run CVs, with lower values obtained when using commercial IQC as compared to IQCs derived from in-

house patient samples. This effect doesn't seem to be explained by a biased use of the type of controls according to the method (Supplemental data, Figure 3). Commercially produced IQCs have the advantage of long term stability and low vial to vial variability. However, the matrix effect on the measurement of analytes may be different from a patient serum and could give erroneously low CVs, which could be detrimental for the interpretation of some patient results, mainly for quantitative assays. For autoantibody assays, this is of high importance considering that the serum to be tested contains high concentrations of IgG, IgM and IgA being non-specific for the autoantigen but potentially influencing the interaction of the antigen-specific autoantibodies to their target. In addition, natural autoantibodies of IgM or IgG isotype may interfere in these immunoassays and their effect on precision should be taken into account [15]. In our analysis we also differentiated data from IQC included in the assay versus IQC independent of the assay manufacturer. Indeed commercial IQC should be bought from a manufacturer different from the assay's provider to ensure an independent assessment of performance [14, 16, 17]. Moreover, positive and negative controls included in commercial assays are frequently ready to use and thus are not treated in the same manner as patient samples. This type of IQC does not reflect the exact uncertainty of measurement and potentially provides a false acceptance limit. For all of these reasons, the use of patient-derived IQC samples has been recently recommended for antinuclear antibody testing by indirect immunofluorescence [18].

Regarding the type of immunoassays, we noticed an over-representation of the fully automated methods, mainly FEIA and CLIA which represented more than half of the immunoassays. This has a significant impact on the proposed acceptable performance, mainly on the 50th percentile performance (inter-run CV lower than 9% at low levels vs 14.6% for ELISA).

To investigate the differences observed between the 50th and the 90th percentile performances, we performed sub-analyses of the results of autoantibody assays according to the immunoassay method. No major difference in performance according to the type of immunoassay was observed except for RF and anti-CL autoantibodies for which higher CVs were observed when using ELISA, as compared to the other types of immunoassay. This difference is not explained by the use of different kinds of automatization or of manual techniques for the same parameter because the data didn't demonstrate lower CVs obtained by manual techniques (data not shown). In addition, this phenomenon seems particularly important when the IQC are at low concentration levels. Indeed, our results show clearly the higher imprecision when the autoantibody levels of the IQC are below 2-fold the positive threshold of the assay. When studying the 90th percentile values, the highest CVs of intra- and inter-run CVs were also observed with low level IQCs. The imprecision observed with low level IQCs is part of the so-called "grey zone" of the autoantibody assays. This zone corresponds to borderline positivity and may have a significant effect on the patient diagnosis and care. That's why it is very important to be aware of the assay performances at these levels. For the diagnosis or classification of several autoimmune diseases, guidelines propose using serological criteria of relatively high autoantibody levels. This applies for the anti-phospholipid syndrome [19], rheumatoid arthritis [20] and coeliac disease [21].

The determination of the positive cut-off, and consequently of the grey-zone, remains debated for autoantibody detection. Indeed, their presence and levels in the normal population don't fit a Gaussian distribution and their prevalence may vary from one autoantibody to another [22]. Due to this imprecision a commentary to the physicians should be added to the results within the "grey zone", in which measurement uncertainty is very high. Indeed, the laboratory should give information about the precision allowing appropriate interpretation of each patient result, at least at the clinically relevant levels.

No reference values for intra- and inter-run CVs are available for autoantibody detection assays in biorepositories. Some data are present on the Westgard website for anti-TPO and anti-TG autoantibodies and for RF, but they are based on only two studies with very few data (33 and 34 samples, respectively). These studies didn't really measured the intra- and inter-run CVs of these parameters but rather the biological variation observed in different samples collected from the same individuals during a short period of time. These values of within-subject variations (anti-TG: 8.5%, anti-TPO: 11.3%, RF: 8.5%) don't inform specifically about the analytical performances of the assays per se [23], [24]. In addition, they are of limited interest in the field of autoimmunity in which for some autoantibody, the levels may vary quickly in patients with active disease

In conclusion, our study revealed that autoantibody measurements exhibit higher imprecision than those observed in clinical chemistry and hematology. However, the imprecision of the autoantibody assays that we report here, is not prejudicial for patient care according to the different clinical guidelines. Though variable according to the different autoimmune diseases, such as in coeliac disease, lupus or vasculitis, [25-28] the significant clinical variations, i.e levels variation that have an impact on the patients diagnostic, prognosis or management, are usually higher than the imprecision of the assays. However, it would be useful for the physicians to be informed about the measurement uncertainty inherent to each autoantibody assay.

This study allows us to propose that an acceptable performance for intra- and inter-run CVs should be below the 90th percentile. Robustness of an assay should thus be re-evaluated if CVs are above the 90th percentile value.

Similar larger studies should be performed by international societies in the field of autoimmunity in order to validate our findings. Nevertheless, regarding the absence of any

available data, our work represents a first step in the analysis of proficiency testing of the CDL implicated in autoantibody diagnostics.

Recommendations:

- IQC must be defined in terms of source and levels of autoantibody according to the positive threshold of the assay.
- IQC should comprise at least one positive sample obtained from a source different from the manufacturer of the assay.
- IQC should comprise at least one positive sample that is to be treated in an identical manner as patient samples.
- Intra- and inter-run CVs should be comprised between the 50th and 90th percentile provided by this study; robustness must be re-evaluated if they are >90th.
- Intra- and inter-run CVs should be calculated from at least 10 values
- The precision of the autoantibody assays must be adapted to their clinical significance and their results must be accompanied by appropriate biological comments.

Acknowledgement

We thank all biologists from the participating French DBL for sharing their data (in alphabetical order) : Biomedical Laboratory of Hospital Ambroise Paré, Paris (Dr J Zhu), Biomedical laboratory of CH Alpes Lemans, Contamine/Arve (Dr B Murienne), Laboratory of Immunology, Hospital Bichat, Paris (Dr P Nicaise), Private Biomedical Laboratory BIO7/Cerballiance, Lisses (Dr M Senant), Private Biomedical Laboratory Bioliance, Nantes (Dr S Le Quere), Private Biomedical Laboratory Biomnis-Eurofins, Ivry (Dr L Guis-Cabanne), Lyon, (Dr G Chyderiotis), Laboratory of Immunology of University hospital of Caen (Dr E Comby), Biomedical Laboratory of Hospital Metropole Savoie, Chambéry (Dr C Dumollard), Biomedical Laboratory of Hospital of Annecy (Dr S Boutruche), Biomedical

Laboratory of Hospital Aulnay (Dr A Grenier), Biomedical Laboratory of Hospital Libourne (Dr C Castang, Dr A Francart), Laboratory of Immunology, Hospital Pontchaillou, Rennes (Dr I Bahun-Riedinger), Laboratory of Immunology, Hospital Cochin, Paris (Dr C Goulvestre), Biomedical Laboratory of Hospital Colmar (Dr F Mathiaux), Laboratory of Immunology, Hospital Pitie-Salpetriere, Paris (Dr P Ghillani-Dalbin), Laboratory of Immunology, University Hospital of Grenoble (Dr C Dumestre-Perard), Laboratory of Immunology, Hospital Georges Pompidou, Paris (Dr M-A Dragon-Durey), Laboratory of Immunology, University Hospital of Lille (Dr S Deleplanque, Dr S Dubucquoi), Laboratory of Immunology, University Hospital of Limoges (Dr G Olombel), Laboratory of Immunology, University Hospital of Lyon (Dr N Fabien, Dr C Lombard, Dr M Dechomet, Dr L Garnier), Laboratory of Immunology, Hospital Henri Mondor, Paris (Dr T Belmondo), Laboratory of Immunology, University Hospital of Nantes (Dr C Hemont), Laboratory of Immunology, Hospital Necker Enfants Malades, Paris (Dr M-A Alyanakian), Laboratory of Immunology, University Hospital of Poitiers (Dr F Jacomet), Laboratory of Immunology, Hospital Robert Debré, Paris (Dr V Guérin), Laboratory of Immunology, University Hospital of Reims (Dr D Giusti), Laboratory of Immunology, University Hospital of Rouen (Dr F Jouen), Private Biomedical Laboratory SCMB12, Grenoble (Dr Guerber), Laboratory of Immunology, Hospital Saint Antoine, Paris (Dr C Johannet), Biomedical Laboratory of St Denis (Dr F Oukil-Fernani), Laboratory of Immunology, University Hospital of St Etienne (Dr A Berger, Dr S Paul), Laboratory of Immunology, University Hospital of Tours (Dr L Decalonne, Dr H Wattier), Biomedical Laboratory of Villefranche/Saone (Dr C Dumont).

Funding

None

Statement of author contributions

Marie Senant collected and analyzed the data. All authors participated in the design of the study, the drafting of the manuscript and provided approval of the final submitted version.

REFERENCES

1. 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 Jan;16(1):81-6.
2. 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 May;17(5):513-7.
3. Damoiseaux J, Andrade LE, Fritzler MJ, Shoenfeld Y. Autoantibodies 2015: From diagnostic biomarkers toward prediction, prognosis and prevention. *Autoimmun Rev.* 2015 Jun;14(6):555-63.
4. Joint Committee for guides in Metrology. International vocabulary of metrology – Basic and general concepts and associated terms (VIM) 3rd edition, JCGM; 2012.
5. 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 Nov;59(7):491-500.
6. 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 Nov-Dec;57(6):685-95.
7. Vis JY, Huisman A. Verification and quality control of routine hematology analyzers. *Int J Lab Hematol.* 2016 May;38 Suppl 1:100-9.
8. Westgard J. *Westgard QC.* 2019.
9. Centers for Medicare and Medicaid Services, . Clinical Laboratory Improvement Amendments of 1988 (CLIA) Proficiency Testing Regulations Related to Analytes and Acceptable Performance. *Federal Register;* 2019. p. 1536-67.

10. Sheldon J, Dellavance A. Strategies for building reference standards for autoantibodies. *Front Immunol*. 2015;6:194.
11. Food and Drug Administration. *Bioanalytical Method Validation Guidance for Industry*. 2018.
12. Damoiseaux J, Olschowka N, Shoenfeld Y. EASI - European Autoimmunity Standardisation Initiative: facing the challenges of diagnostics in autoimmunity. *Clin Chem Lab Med*. 2018 Sep 25;56(10):1620-3.
13. 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.
14. COFRAC. SH GTA 06 : Guide technique d'accréditation : contrôle de qualité en biologie médicale. 2012.
15. Maddur MS, Lacroix-Desmazes S, Dimitrov JD, Kazatchkine MD, Bayry J, Kaveri SV. Natural Antibodies: from First-Line Defense Against Pathogens to Perpetual Immune Homeostasis. *Clin Rev Allergy Immunol*. 2019 Jun 3.
16. Kinns H, Pitkin S, Housley D, Freedman DB. Internal quality control: best practice. *J Clin Pathol*. 2013 Dec;66(12):1027-32.
17. Clinical and laboratory standards institute. *Statistical Quality Control for Quantitative Measurement Procedures : Principles and definitions* 2016.
18. Bogaert L, Van den Bremt 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 Jun 26;57(7):990-8.

19. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost.* 2006 Feb;4(2):295-306.
20. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010 Sep;62(9):2569-81.
21. Lebowitz B, Sanders DS, Green PHR. Coeliac disease. *Lancet.* 2018 Jan 6;391(10115):70-81.
22. 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 Mar 9;347(9002):693.
23. Jensen E, Petersen PH, Blaabjerg O, Hegedus L. Biological variation of thyroid autoantibodies and thyroglobulin. *Clin Chem Lab Med.* 2007;45(8):1058-64.
24. Jimenez CV. Usefulness of reference limits and evaluation of significant differences. An example of the biological variation of serum rheumatoid factors. *Ann Biol Clin (Paris).* 1994;52(7-8):529-33.
25. Petroff D, Wolf J, Richter T, Auth MKH, Uhlig HH, Laass MW, et al. Antibody Concentrations Decrease 14-Fold in Children With Celiac Disease on a Gluten-Free Diet but Remain High at 3 Months. *Clin Gastroenterol Hepatol.* 2018 Sep;16(9):1442-9 e5.
26. Babaoglu H, Li J, Goldman D, Magder LS, Petri M. Predictors of predominant Lupus Low Disease Activity State (LLDAS-50). *Lupus.* 2019 Dec;28(14):1648-55.
27. Damoiseaux JG, Slot MC, Vaessen M, Stegeman CA, Van Paassen P, Tervaert JW. Evaluation of a new fluorescent-enzyme immuno-assay for diagnosis and follow-up of ANCA-associated vasculitis. *J Clin Immunol.* 2005 May;25(3):202-8.

28. Osman MS, Tervaert JWC. Anti-neutrophil Cytoplasmic Antibodies (ANCA) as Disease Activity Biomarkers in a "Personalized Medicine Approach" in ANCA-Associated Vasculitis. *Curr Rheumatol Rep*. 2019 Dec 26;21(12):76.

Table I: 90th and 50th percentiles CVs for each analyte, each level of IQC and for intra- and inter-run variation. The colors indicate the lowest (in green) and the highest (in red) values of acceptable CVs.

	CV90 (90th percentile)						CV50 (50th percentile)					
	Intra-run CV (%)			Inter-run CV (%)			Intra-run CV (%)			Inter-run CV (%)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
RF	10.0	8,2	8.8	13.2	14.3	11.0	3.8	3.9	3.9	2.8	6.4	6.9
Anti-dsDNA	10.7	6.5	7.5	19.6	12.9	15.9	5.7	4.6	4.6	13.2	7.9	7.0
Anti-TG	14.5	10.1	10.9	12.3	10.0	16.0	4.3	6.8	3.9	6.6	4.7	5.8
Anti-TPO	7.7	6.9	7.0	14.0	18.0	9.4	5.0	4.7	4.0	10.2	7.4	3.9
Anti-TSH-R	10.0*	3.3*	7.0*	10.7*	10.7*	10.7*	8.0*	10.7*	7.0*	7.8*	7.8*	7.8*
Anti-TTG IgA	16.2	6.0	6.2	15.2	13.4	11.8	5.5	4.6	5.1	8.4	8.8	6.8
Anti-B2GP1 IgM	7.8	5.4	10*	13.6	6.7	13.0	3.3	2.9	7.0*	9.2	4.4	10.5
Anti-B2GP1 IgG	10.6	6.2	6.9	23.2	13.0	11.6	5.7	5.3	3.0	11.2	6.1	8.0
Anti-CL IgM	7.9	10.6	6.0*	20.2	11.1	15.0	3.1	7.4	5.0*	7.9	6.7	10.8
Anti-CL IgG	11.5	12.4	12.6	20.3	16.1	17.4	5.0	6.7	8.1	9.4	7.9	8.0
Anti-CCP IgG	11.6	6.7	7.8	14.8	11.8	21.3	4.5	4.8	5.0	7.8	7.4	9.3
MPO-ANCA	9.9	10.3	6.2	13.3	15.1	10.9	5.2	3.9	4.0	10.0	8.5	7.5
PR3-ANCA	12.3	10.5	11.0	14.9	15.4	12.1	6.0	3.4	7.0	10.4	9.2	8.0
Anti-GBM	11.7	8.0*	6.3	16.4*	16.4*	16.4*	4.1	8.0*	5.2	10.1*	10.1*	10.1*

* : results calculated from less than 5 values.

RF: rheumatoid factor, dsDNA: double stranded-DNA, TG: thyroglobulin, TPO: thyroperoxidase, TSH-R: TSH-receptor, TTG : tissue transglutaminase, B2GP1: beta 2 glycoprotein 1, CL: anti-cardiolipin, CCP: cyclic citrullinated peptides, MPO: myeloperoxidase, PR3: proteinase 3, ANCA: antineutrophil cytoplasmic antibodies, GBM: glomerular basement membrane.

Table 2: Statistical description of the Inter-run CVs results, according to the type of immunoassay and the IQC levels.

Method	Level of IQC	Number of values	Median	IQR	Ranges
FEIA	Low	42	9.30	7.2-12.47	4.40-20.1
	Medium	117	7.42	5.98-10.45	2.90-33.3
	High	75	8.00	6.00-10.30	3.36-32.95
CLIA	Low	57	7.73	6.15-10.82	2.39-10.82
	Medium	69	6.68	4.78-9.91	1.40-14.56
	High	41	3.70	3.46-7.19	1.90-13.5
ELISA	Low	33	14.6	11.28-20.93	1.40-36.6
	Medium	69	10.05	5.98-14.90	1.50-23.00
	High	53	10.00	6.77-15.00	3.58-26.40
ALBIA	Low	5	5.10	4.1-12.71	4.10-14.61
	Medium	16	7.40	6.10-8.93	5.10-12.66
	High	9	7.95	7.42-10.53	4.90-13.50
Nephelo/Turbidimetry	Low	16	2.38	2.16-4.11	1.80-14.9
	Medium	8	1.77	1.72-7.64	1.70-17.00
	High	6	4.07	3.49-7.59	1.97-8.60

FEIA: fluorescent-enzyme immunoassay, CLIA: chemiluminescent immunoassay, ELISA: enzyme-linked immunosorbent assay, ALBIA: addressable laser bead immunoassay, IQC: internal quality control; IQR: Inter-quartile ranges.

Legends of the figures.

Figure 1: (A) Pre-defined Excel sheet sent to 88 French clinical diagnostic laboratories.

The table was ready to be completed for the different items by unchangeable drop-down menus. (B) **Number of coefficients of variation collected for each autoantibody assay.**

Figure 2: Combined results of intra- and inter-run coefficients of variation according to the number of measurements used for their determination. No statistical difference was observed between the 4 groups.

Figure 3: Levels of CVs according to the source of IQC used (commercial control different from the manufacturer of the assay, commercial control included in the kit or patients' serum or plasma derived samples,). Lower CVs of intra-run variation were obtained with the commercial controls. NS : $P > 0.05$, ****: $P \leq 0.0001$.

Figure 4: (A) CVs for intra-run variation according to the levels of autoantibodies of the IQC and type of immunoassay. Dotted lines indicate acceptable limits corresponding to 50th percentile and plain lines acceptable limits corresponding to 90th percentile. Table (B) resumes the values corresponding to 90th percentile (i.e the lowest intra-run CV value obtained by 90% of the laboratories). *: Not applicable, only one value reported.

Figure 5: (A) CVs for inter-run variation according to the levels of autoantibodies of the IQC and type of immunoassay. Dotted lines indicate acceptable limits corresponding to 50th percentile and plain lines acceptable limits corresponding to 90th percentile. Table (B) resumes the values corresponding to 90th percentile (i.e the lowest inter-run CV value obtained by 90% of the laboratories). *: Acceptable limits calculated from less than 5 values.

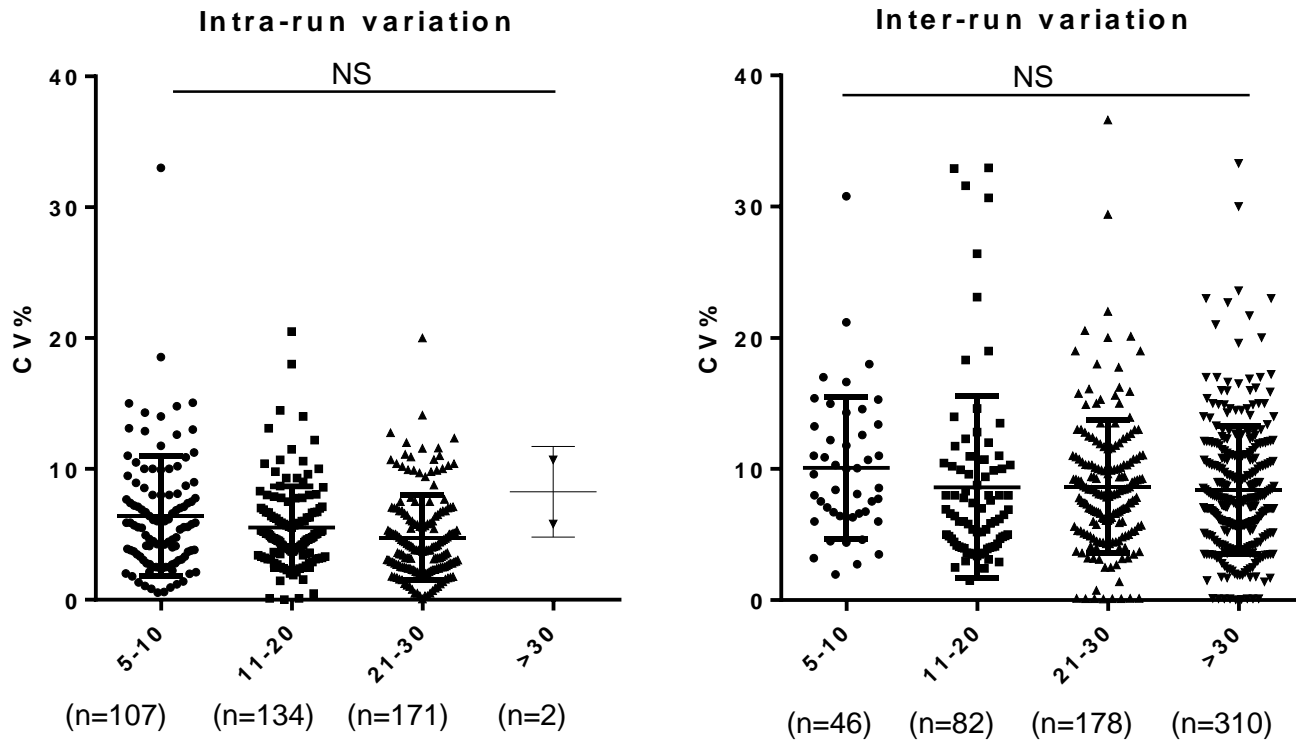
A.

Name of Analyte	Immunoassay	Method of experiment	Number of values	IQC levels	Nature of the sample
Anti-dsDNA	ELISA	Manual	<5	Low (<2x Cut off)	Serum/plasma
Anti-CCP-IgG	CLIA	Semi-automatization	5-15	Medium (2-5x Cut off)	Internal commercial control
RF	FEIA	Full automatization	15-30	High (>5x Cut off)	External commercial control
Anti-CL IgG	ALBIA		>30		
Anti-CL IgM	Nephelo/Turbidimetry				
Anti-B2GP1 IgG	Other				
Anti-B2GP1 IgM					
Anti-TTG-IgA					
Anti-MPO					
Anti-PR3					
Anti-GBM					
Anti-TPO					
Anti-TG					
Anti-TSH-R					

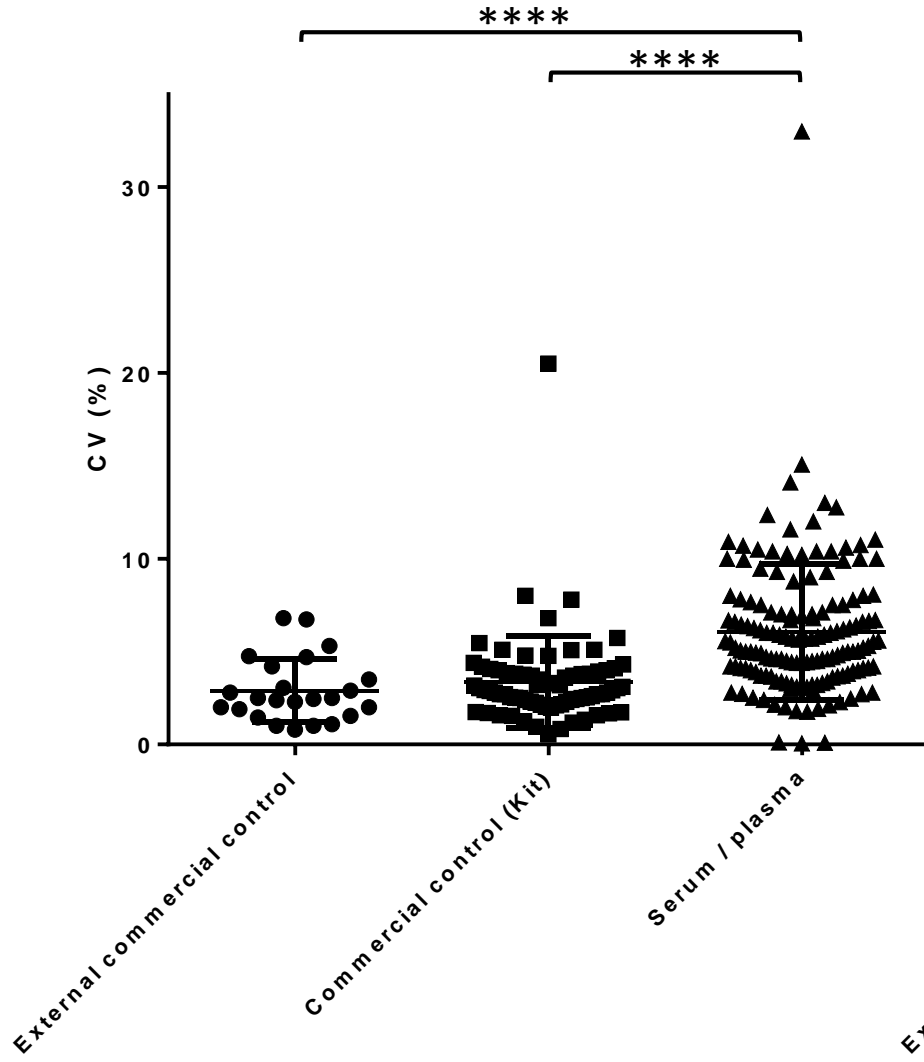
B.

	Intra-run variation	Inter-run variation
anti-CCP	53	68
RF	49	60
anti-TPO	40	57
anti-TG	36	61
anti-dsDNA	34	57
anti-PR3	34	47
anti-TTG IgA	32	53
anti-CL IgG	31	47
anti-MPO	30	45
anti-B2GP1 IgG	26	43
anti-CL IgM	26	40
anti-GBM	20	25
anti-B2GP1 IgM	18	34
anti-TSH-R	7	14

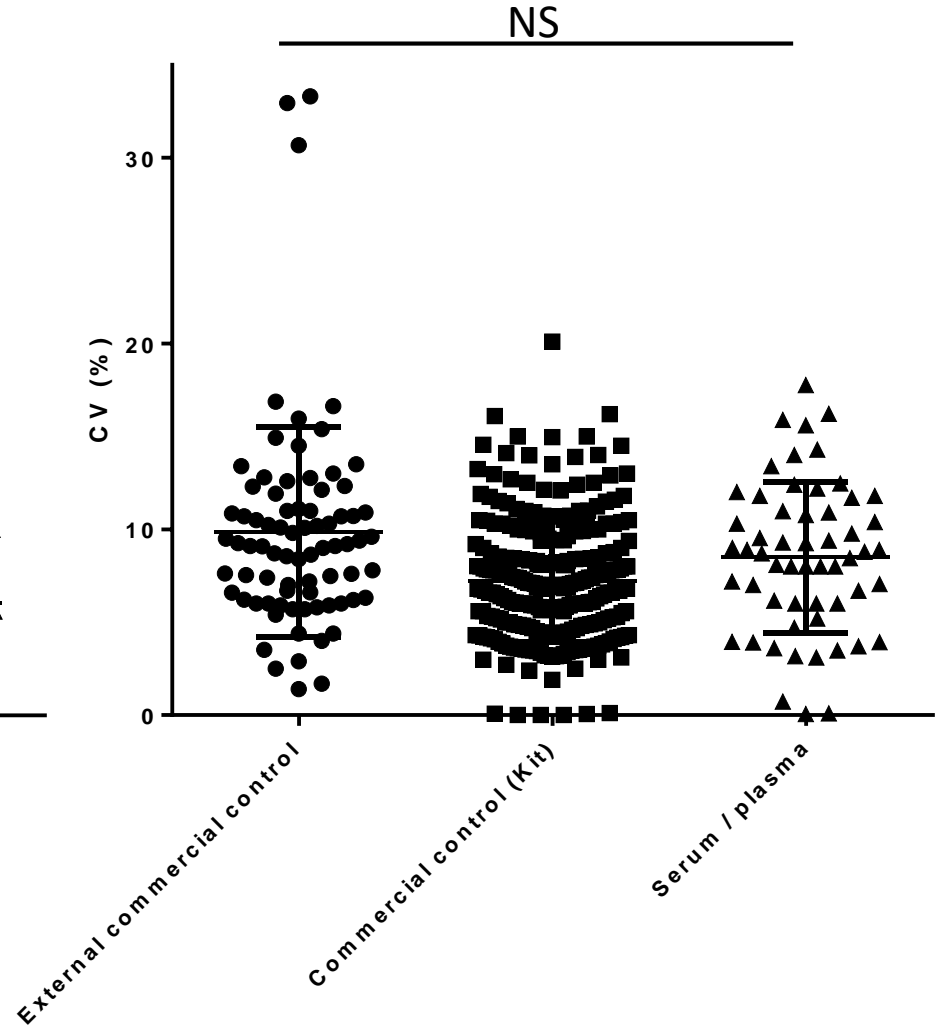
Number of measurements used for CVs calculation



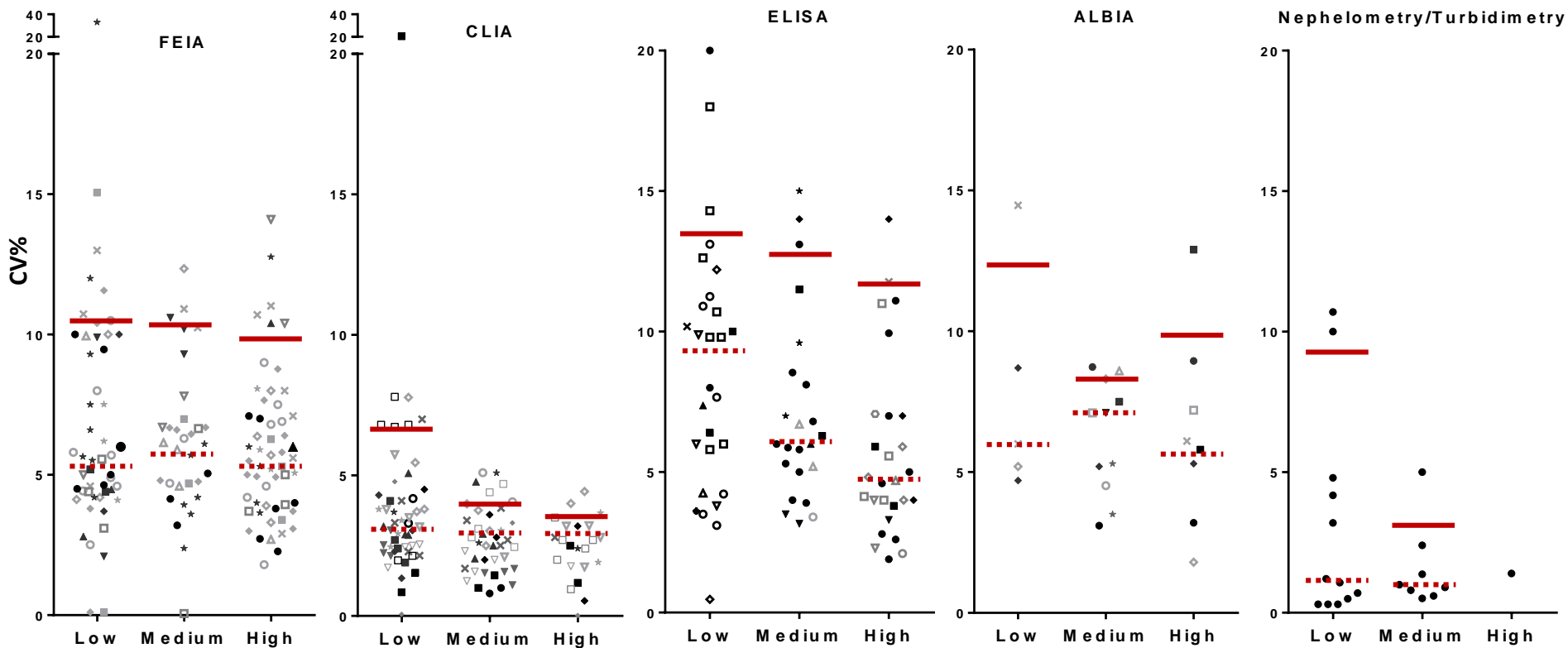
Intra-run CVs



Inter-run CVs



A.

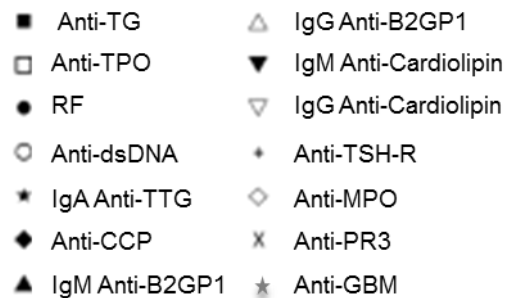
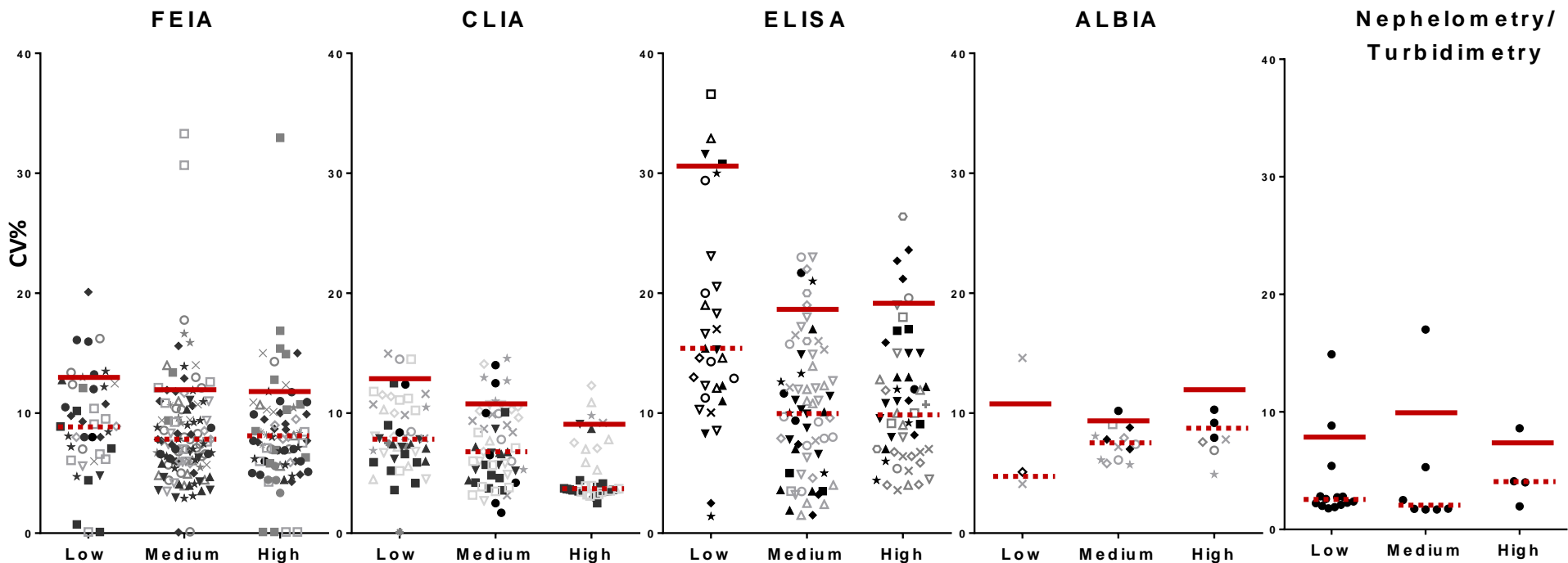


B.

- Anti-TG
- Anti-TPO
- RF
- Anti-dsDNA
- * IgA Anti-TTG
- ◆ Anti-CCP
- ▲ IgM Anti-B2GP1
- △ IgG Anti-B2GP1
- ▼ IgM Anti-Cardiolipin
- ▽ IgG Anti-Cardiolipin
- + Anti-TSH-R
- ◇ Anti-MPO
- × Anti-PR3
- ☆ Anti-GBM

	CV90 (90th percentile)			CV50 (50th percentile)		
	Low	Medium	High	Low	Medium	High
FEIA	10.8	10.2	9.8	5.5	6.1	5.5
CLIA	6.8	4.4	3.7	3.2	2.6	2.7
ELISA	13.5	12.8	11.4	8.9	6.1	4.7
ALBIA	12.2	8.6	10.1	6.0	7.1	6.0
Nephelometry/ Turbidimetry	8.9	3.2	-*	1.1	1.0	-*

A.



B.

	CV90 (90th percentile)			CV50 (50th percentile)		
	Low	Medium	High	Low	Medium	High
FEIA	13.5	12.7	12.5	9	7.4	7.8
CLIA	12.4	10.8	9.3	7.6	6.7	3.7
ELISA	30.8	18	18.9	14.6	9.9	10.0
ALBIA	10.8	9.2	11.2	4.1	6.5	7.9
Nephelometry/ Turbidimetry	7.5	10.0	7.3*	2.4	1.8	4.1*