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**Artikelen**

# Evaluation of immunoturbidimetric assays for the determination of C3, C4, haptoglobin, IgA, IgG and IgM on clinical-chemistry analyzer Aeroset® and comparison with other assays

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**We evaluated new immunoturbidimetric assays for the determination of C3, C4, haptoglobin, IgA, IgG and IgM on the clinical-chemistry analyzer Aeroset® from Abbott Diagnostics. The assays showed low imprecisions with coefficients of variation < 3.2% for all analytes investigated. The biases of all analytes were < 4.0%, except for haptoglobin that showed a bias of +8.6%. The linearities were comparable to those of established assays, but the linearity range of the haptoglobin assay was significantly smaller. An assay comparison with immunoturbidimetric assays on Roche´s Hitachi 717® and immunonephelometric assays on Beckman-Coulter´s Immage®, performed with 102 patient samples, showed good correlations (r = 0.948 to 0.997), excepted C4 assay compared to the immunonephelometric assay (r = 0.908). The slopes of the correlation graphs ranged from 0.826 to 1.153. In summary, the new Aersoset® assays were comparable or even superior to established assays with respect to analytical and performance results for medical requirements. However, the linearity of the haptoglobin assay has to be improved.**

*Key words: complement, haptoglobin, immunoglobulins, Aeroset® Analyzer*

The serum proteins examined in this study are measured routinely in many laboratories for different diagnostic reasons: The determination of the complement components C3 and C4 serves to assess the activation status of the complement system in different autoimmune diseases, inspecific infections or complement defects (1). Haptoglobin is determined especially in order to verify hemolytic diseases (2), whereas the immunoglobulins IgA, IgG and IgM are measured in the diagnostics of monoclonal gammopathies (3) and, as conductors of humoral infection defense, in subacute and chronic infections (4) as well as in antibody-deficiency syndromes (5). Methods for the quantitative determination of immunoglobulins are radial immunodiffusion (RID), nephelometry and turbidimetry (6). However, all specific proteins mentioned are usually analyzed with automated immunoturbidimetric and immunonephelometric methods on different analyzers with or without pre-analytical dilution steps. After evaluation of its clinical-chemistry analyzer Aeroset® for analytes measured by ISE and photometry (7), the company Abbott Diagnostics (Irving, Texas, U.S.A.) has recently developed new immunoturbidimetric assays for the determination of the proteins mentioned above on this analyzer. These assays are designed to detect immunocomplexes with high reliability and high speed at wavelengths of 340 nm (IgM), 604 nm (C3, C4, haptoglobin) and 700 nm (IgA, IgG), applying specific monoclonal antibodies. After installation of the clinical-chemistry analyzer Aeroset® (8), we evaluated the analytical performance of these assays with respect to imprecision, bias, limit of detection (LOD), limit of quantitation (LOQ) and linearity under routine conditions of a university hospital laboratory. Furthermore, we carried out assay comparisons with immunoturbidimetric and immunonephelometric assays established on the routine analyzers Hitachi 717® (Roche, Mannheim, Germany) and Immage® (Beckman-Coulter, Krefeld, Germany).

## Material and Methods

Over a period of two months we collected sera obtained from many different patients of our university hospital. Aliquots of the samples and controls were stored in 2 ml plastic cups (Eppendorf, Hamburg, Germany) at -30 °C until analysis. All assays and reagents were applied to the analyzers following the instructions of the manufacturers exactly. Calibrations were performed in accordance with the manufacturer's instructions. Daily quality control was performed according to the German guidelines for quality control (9). The within-day and between-day

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*Abbreviations*: CRM, certified matrix-reference material; LOD, limit of detection; LOQ, limit of quantitation; NCCLS, National Committee for Clinical Laboratory Standards (U.S.A.); RID, radial immunodiffusion

imprecisions were determined for Aeroset® protein assays as well as for Immage® assays according to the NCCLS (National Committee for Clinical Laboratory Standards, U.S.A.) protocol EP5-A (10). To this end, we analyzed 20 replicates each of two specimen ("low" and "high") of pooled patient sera for the determination of the within-day imprecisions. Between-day imprecisions were determined by measuring Bio-Rad Liquicheck® immunology control levels 1 and 3 (Bio-Rad, Munich, Germany) on 20 working days. The biases of all assays were examined by measuring the CRM 470 reference standard (Institute for Reference Materials and Measurements, Geel, Belgium) (11) four times a day. Limits of detection (LODs) and limits of quantitation (LOQs) were evaluated for all assays on the Aeroset®, Hitachi 717® and Immage®. The LODs of the assays were determined statistically by measuring 20 replicates of patient samples containing specific protein concentrations near the detection limit according to Long et al. (12). LODs are calculated then by adding the 2-fold SD of the results for the replicates to zero. LOQs were determined empirically by repeated measuring of different samples containing specific protein concentrations near the quantitation limit as described by Armbruster et al. (13). From the extrapolated curve the LOQ concentration is taken from the point at which CV is 20%. Linearities were evaluated according to the NCCLS protocol EP6-P (14). To this end, samples with high concentrations of specific proteins were diluted with saline to five lower concentration levels covering the linearity ranges stated in the assay sheets. The assay comparisons of the Aeroset® assays with the assays on the routine analyzers Hitachi 717® and Immage® were performed following NCCLS protocol EP9-A (15). 102 patient samples (60 normals and 42 abnormals including 25 sera with extragradients detected by serum electrophoresis) were measured with each of the three assays on the different routine analyzers. Pre-analytical centrifugation of all samples was carried out at 4,000 rpm (radius of centrifuge: 10 cm). The measurements on the analyzers were performed within two hours to avoid influences by different storage. The results were evaluated using the biometric method according to Passing-Bablok (16).

**Results and Discussion**

The results of the measurements for imprecision in patient and control samples are shown in table 1. Within-day imprecisions of the Aeroset® assays in pooled patient sera ranged from 0.7 to 3.2% (mean 1.6%). The imprecisions determined were slightly higher than those presented for Bio-Rad immunochemistry controls by Duly et al. (17). In comparison, the assays on Immage® showed within-run CVs from 1.0 to 5.9% (mean 2.3%) that were slightly higher compared to those described for haptoglobin and immunoglobulins in control sera by Giraudeaux et al. (18). Between-day imprecisions of Aeroset® assays were determined in Liquicheck® controls and ranged from 0.8 to 2.5% (mean 1.5%), whereas the imprecisions of the Immage® assays showed values in the range of 1.7 to 4.0% (mean 2.6%). Hence it follows, that imprecisions investigated are smaller in more stable control than in patient samples and complied in both cases with analytical and medical needs (9, 19). However, the ranges of the CVs of the Aeroset® assays were significantly smaller and the means significantly lower than those of the Immage® assays.

All investigated Aeroset® assays passed two proficiency testings from the German Reference Institute

**Table 1.** Mean values of 20 measurements and within-day imprecisions determined in two different pooled patient sera and in two levels of Bio-Rad Liquicheck® controls with the Aeroset® compared to the Immage® methods

| Analyte | Aeroset® | | Immage® | | Aeroset® | | Immage® | |
|---|---|---|---|---|---|---|---|---|
| | Mean (g/l) | CV (%) | Mean (g/l) | CV (%) | Mean (g/l) | CV (%) | Mean (g/l) | CV (%) |
| | Serum 'low' | | | | Serum 'high' | | | |
| C3 | 0.56 | 2.0 | 0.38 | 1.0 | 2.01 | 1.1 | 2.09 | 2.2 |
| C4 | 0.32 | 1.3 | 0.29 | 2.1 | 0.50 | 1.0 | 0.52 | 1.1 |
| Haptoglobin | 0.39 | 0.9 | 0.46 | 1.0 | 3.59 | 3.2 | 3.34 | 1.9 |
| IgA | 2.64 | 1.1 | 2.89 | 1.9 | 3.93 | 1.6 | 4.68 | 3.1 |
| IgG | 11.46 | 1.1 | 12.00 | 1.5 | 19.11 | 1.6 | 18.20 | 3.1 |
| IgM | 0.40 | 2.1 | 0.41 | 5.9 | 1.26 | 0.7 | 1.61 | 3.3 |
| | Level 1 | | | | Level 3 | | | |
| C3 | 0.78 | 1.5 | 0.78 | 2.8 | 1.83 | 1.4 | 1.85 | 3.4 |
| C4 | 0.15 | 1.0 | 0.14 | 4.0 | 0.39 | 2.5 | 0.40 | 1.7 |
| Haptoglobin | 0.62 | 1.8 | 0.58 | 2.2 | 1.87 | 1.7 | 1.73 | 1.7 |
| IgA | 1.17 | 1.0 | 1.29 | 1.9 | 3.09 | 1.4 | 3.69 | 3.4 |
| IgG | 9.24 | 0.9 | 9.57 | 2.8 | 29.84 | 2.4 | 28.30 | 2.4 |
| IgM | 0.57 | 0.8 | 0.62 | 2.8 | 1.67 | 1.3 | 1.99 | 2.6 |

**Table 2.** Biases determined in CRM 470 standard

| Analyte | Target value (g/l) | Deviation (%) | | |
|---|---|---|---|---|
| | | Aeroset® | Hitachi 717® | Immage® |
| C3 | 1.09 | – 1.2 | + 17.6 | – 1.2 |
| C4 | 0.15 | + 1.2 | + 7.9 | – 11.1 |
| Haptoglobin | 0.89 | + 8.6 | – 14.9 | – 2.3 |
| IgA | 1.96 | + 3.9 | + 14.7 | + 7.4 |
| IgG | 9.68 | + 3.9 | + 3.2 | + 4.0 |
| IgM | 0.80 | – 3.3 | + 7.0 | + 7.5 |

**Table 3.** Limits of detection and limits of quantitation determined for IgA and IgM

| Analyte | Aeroset® (g/l) | Hitachi 717® (g/l) | Immage® (g/l) |
|---|---|---|---|
| LOD: | | | |
| IgA | 0.002 | 0.013 | 0.067* |
| IgM | 0.008 | 0.017 | 0.042* |
| LOQ: | | | |
| IgA | 0.004 | 0.050 | 0.067* |
| IgM | 0.016 | 0.055 | 0.042* |

*minimum value displayed by the analyzer

**Table 4.** Method comparisons with 102 patient samples of the Aeroset® versus the Hitachi 717® and the Immage® methods **(bold numbers:** rejection of null-hypothesis of slope and/or intercept)

| Aeroset® | | compared to Hitachi 717® | | | compared to Immage® | | |
|---|---|---|---|---|---|---|---|
| | Mean (g/l) (min-max) | (y-x)% of x mean (± SD) | Regression eq. y=ax + b | Correlation factor r | (y-x)% of x mean (± SD) | Regression eq. y=ax + b | Correlation factor r |
| C3 | 1.36 (0.27-2.30) | –15.8 (± 4.1) | y = **0.826** x +0.030 | 0.988 | – 8.9 (± 8.3) | y = **0.864** x +**0.065** | 0.948 |
| C4 | 0.26 (0.04-0.64) | – 2.7 (± 7.1) | y = **1.063** x –0.018 | 0.992 | – 1.6 (±16.5) | y = **0.947** x +0.014 | 0.908 |
| Haptoglobin | 1.45 (0.02-4.21) | +10.7 (±14.2) | y = **1.153** x –0.049 | 0.981 | + 4.6 (±15.0) | y = 1.036 x –0.008 | 0.963 |
| IgA | 2.54 (0.67-9.28) | – 8.5 (± 4.5) | y = 0.961 x –**0.112** | 0.997 | –15.1 (± 7.3) | y = **0.871** x –0.040 | 0.981 |
| IgG | 13.9 (4.95-47.36) | + 3.2 (± 4.5) | y = 0.985 x +**0.416** | 0.992 | – 9.8 (± 6.9) | y = **0.895** x +0.008 | 0.987 |
| IgM | 1.28 (0.17-10.93) | – 1.1 (± 7.9) | y = 1.033 x –0.048 | 0.997 | –21.8 (±10.3) | y = **0.771** x +0.002 | 0.987 |

for Bioanalysis (DGKC, Bonn, Germany). The investigation of bias versus the CRM 470 reference standard (table 2) resulted in deviations of -1.2 to +8.6% (mean +2.2%). The biases for Hitachi 717® assays were determined with -14.9 to +17.6% (mean +5.9%) and with -11.1 to +7.5% (mean -0.7%) for Immage® assays. Deviations of the protein measurements with Aeroset® assays compared with CRM 470 standard were <4%, except for haptoglobin that showed a bias of +8.6%. Therefore, the biases of the Aeroset® assays were found to be lowest comparing all three instruments. Additionally, we compared the results of Bio-Rad Liquicheck® controls with the target values for the immunoturbidimetric Hitachi 717® assays. All assays showed biases ranging from -14.4 to +18.8% (mean -4.7%).

The results of the determinations of the LODs and LOQs for IgA and IgM assays are presented in table 3. The values shown for Immage® assays represent the lowest concentration results displayed by the instrument, whereas the other values were obtained from analysis in the way described above. The LODs and LOQs of the Aeroset® assays were signicantly lower than those of Hitachi 717® and Immage® assays. Furthermore, the Aeroset® assays required 2.0 to 6.5 µl of patient sample for a single determination, whereas the Hitachi 717® and Immage® assays required 2 to 10 µl and 0.1 to 0.6 µl, respectively. Therefore, the Aeroset® assays improve the ability to detect low concentrations of IgA or IgM reliably without assay modification at low serum-sample volumes. The high sensitivity might also offer the option to analyze low concentrations of immunoglobulins in cerebrospinal fluid.

Linearities were confirmed with regression coefficients of r = 0.993-1.000 for C3 (0.01-2.26 g/l), C4 (0.07-0.70 g/l), haptoglobin (1.05-2.11 g/l), IgA (0.04 to 7.00 g/l), IgG (3.72-37.15 g/l), and IgM (0.04-3.11 g/l). The upper limits of the linearity ranges for all analytes depended on the highest specific protein concentration in the calibrator batch stated by the manufacturer. The linearity ranges of all Aeroset® assays were comparable to the stated initial measuring ranges of Hitachi 717® and Immage® assays. However, the haptoglobin assay showed a lower linearity compared with the assays established and the linearity range was smaller than described by Duly et al. (17) (linearity range 0.11-2.77 g/l), too.

Table 4 shows the results of the assay comparisons. Significant differences with rejection of null-hypothesis for the regression line slopes and/or intercepts were observed between most of the Aeroset® compared with Hitachi 717® or Immage® assays (bold numbers in table 4). Decreased and increased slopes and intercepts in these assay comparisons should result in larger differences between the biases of two

methods in table 2, too. In this point of view, the comparison of Aeroset® with Hitachi 717® assays showed good agreement, but as compared with Immage® assays the slopes seem to be somewhat lower than assumpted from bias determinations. On average, the Aeroset® measures C3 slightly lower than Hitachi 717® ((y-x)% of x: -15.8, slope 0.826) and Immage® ((y-x)% of x: -8.9, slope 0.864) assays. Haptoglobin is measured >10% higher than with the corresponding Hitachi 717® assay ((y-x)% of x: +10.7, slope 1.153). Slightly lower values were also determined with Aeroset® immunoglobulin assays as compared with Immage® assays ((y-x)% of x: -21.8 to -9.8, slope 0.771 to 0.895). Overall, the slopes of the regression equations were compatible with the different reference ranges, that differ in the differentiation of age and sex, the mean value and ranges, that were stated for the three instruments by the manufacturers. Differences regarding regression equations and the reference ranges might be explained with the different binding specificity and strength of applied reagent's antibodies, but also with possible lipemia.

The investigated Aeroset® and Immage® assays for complement factors and immunoglobins use antibodies obtained from goat serum, whereas the Hitachi 717® assays involve reagents with antibodies obtained from rabbit serum. Additional information about the antibody-binding capabilities was not available. The C3 assays on the Aeroset® and the Hitachi 717® should measure similar or even higher concentrations compared to the Immage assay, because they promise to detect inactive C3 and the activated degradation products C3a, C3b, C3c and C3d, whereas the Immage® assay promises to measure native C3 and the two main products in complement activation C3c and C3d only. However, this cannot be confirmed in these assay comparisons and is perhaps also due to differences in binding specificity and binding strength of the antibodies applied.

The absence of prozone effect was checked by Abbott Diagnostics for all of the investigated assays up to the 2- to 10-fold of the upper limits of linearity. Nevertheless, paraproteins are stated as interferents for all Aeroset® assays as well as for complement and immunoglobulin assays on the Hitachi 717® and immunoglobulin assays on the Immage®. The Aeroset® offered the option to uncover and handle antigen excess in immunoglobulin determinations, e.g. for IgA and IgM, to avoid additional time-consuming electrophoresis of a patient sample to prove for such interferences. Two measuring methods ("diluted" and "undiluted") were carried out by the instrument parallel and the operator was assisted by suggestions for sample rerun options in comparing the ratio of these two determinations with acceptability criteria. In the assay comparisons of this study, including 25 patient samples with monoclonal peaks in the gamma-fraction of serum electrophoresis, this assistance worked well except for one IgA determination in a sample from a patient with known IgA plasmocytoma. With some single measurements we confirmed interference declarations for bilirubin, hemoglobin and triglycerides that were determined by Abbott Diagnostics at medical decision levels according to NCCLS protocol EP7-P (20). Negligible interferences of <10% were observed for bilirubin concentrations of up to 1,026 µmol/l, for hemoglobin concentrations of up to 5,000 mg/l and for triglyceride concentrations of up to 8.5 mmol/l.

## Conclusions

The application of the new specific protein assays in the clinical-chemistry analyzer Aeroset® improves laboratory efficiency by using low sample and reagent volumes. Good results were obtained regarding imprecisions, biases, limits or detection and limits of quantitation of the Aeroset® assays in comparison with established assays. From imprecision measurements we can conclude that calibration and reagent stabilities were >25 days for the Aeroset® assays, which was in accordance with the stated time of Abbott Diagnostics of 23 to 41 days. Especially the low limits of detection and limits of quantitation of the Aeroset® assays, together with low imprecisions for the IgA and IgM assays, improve the ability to detect reliably hypo-gamma-globulinemias. With the analytical and instrumental qualities shown, it seems to be possible to apply the assays to cerebrospinal fluid, also. Linearities show qualities comparable to or even better than the assays established on Hitachi 717® and Immage® analyzers, but linearity of the haptoglobin assay still needs to be improved. In the assay comparisons the results of the Aeroset® assays differ from those of Hitachi 717® and Immage® assays, but due to good correlation factors results can be adapted to these assays.

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**Literature**

1. Frank MM. Detection of complement in disease. J Allergy Clin Immunol 1992; 89: 641-648.
2. Marchand A, Galen RS, Lente F van. The predictive value of serum haptoglobin in hemolytic disease. JAMA 1980; 243: 1909-1911.
3. Legras B, Gaudin M, Ruelland A, Cottencin M, Cloarec L. Revue critique de 243 immunoglobulinopathies monoclonales. Ann Biol Clin (Paris) 1984; 42: 211-216.
4. Gross S, Blaiss MS, Herrod HG. Role of immunoglobulin subclasses and specific antibody determinations in the evaluation of recurrent infection in children. J Pediadr 1992; 121: 516-522.
5. Hanson LA, Soederstroem T, Oxelius VA, eds. Immunoglobulin subclass deficiencies. Monographs in Allergy 1986: Basen, Karger, Vol. 20.
6. Thomas L. Quantitative immunchemische Plasmaproteinbestimmung mittels Nephelometrie und Turbidimetrie. Lab Med 1990; 14: 313-320.
7. Sanders E, van Wijk EM, Eijkman-Rotteveel RC. Analytische evaluatie van de Abbott Aeroset analyzer. Ned Tijdschr Klin Chem 1999; 24: 275-280.
8. Sturm B. Aeroset® CC Analysatoren im Routineeinsatz. Abbott Times 2001; 2: 34-35.
9. Bundesaerztekammer. Qualitaetssicherung der quantitativen Bestimmungen im Laboratorium. Deutsches Aerzteblatt 1988; 85: A697-712.

10. National Committee for Clinical Laboratory Standards. Evaluation of imprecision performance of clinical chemistry devices; approved guideline EP5-A. Wayne, PA: NCCLS 1999: 1-43.
11. Baudner S, Bienvenu J, Blirup-Jensen S, Carlström A, Johnson AM, Milford Word A, *et al*. The certification of a matrix reference material for immunochemical measurement of 14 human serum proteins - CRM 470 (EUR 15243). Luxembourg: Commision of the European Communities, 1993.
12. Long GL, Winefordner JD. Limit of detection: a closer look at the IUPAC definition. Anal Chem 1983; 55: 712A-724A.
13. Armbruster DA, Tillman MD, Hubbs LM. Limit of detection (LOD)/Limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs. Clin Chem 1994; 40: 1233-1238.
14. National Committee for Clinical Laboratory Standards. Evaluation of the linearity of quantitative analytical methods; proposed guideline EP6-P. Wayne, PA: NCCLS 1986: 509-575.
15. National Committee for Clinical Laboratory Standards. Method comparison and bias estimation using patient samples; approved guideline EP9-A. Wayne, PA: NCCLS 1995, 1-36.
16. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J Clin Chem Clin Biochem 1983; 21: 709-720.
17. Duly EB, Barnes G, Grimason S, Trinick TR. Analytical performance of specific-protein assays on Abbott Aeroset® system [Short communication]. Clin Chem 2001; 47: 1709-1710.
18. Giraudeaux V, Fayard MF, Zerbani A. Evaluation analytique de l'Immage pour dix proteines. Ann Biol Clin (Paris) 1998; 56: 593-598.
19. Fraser CG, Petersen CG. Desirable standards for laboratory tests if they are to fulfill medical needs. Clin Chem 1993; 39: 1447-1455.
20. National Committee for Clinical Laboratory Standards. Interference testing in clinical chemistry (EP7-P); proposed guideline EP7-P. Wayne, PA: NCCLS 1986; 45: 62-67.