# COMPARISON OF SERUM IMMUNOGLOBULIN AND COMPLEMENT ASSAYS ACROSS THREE AUTOMATED IMMUNOCHEMISTRY PLATFORMS

**Vu Thi Tu Uyen[1,2], Tran Minh Dien[1], Tran Huy Thinh[2]**
**Ngoc Thu Thao[1], Luong Minh Tan[3] and Tran Thi Chi Mai[1,2,✉]**
*[1]Vietnam National Children's Hospital*
*[2]Hanoi Medical University*
*[3]Hanoi University of Public Health*

*Serum immunoglobulins (IgA, IgG, IgM) and complement components (C3, C4) are essential biomarkers widely used in clinical practice to evaluate immune function and support the diagnosis of inflammatory and immunological disorders. This study assessed the concordance of quantitative results for these markers across three automated analytical platforms Cobas Pro (Roche Diagnostics), Atellica Solution (Siemens Healthineers), and AU5800 (Beckman Coulter), using pediatric serum samples. Correlation analysis revealed very strong linear relationships between the systems (r = 0.90 - 0.995). However, significant proportional biases were observed, particularly for C4, with slope values of 1.3226 (Cobas Pro vs. AU5800) and 1.3141 (Atellica vs. AU5800), indicating a trend of higher C4 values reported by AU5800. Bland-Altman analysis indicated the highest absolute bias for C4, followed by C3 and IgM, confirming the presence of clinically relevant and systematic differences. These findings suggest that results are not directly interchangeable without proper adjustment. Establishing system-specific reference intervals or applying validated conversion factors is necessary to ensure accuracy in clinical interpretation.*

**Keywords: Immunoglobulin, complement, immunoturbidimetry, method comparison, children.**

## I. INTRODUCTION

Serum immunoglobulins (Ig) and complement components are fundamental biomarkers widely used in clinical laboratories to assess immune status, diagnose immunological disorders, and monitor inflammatory conditions. Immunoglobulins-including IgG, IgA, and IgM-are central to humoral immunity, playing roles in antigen neutralization, opsonization, and immune memory.[1] Meanwhile, complement proteins such as C3 and C4 form part of the innate immune system, acting as key mediators in the clearance of pathogens and immune complexes, and modulating inflammation.[2,3] Quantitative assessment of these analytes is indispensable in the evaluation of patients with primary immunodeficiencies, autoimmune diseases (such as systemic lupus erythematosus and rheumatoid arthritis), and infectious or chronic inflammatory conditions .[4]

Advancements in automated clinical chemistry platforms have enabled the high-throughput and reproducible measurement of serum proteins, replacing manual methods such as radial immunodiffusion or enzyme-linked immunosorbent assays (ELISA).[5] Most platforms rely on immunoturbidimetric or nephelometric detection principles, using reagent kits optimized for speed, precision,

*Corresponding author: Tran Thi Chi Mai*
*Vietnam National Children's Hospital*
*Email: tranchimai@hmu.edu.vn*
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and operational efficiency. Immunoturbidimetry is a method for measurement of Ig based on the decrease of light intensity caused by the formation of antigen-antibody complexes in fluid (optical detection). This allows for the automated and rapid quantification of analytes targeted in an assay.[6] Despite their widespread use immunoturbidimetric assays are subject to various sources of analytical variability. Deference in reagent composition, antibody specificity, detection sensitivity, and calibration traceability among different analyzer systems may lead to variability in results.[7] These methodological divergences are particularly concerning in clinical settings, where diagnostic interpretation often depends on strict decision thresholds or subtle shifts in biomarker concentrations.

Several studies have confirmed inter-platform discrepancies in the quantification of serum immunoglobulins and complement components, emphasizing the necessity of method comparison and validation. Denham et al. reported that immunoturbidimetric assays on the Architect ci8200 system, although demonstrating acceptable precision, produced systematic differences when compared with nephelometric measurements, particularly for complement proteins such as C3 and C4.[8] Similarly, a multicenter evaluation by Qin et al. revealed clinically relevant variability in protein measurements - most notably for C4 - between different analytical platforms, reinforcing the need for platform – specific reference intervals.[9] Furthermore, guidance from the Clinical and Laboratory Standards Institute (CLSI) emphasizes the importance of method comparison, bias estimation, and result harmonization before introducing new assays or instruments into routine use.[10]

Given the clinical importance of accurate immunoglobulin and complement quantification, this study aims to compare the analytical performance of three widely used automated immunoturbidimetric analyzers, including Cobas Pro (Roche Diagnostics), Atellica Solution (Siemens Healthineers), and AU5800 (Beckman Coulter), in measuring serum IgA, IgG, IgM, C3, and C4. The findings will contribute to evaluating consistency between systems and ensuring result comparability in clinical practice.

## II. MATERIALS AND METHODS

### 1. Sample collection and study design

This method comparison study was conducted using surplus serum samples collected from patients who underwent routine testing for immunoglobulins (IgA, IgG, IgM) and complement components (C3, C4) at the National Children's Hospital between September 2024 and January 2025. All samples were de-identified prior to analysis and stored at -80°C until testing. Laboratory technicians directly observed each serum sample to identify if it had any visual signs of hemolysis, icterus, or lipemia (reddish or yellowish discoloration, milky or cloudy serum). These signs would interfere and cause inaccurate optical measurements. Therefore, only samples with sufficient volume and confirmed to be free of hemolysis, icterus, or lipemia, as determined by both visual inspection and the LIH index results automatically obtained from the analyzer, were included in the analysis.

The method comparison followed the CLSI EP09-A3 guidelines[10]. Briefly, the comparison protocol comprised seven major steps: 1) Defining the purpose and scope of the study, 2) Selecting and preparing appropriate samples, 3) Choosing the measurement procedures to be compared, 4) Performing paired measurements under specified conditions, 5) Analyzing the

data using appropriate statistical methods, 6) Estimating bias and evaluating agreement between methods, 7) Interpreting the results and preparing the comparison report.

The parameters compared between platforms included immunoglobulins (IgA, IgG, IgM, in g/L) and complement components (C3, C4, in g/L). Measurements were performed in parallel on three fully automated immunochemistry platforms: Cobas Pro (Roche Diagnostics), Atellica Solution (Siemens Healthineers), and AU5800 (Beckman Coulter). While all platforms use immunoturbidimetric methods, they differ in reagent formulations, calibration protocols, and detection optics. Each system was operated according to the manufacturer's instructions. Internal quality control (QC) materials at two levels (normal and pathological) were run daily on all platforms, and all results met the laboratory-defined QC acceptance criteria.

**Table 1. Overview of method details for analytes used for the comparison study across Cobas Pro, Atellica Solution, and AU5800 platforms**

| Test | Parameter | Cobas Pro (Roche) | Atellica Solution (Siemens) | AU5800 (Beckman Coulter) |
|---|---|---|---|---|
| IgG | Linearity range | 3.00 - 50.0 g/L | 1.40 - 34.0 g/L | 0.75 - 30.0 g/L |
| | Traceability | CRM 470 | CRM 470 | CRM 470 |
| IgA | Linearity range | 0.50 - 8.0 g/L | 0.33 - 5.40 g/L | 0.1 - 7.0 g/L |
| | Traceability | CRM 470 | CRM 470 | CRM 470 |
| IgM | Linearity range | 0.25 - 6.5 g/L | 0.21 - 3.3 g/L | 0.2 - 5.0 g/L |
| | Traceability | CRM 470 | CRM 470 | CRM 470 |
| C3 | Linearity range | 0.04 - 5.0 g/L | 0.01 - 5.0 g/L | 0.15 - 5.0 g/L |
| | Traceability | CRM 470 | CRM 470 | CRM 470 |
| C4 | Linearity range | 0.02 - 1.0 g/L | 0.01 - 1.4 g/L | 0.08 - 1.5 g/L |
| | Traceability | CRM 470 | CRM 470 | CRM 470 |

*CMR: Certified Reference Material*

***Statistical* Analysis**

For each analyte, results from the three platforms were compared using Passing–Bablok regression to assess systematic and proportional bias, and Bland–Altman analysis to evaluate agreement and visualize mean differences with 95% limits of agreement. All statistical analyses were performed using MedCalc Statistical Software, version 23.2.1.

**3. Research ethics**

This study is part of the research project entitled *"Establishment of Reference Values for Blood Biomarkers in Vietnamese Children"*, funded by the VINIF Innovation Foundation. The study was approved by the Ethics Committee of the Vietnam National Children's Hospital (Approval No. 3054/BVNTW-HĐĐĐ, dated November 30, 2023).

## III. RESULTS

The sample sizes for each analyte were consistent across the three platforms, providing a reliable basis for comparison. Median concentrations of immunoglobulins (IgA, IgG, IgM) were comparable among the Cobas Pro, Atellica Solution, and AU5800 systems, indicating consistent quantification across

platforms. For complement components, the Atellica Solution tended to report slightly lower median values for C3 and C4 compared to the Cobas Pro, while the AU5800 yielded the highest median value for C4.

Despite these variations, measured concentrations for all analytes spanned wide ranges, reflecting the clinical diversity of the study population. Importantly, all observed values fell within the validated linearity ranges of each platform, covering clinically relevant intervals. For example, IgG concentrations (0.9 - 49.4 g/L) were well within the analytical measurement ranges of the Cobas Pro (3.0 - 50.0 g/L), Atellica Solution (1.4 - 34.0 g/L), and AU5800 (0.75 - 30.0 g/L). Similar compatibility was observed for IgA, IgM, C3, and C4, confirming the suitability of the methods and minimizing the risk of measurement errors due to extrapolation beyond validated ranges.

**Table 2. Descriptive statistics of concentration of serum immunoglobulin and complement measurements across Cobas Pro, Atellica Solution, and AU5800 platforms**

| Platforms | Analyte / Statistics | IgA | IgG | IgM | C3 | C4 |
|---|---|---|---|---|---|---|
| *Cobas Pro* | Sample size | 67 | 129 | 84 | 60 | 60 |
| | Median | 1.16 | 7.9 | 1.075 | 1.505 | 0.245 |
| | Lowest value | 0.11 | 1.7 | 0.29 | 0.25 | 0.05 |
| | Highest value | 4.24 | 49.4 | 3.53 | 2.17 | 0.47 |
| *Atellica Solution* | Sample size | 67 | 129 | 84 | 60 | 60 |
| | Median | 1.12 | 7.84 | 1.075 | 1.215 | 0.22 |
| | Lowest value | 0.22 | 1.55 | 0.23 | 0.13 | 0.03 |
| | Highest value | 3.83 | 44.38 | 3.28 | 1.92 | 0.47 |
| *AU5800* | Sample size | 67 | 128 | 84 | 59 | 59 |
| | Median | 1.08 | 7.125 | 1.0 | 1.32 | 0.31 |
| | Lowest value | 0.03 | 0.9 | 0.06 | 0.19 | 0.06 |
| | Highest value | 4.05 | 42.01 | 3.49 | 2.06 | 0.6 |

The comparative analysis demonstrated consistently high Spearman correlation coefficients (rho ≥ 0.90, $p < 0.0001$) across all three platforms for all analytes evaluated (IgA, IgG, IgM, C3, and C4), indicating strong and statistically significant inter-method agreement.

For immunoglobulins (IgA, IgG, and IgM), correlations were particularly strong (rho ranging from 0.93 to 0.995), with regression parameters showing slopes close to 1 and intercepts near 0 across most platform pairs. This suggests excellent agreement in quantification among the systems. In contrast, the complement components (C3 and C4) exhibited greater variability, particularly in comparisons involving the AU5800. Notably, C4 showed substantial proportional bias, with slope values reaching 1.3226 (Cobas Pro vs. AU5800) and 1.3141 (Atellica vs. AU5800), indicating that the AU5800 tends to report markedly higher C4

concentrations compared to the other platforms (Table 3 and Chart 1).

Residual Standard Deviations (RSDs) and the ±1.96 RSD ranges were within acceptable limits for all analytes. Additionally, the Cusum test results ($p > 0.05$) confirmed that the linear regression models adequately fit the data, with no evidence of systematic deviation (Table 3).

**Table 3. Spearman Correlation and Passing-Bablok Regression Analysis of method comparison for serum immunoglobulin and complement measurements across Cobas Pro, Atellica Solution, and AU5800 platforms**

| Platforms | Analyte / Statistics | IgA | IgG | IgM | C3 | C4 |
|---|---|---|---|---|---|---|
| *Cobas Pro and Atellica* | Spearman's rho | 0.985 | 0.994 | 0.982 | 0.90 | 0.945 |
| | p-value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | Intercept (95% CI) | 0.0108 (-0.0469 to 0.0676) | -0.2613 (-0.4022 to -0.1658) | -0.0532 (-0.0790 to -0.0247) | -0.1322 (-0.2340 to -0.0350) | -0.0300 (-0.0459 to 0.0139) |
| | Slope (95%CI) | 0.9481 (0.9118 to 0.9862) | 1.0043 (0.9882 to 1.0217) | 1.0795 (1.0541 to 1.1059) | 0.8706 (0.8000 to 0.9492) | 1.0000 (0.9394 to 1.0909) |
| | Residual Standard Deviation (RSD) | 0.1151 | 0.5027 | 0.0934 | 0.1101 | 0.02 |
| | ± 1.96 RSD | -0.2257 to 0.2257 | -0.9852 to 0.9852 | -0.1831 to 0.1831 | -0.2159 to 0.2159 | -0.0392 to 0.0392 |
| | Cusum Test | p = 0.63 | p = 0.29 | p = 0.41 | p = 0.56 | p = 0.78 |
| *Cobas Pro and AU5800* | Spearman's rho | 0.984 | 0.9721 | 0.939 | 0.906 | 0.992 |
| | p-value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | Intercept (95% CI) | -0.0175 (-0.0700 to 0.0105) | 0.0918 (-0.0138 to 0.2016) | -0.0535 (-0.0899 to -0.0235) | -0.0773 (-0.1700 to 0.0021) | -0.0210 (-0.0327 to -0.0106) |
| | Slope (95%CI) | 0.9549 (0.9180 to 0.9855) | 0.8986 (0.8838 to 0.9150) | 1.0127 (0.9867 to 1.0459) | 0.9091 (0.8451 to 1.0000) | 1.3226 (1.2813 to 1.3636) |
| | Residual Standard Deviation (RSD) | 0.0974 | 1.1202 | 0.1309 | 0.1005 | 0.0089 |

| Platforms | Analyte / Statistics | IgA | IgG | IgM | C3 | C4 |
|---|---|---|---|---|---|---|
| *Cobas Pro and AU5800* | ± 1.96 RSD | -0.1910 to 0.1910 | -2.1955 to 2.1955 | -0.2566 to 0.2566 | -0.1971 to 0.1971 | -0.0174 to 0.0174 |
| | Cusum Test | p = 0.63 | p = 0.09 | p = 0.77 | p = 0.35 | p = 0.54 |
| *Atellica and AU5800* | Spearman's rho | 0.995 | 0.9695 | 0.930 | 0.973 | 0.964 |
| | p-value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | Intercept (95% CI) | -0.0400 (-0.0656 to -0.0176) | 0.3518 (0.2016 to 0.4924) | -0.0111 (-0.0285 to 0.0099) | 0.0954 (0.0382 to 0.1468) | 0.0149 (-0.0020 to 0.0312) |
| | Slope (95%CI) | 1.0000 (0.9780 to 1.0177) | 0.8978 (0.8765 to 0.9204) | 0.9442 (0.9204 to 0.9655) | 1.0168 (0.9737 to 1.0633) | 1.3141 (1.2353 to 1.4000) |
| | Residual Standard Deviation (RSD) | 0.0646 | 1.1664 | 0.1223 | 0.0451 | 0.0178 |
| | ± 1.96 RSD | -0.1267 to 0.1267 | -2.2861 to 2.2861 | -0.2397 to 0.2397 | -0.0883 to 0.0883 | -0.0348 to 0.0348 |
| | Cusum Test | p = 0.46 | p = 0.53 | p = 0.77 | p = 0.94 | p = 0.94 |

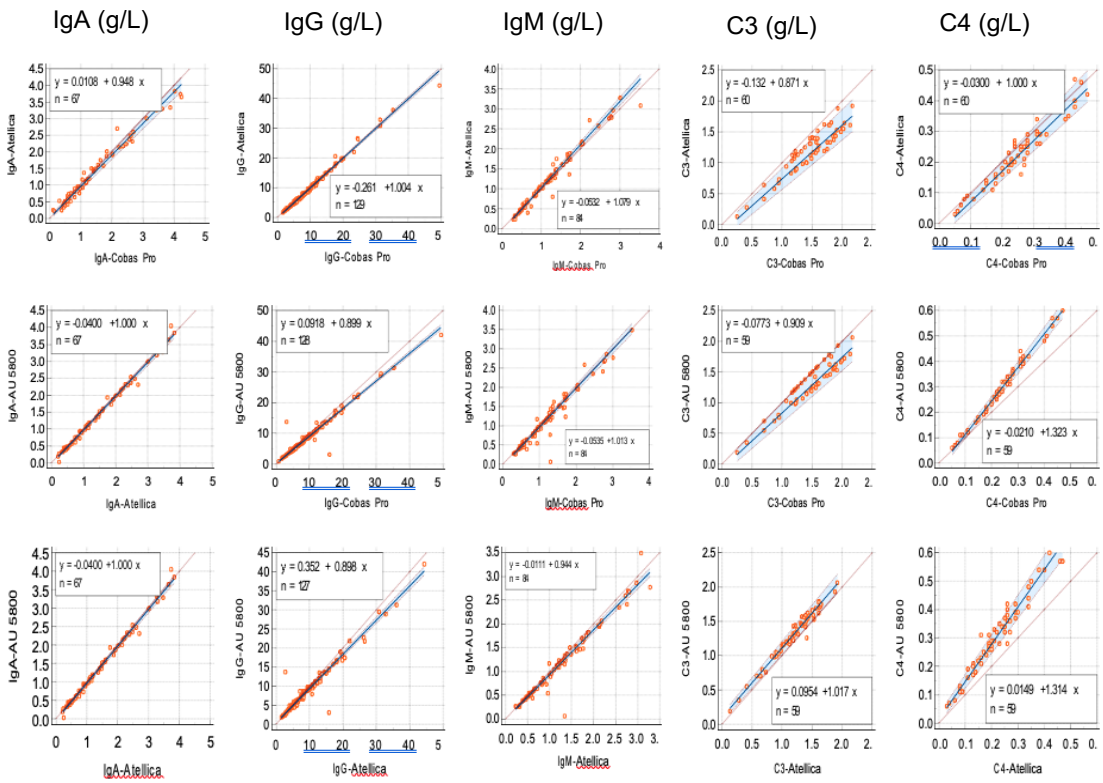


**Chart 1. Passing-Bablok plots comparing the serum immunoglobulin and complement measurements across the Cobas Pro, Atellica Solution, and AU5800 systems**

**Table 4. Bland-Altman analysis of method comparison for serum immunoglobulin and complement measurements across Cobas Pro, Atellica Solution, and AU5800 platforms**

| Platforms | Analyte / Statistics | IgA | IgG | IgM | C3 | C4 |
|---|---|---|---|---|---|---|
| *Cobas Pro and Atellica* | Mean bias (95% CI) | 0.0512 | 0.1963 | -0.0256 | 0.2685 | 0.0255 |
| | Mean bias (%) (95% CI) | 2.01 | 3.20 | 0.012 | 21.59 | 13.50 |
| | LOA | -0.2965 to 0.3989 | -1.1872 to 1.5797 | -0.2882 to 0.237 | -0.0264 to 0.5634 | -0.0287 to 0.0797 |
| | LOA (%) | -0.3387 to 0.3789 | -0.0825 to 0.1465 | -0.2324 to 0.2327 | -0.0349 to 0.4666 | -0.3387 to 37.89 |

| Platforms | Analyte / Statistics | IgA | IgG | IgM | C3 | C4 |
|---|---|---|---|---|---|---|
| *Cobas Pro and AU5800* | Mean bias (95% CI) | 0.0909 | 0.8242 | 0.0682 | 0.1559 | -0.0605 |
| | Mean bias (%) (95% CI) | 8.76 | 7.94 | 9.44 | 11.71 | -20.59 |
| | LOA | -0.2052 to 0.387 | -2.617 to 4.2655 | -0.2888 to 0.4252 | -0.1061 to 0.418 | -0.1254 to 0.0043 |
| | LOA (%) | -0.1384 to 0.4085 | -0.2949 to 0.4702 | -0.2676 to 0.4263 | -0.3588 to 0.5476 | -0.0683 to 0.3025 |
| *Atellica and AU5800* | Mean bias (95% CI) | 0.0397 | 0.6364 | 0.0938 | -0.1092 | -0.0859 |
| | Mean bias (%) (95% CI) | 6.26 | 4.96 | 9.40 | -9.92 | -33.85 |
| | LOA | -0.1381 to 0.2175 | -2.8982 to 4.171 | -0.2418 to 0.4295 | -0.2331 to 0.0148 | -0.1612 to -0.0106 |
| | LOA (%) | -0.3296 to 0.4549 | -0.3199 to 0.4192 | -0.3217 to 0.5097 | -0.2365 to 0.0382 | -0.5513 to -0.1258 |
| *Acceptable Bias (%)* [11] | Optimal | 4.2 | 2.2 | 6.1 | 2 | 3.2 |
| | Desirable | 8.3 | 4.4 | 12.2 | 4 | 6.4 |
| | Minimum | 12.5 | 6.5 | 18.3 | 6 | 9.5 |

*Note: LOA - Limit of agreement*

Bland-Altman analysis among the three systems-Cobas Pro, Atellica Solution, and AU5800- revealed that the mean bias varied considerably across analytes. For IgA, the mean bias ranged from 2.01% (Cobas Pro vs. Atellica) to 8.76% (Cobas Pro vs. AU5800), all within the minimum allowable limit based on biological variation (< 12.5%). However, only the Cobas-Atellica comparison met the optimal performance criteria.

For IgG, the highest bias was observed between Cobas Pro and AU5800 (7.94%), approaching the minimum allowable threshold (6.5%) and exceeding the desirable limit. This suggests that adjustments may be necessary when comparing results between these platforms.

For IgM, all three comparisons showed biases within the allowable range (< 18.3%). Nevertheless, only the Cobas-Atellica comparison met the optimal criterion, while the other comparisons exceeded the desirable limit (6.1%).

Notably, substantial discrepancies were observed in the measurements of C3 and C4. The bias for C3 between Cobas and Atellica reached 21.59%, and for C4 between Atellica and AU5800, it was -33.85%, both far exceeding the minimum specifications (C3 < 6%, C4 < 9.5%). In addition, the limits of agreement (LOA) for IgG, C3, and C4 were wide-particularly for C4 between Atellica and AU5800 (-55.13% to -12.58%)-indicating not only systematic bias but also considerable sample-to-sample variability.

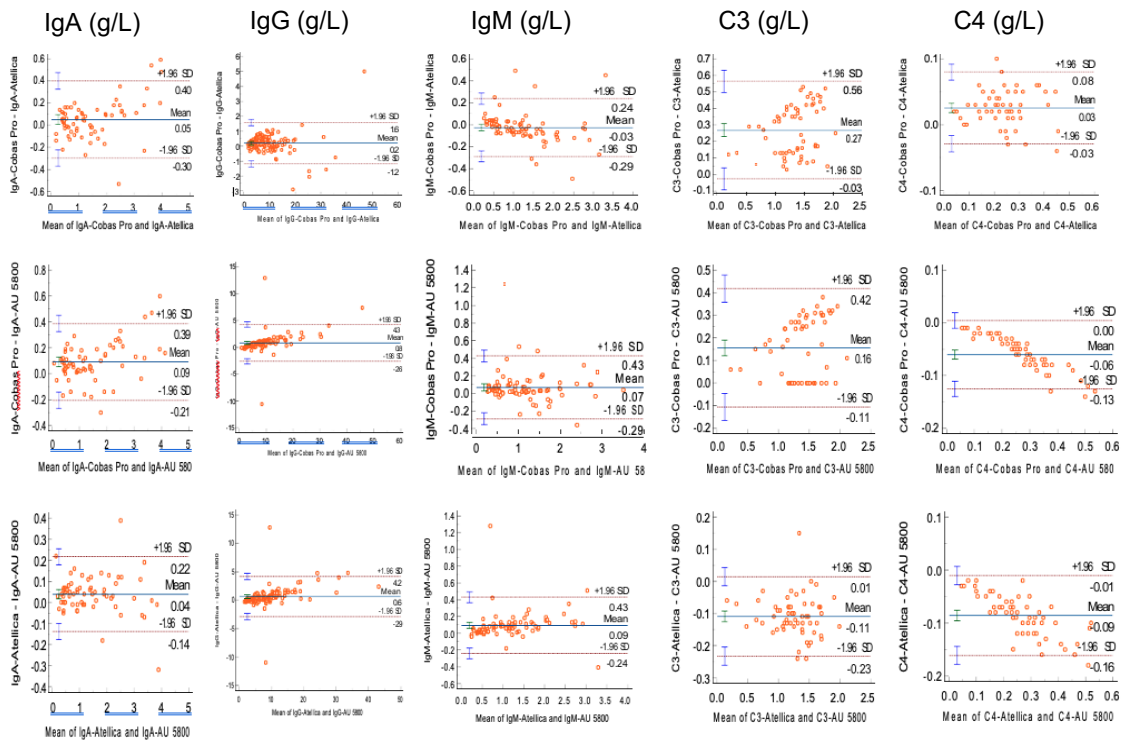


**Chart 2. Passing-Bablok and Bland-Altman plots comparing the serum immunoglobulin and complement measurements across the Cobas Pro, Atellica Solution, and AU5800 systems**

## IV. DISCUSSION

This study evaluated the concordance of serum IgA, IgG, IgM, C3, and C4 quantification results across three automated analytical systems: Cobas Pro (Roche Diagnostics), Atellica Solution (Siemens Healthineers), and AU5800 (Beckman Coulter), using 59 to 129 pediatric serum samples per analyte. All systems employed the immunoturbidimetric method and were traceable to the same international reference material to ensure consistency in calibration and measurement principles.

Correlation analysis in our study revealed a strong linear relationship between Cobas Pro, Atellica Solution, and AU5800 in the quantification of serum IgA, IgG, IgM, C3, and C4, with Spearman correlation coefficients ranging from 0.898 to 0.9961 ($p < 0.0001$). This suggests that analyte ranking across platforms was generally consistent. However, correlation alone does not imply quantitative agreement. As emphasized by the Clinical and Laboratory Standards Institute (CLSI), method comparison should evaluate both correlation and systematic bias.[10]

Passing-Bablok regression revealed notable proportional differences between certain platform pairs. For example, the slope of 1.3141 for C4 between AU5800 and Atellica indicated that AU5800 tended to report approximately 30% higher values. Similar proportional biases were observed for IgA and C3. Bland-Altman analysis further confirmed these discrepancies, showing a mean bias of -33.85% for C4 with wide limits of agreement (-55.13% to -12.58%). Comparable deviations were observed for IgM and C3, underlining the presence of significant systematic errors.

To assess the clinical relevance of these observed differences, analytical quality specifications (AQS) derived from biological variation were applied. According to the EFLM Biological Variation Database, the minimum allowable bias for C4 is 9.5%, while the desirable and optimal thresholds are 6.4% and 3.2%, respectively.[11] The observed bias for C4 in this study far exceeded these thresholds, highlighting the need for careful clinical interpretation and system-specific validation.

These results are consistent with previous reports. Qin et al documented meaningful differences in immunoglobulin and complement quantification between nephelometric systems.[9] Likewise, Mali et al found that analytical bias between turbidimetric and nephelometric methods, especially for C4 and IgM, often exceeded biological variation thresholds.[13] Denham et al also reported significant proportional bias between immunoturbidimetric methods on the Architect ci8200 and nephelometry, despite strong correlation.[14]

Although all three platforms in our study employed immunoturbidimetric assays traceable to a common international reference material, such as CRM 470, differences in results were largely attributable to system-specific technical factors. These include antibody specificity, epitope recognition, optical signal processing, calibration modeling, and matrix interactions-factors that have been extensively discussed in prior evaluations of immunodetection variability.[7,8,15] Importantly, C4 is a low-abundance protein with known structural heterogeneity and susceptibility to partial degradation, making it particularly sensitive to differences in reagent design and assay configuration. In addition, manufacturers may adopt distinct calibration strategies - such as matrix-based versus purified protein calibrators - which can further contribute to systematic discrepancies across platforms. These technical and methodological factors likely underlie the observed proportional biases for C4.

These concerns are further supported by findings from a large External Quality Assessment Scheme, which demonstrated that inter-system variability persists even under harmonized calibration standards, particularly for serum proteins such as immunoglobulins and complement components.[12] The report emphasized the need for methodological harmonization and careful interpretation when comparing results across platforms.

Despite strong correlations among the systems, the observed proportional and systematic differences, as well as nonlinearity in certain concentration ranges, suggest that results from these platforms should not be used interchangeably without proper adjustment. Therefore, caution is warranted when interpreting results in clinical settings, especially in pediatric populations where reference intervals vary by age and physiological status. As the study was limited to pediatric patients, the findings may not be generalizable to other age groups. Laboratories employing multiple analytical systems are advised to establish system-specific reference intervals or validated internal conversion factors. Independent validation of these conversion factors in adult populations is also recommended to ensure diagnostic accuracy and safety.

## V. CONCLUSION

The study demonstrated that the three automated analytical systems Cobas Pro (Roche Diagnostics), Atellica Solution (Siemens Healthineers), and AU5800 (Beckman Coulter) exhibited very strong correlations (rho = 0.90 - 0.995) in the quantification of serum IgA, IgG, IgM, C3, and C4 in pediatric patients. Despite

this strong correlation, notable proportional differences were observed, particularly in C4 measurements. The slope between Cobas Pro and AU5800 was 1.3226, and 1.3141 between Atellica and AU5800, indicating that AU5800 consistently reported higher C4 values. Bland-Altman analysis further confirmed clinically significant discrepancies and systematic bias. These findings suggest that results are not directly interchangeable without proper adjustment. Establishing system-specific reference intervals or applying validated conversion factors is necessary to ensure accuracy in clinical interpretation.

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