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Research Article

Rheumatoid Factor and ASO assessment by immunoturbidimetry and immunonephelometry

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Abstract

Objectives: In this study, ASO and RF immunoturbidimetric assays determined on the Roche Cobas analyzer were evaluated against an immunonephelometric method.

Methods: ASO and RF assays were performed with the immunonephelometric method using the Beckman Coulter Immage 800 analyzer and the immunoturbidimetric method using the Cobas c501 analyzer. Precision values of both assays were calculated using internal quality control (IQC) samples provided by the test manufacturers. In addition, to assess bias, IQC and external quality control (EQA) data were used. Method comparison studies were performed using serum specimens randomly selected from routine hospital orders.

Results: Both assays demonstrated good precision for ASO, with precision values of 3.2% CV in the immunoturbidimetric assay and 5.0% CV in the immunonephelometric assay. Although the immunoturbidimetric assay for RF showed good precision, the precision of RF exceeded the desired limits in the immunonephelometric assay. Bias obtained from EQA data was excellent in both ASO and RF for the immunoturbidimetric assay. The Passing–Bablok regression equation was obtained as y=1.65x - 20, r=0.98 for ASO, and as y=1.02x - 10.9, r=0.85 for RF.

Conclusion: In conclusion, ASO and RF tests on the Cobas analyzer are suitable for routine use because they meet the requirements for accuracy and precision. The imprecision of the RF assay should be improved, especially for the immunonephelometric assay.

Keywords: Antistreptolysin-O, nephelometry, rheumatoid factor, turbidimetry

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The utilization of specific protein tests to predict risks associated with infection and autoantibody presence has increased over the last few years. Antistreptolysin O (ASO) and rheumatoid factor (RF) tests are among the most requested tests. ASO antibodies are produced by the host after an infection with group A beta-hemolytic streptococcus. ASO is used as a serological marker to indicate a past infection, even though evidence of its usefulness is limited. ASO titers are frequently requested, especially in cases of acute tonsillitis in the pediatric population [1]. RF is an autoantibody directed against gamma globulins. Rheumatoid arthritis (RA) patients have high RF levels in serum [2]; its titer mostly correlates with disease severity and predicts a poor prognosis [3, 4].

Specific proteins are analyzed using specialized methods such as radial immunodiffusion, immunoelectrophoresis, ELISA, dedicated immunonephelometers, or immunoturbidimeters [5]. Nephelometry has traditionally been considered a reference method. The most frequently used methods for the routine measurement of serum ASO and RF are based on immunonephelometry or immunoturbidimetry. Turbidimetry and nephelometry are photometric assays commonly used to quantify immune-complex precipitates by their ability to interact with incident light [6]. A special analyzer is required for nephelometric measurements, whereas turbidimetric measurements can be easily performed on a clinical chemistry analyzer. Thus, clinical laboratories may opt to shift some analyses

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to the clinical chemistry analyzer for cost savings. Some studies in the literature compare specific protein levels between turbidimetry and nephelometric methods [7, 8]. These studies compared the turbidimetric system of the Abbott Architect ci8200 with the nephelometric systems of Beckman Immage or Dade Behring. To the best of our knowledge, there is no comparative study in the literature with the turbidimetric assay of the Roche Cobas analyzer, so it is necessary to evaluate the performance of specific protein assays on the Cobas system.

In this study, we aim to evaluate the analytical performance of ASO and RF assays conducted on the Cobas analyzer and to compare them with nephelometric assays.

Materials and Methods

Analyzers and assays

Assays for ASO and RF were performed with the immunonephelometric method using the Beckman Coulter Immage 800 (Beckman Coulter Inc., USA) analyzer and the immunoturbidimetric method using the Cobas c501 (Roche Diagnostics GmbH, Mannheim, Germany) analyzer. The Cobas c501 module performs photometric assays, with a throughput of up to 1000 tests per hour for a combination of photometric and ion-selective electrode (ISE) tests. The Immage 800 is a nephelometer that performs approximately 180 tests per hour. Reagents, calibrators, and quality control (QC) materials were of the same origin as the instruments. The sample volume is 2 µL for ASO and 3 µL for RF in the Cobas system, while it is 3.5 µL for ASO and 5 µL for RF in the Immage system. The analytical ranges were 20–600 IU/mL (Cobas) and 25–800 IU/mL (Immage) for ASO, and 10–130 IU/mL (Cobas) and 20–800 IU/mL (Immage) for RF. The declared within-run imprecision for Cobas was below 2% for the ASO assay and below 1% for the RF assay, whereas for Immage, it was above 2% for both the ASO and RF assays.

Precision and bias

Precision study was performed according to the CLSI EP05-A3 protocol using the manufacturers internal quality control (IQC) (low and high levels) materials [6]. The cumulative coefficient of variation (CV%) measured on different days (20 days/month, twice a day), was calculated for both IQC levels, designated as CV1% (low-level) and CV2% (high-level). Total CV% was calculated as the following formula:

$$CV\% = \sqrt{(CV1^2 + CV2^2)}$$

Biases were calculated from the difference between the laboratory results and the target values of the IQC samples in the control inserts. To obtain bias of the turbidimetric method, the external quality assurance (EQA) program data were collected from BIORAD EQAS schemes (Monthly specific proteins) in 2023 year. Bias% values were calculated as following formula:

Bias% = Lab EQAS result- Peer group mean / Peer group mean

The desirable specifications for imprecision and bias were presented as 4.3% and 6.5% for RF test [10]. Hoewever, there were no desirable specifications in the literature for ASO test.

Method comparison

For comparison, fresh serum samples from patients whose ASO and RF levels were ordered in the routine laboratory were used. No additional samples were collected, no medical records were reviewed, and no contact with patients was made. For method comparison experiments, samples were analyzed on the two analyzers on the same day. Abnormal samples, such as those indicating hemolysis, icterus, or lipemia, were excluded.

Statistical analysis

The MedCalc for Windows statistical package (MedCalc Software, Ostend, Belgium) was used to perform method comparison. Method comparison results were analyzed using Passing-Bablok regression analysis and presented as y=bx+a. Intercept (a) and slope (b) values were considered significantly different from 0 and 1, respectively.

Results

The results of the precision and bias studies are summarized in Table 1.

We compared random patient samples, which included 99 for ASO and 61 for RF results. The patient sample-based method comparison data is presented in Figures 1 and 2. Strong correlations were determined between the turbidimetric and nephelometric methods for ASO and RF (r=0.977 and r=0.854, respectively). Passing-Bablok regression analysis gave a slope of 1.64 and an intercept of -20.0 for ASO, and a slope of 1.02 and an intercept of -10.9 for RF (Figs. 1, 2).

Discussion

Recently, growing test volumes for infection and autoantibody detection, along with the need for rapid turnaround times, have led to the increased use of turbidimetric analyzers rather than nephelometric analyzers. Turbidimetric measurements are easily performed on photometers or spectrophotometers and require minimal optimization [11]. In this study, we found that the imprecision of the ASO and RF assays on the Cobas analyzer was acceptable. Tur-

Table 1. Performances obtained IQC and EQA results of two methods

Test	CV1%	CV2%	Total CV%	Bias% (IQC)	Bias% (EQA)
ASO					
Turbidimetry	2.5	2.0	3.2	-3.6	-1.6
Nephelometry	3.8	3.3	5.0	-1.1	NA
RF					
Turbidimetry	2.0	1.5	2.5	1.4	-3.6
Nephelometry	5.6	3.9	7.3	1.6	NA

IQC: Internal quality control; EQA: External quality assurance; CV: Coefficient of variation; ASO: Antistreptolysin O; RF: Rheumatoid factor; NA: Not available

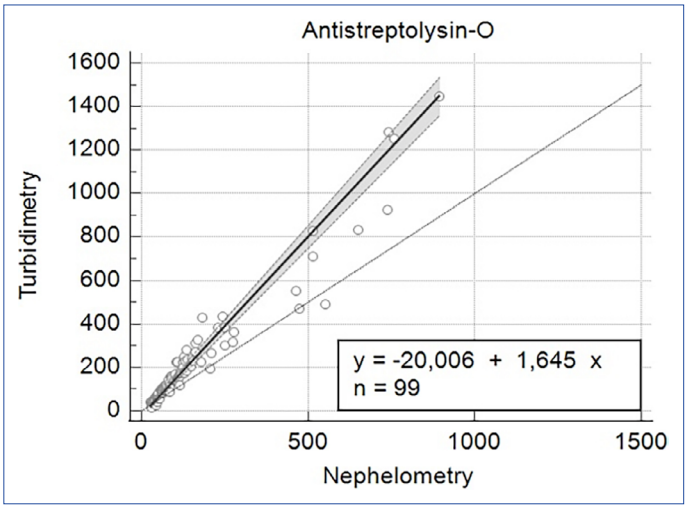

Figure 1. Comparison of ASO results using regression analysis.
ASO: Antistreptolysin O.

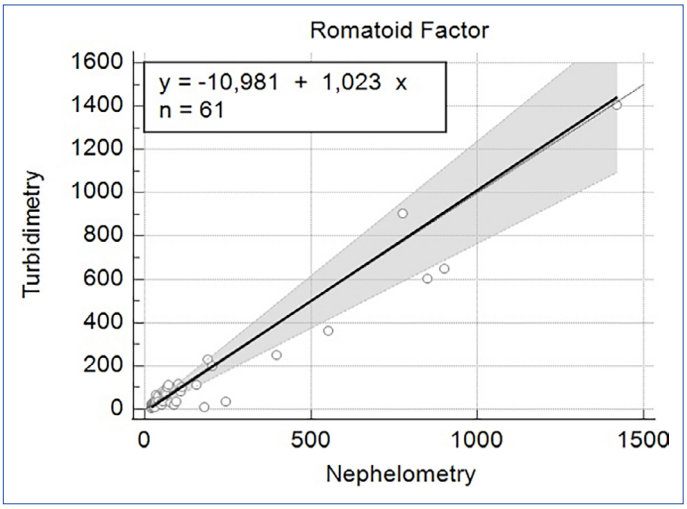

Figure 2. Comparison of RF results using regression analysis.
RF: Rheumatoid factor.

bidimetry appeared to perform well in ASO and RF assays when also evaluated for accuracy. When comparing the two systems, imprecision was lower with the turbidimetry-based instrument than with nephelometry. Our results align with studies reporting that turbidimetric assays are rapid, automated, applicable, and more reproducible than nephelometric assays [12, 13]. However, considering the acceptability of analytical imprecision, RF nephelometric analysis fell outside the desirable range.

The ASO and RF turbidimetric tests compared well with the corresponding nephelometric assays, based on the observed correlation coefficients. Interestingly, although the method bias for ASO and RF was within acceptable ranges, the slope performance indicated a proportional bias for ASO and a constant bias for RF. The regression line slopes from method comparison studies were 1.65 for ASO and 1.02 for RF. Specifically, the slope for ASO was outside the acceptable range; however, this was not evaluated as unexpected, due to antibody specificity in immune measurements based on antigen-antibody complexes. For this reason, the reference ranges declared by the two systems for these tests may differ. The reference interval of the Immage analyzer for ASO is 25–116 IU/mL, while the reference interval of the Cobas analyzer is higher (20–150 IU/mL).

In this comparison, we initially accepted nephelometry as the reference method for serum ASO and RF determination. However, by the end of our study, we believe that this traditional acceptance in the measurement of specific proteins should be reconsidered. Some studies in the literature comparing these two methods already express doubt about which one is more accurate [14–16]. Therefore, we suggest that further improvement in the standardization of nephelometric methods is beneficial, specifically for the RF assay.

There are limitations to our study, as we did not investigate the effects of interference and different clinical conditions, including normal and pathological levels.

Conclusion

In conclusion, ASO and RF tests on the Cobas analyzer are suitable for routine use, as they meet the requirements for accuracy and precision. The imprecision of the RF assay should be improved, especially for the immunonephelometric assay.

Ethics Committee Approval: The study was approved by The Zonguldak Bulent Ecevit University Non-interventional Clinical Research Ethics Committee (No: 2023/09, Date: 03/05/2023).

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