

Standardisation of PR3-ANCA and MPO-ANCA

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Standardisation of PR3-ANCA and MPO-ANCA: evaluation of certified reference materials

Antineutrophil cytoplasmic antibodies (ANCA) are important laboratory markers to support the diagnosis of ANCA-associated vasculitis. A 2017 international consensus states that high-quality immunoassays for proteinase-3 (PR3)-ANCA and myeloperoxidase (MPO)-ANCA can be used as the primary screening method for patients suspected of having granulomatosis with polyangiitis or microscopic polyangiitis.¹

Efforts have been undertaken to standardise PR3-ANCA and MPO-ANCA measurements. More than 10 years ago, PR3-ANCA and MPO-ANCA reference standards have been prepared (from single patients) under the umbrella of the International Union of Immunological Societies (IUIS) and are available through the Autoantibody Standardization Committee (www.AutoAb.org). Several companies are using these reference standards (assigned value: 100 IU/mL) to calibrate their assays, such as Svar Life Science (ELISA), Thermo Fisher Scientific (fluoroenzyme immunoassay (FEIA)), Medipan (CytoBead assay) and Inova Diagnostics (chemiluminescence assay (CLIA)).

The Institute for Reference Materials and Measurements (IRMM) recently released certified reference materials for MPO-ANCA (ERM-DA476/IFCC) and for PR3-ANCA (ERM-DA483/IFCC).^{2,3} Both reference materials are based on plasma-pheresis samples from single patients. We evaluated whether using the IRMM-certified reference materials aligns results for PR3-ANCA and MPO-ANCA across assays.

A random selection of 98 serum samples covering low, medium and high PR3-ANCA or MPO-ANCA levels from the Leuven and the Maastricht cohort included in the international EUVAS study⁴ were distributed to five manufacturers, together with the PR3-ANCA and MPO-ANCA certified reference materials and dilutions thereof (obtained from I. Zegers, IRMM). The manufacturers performed the analyses using the IRMM reference

materials as standards and reported results in mass units ($\mu\text{g/L}$). The immunoassays included were from Inova (QUANTA Flash (CLIA)) and (QUANTA Lite (ELISA)), Euroimmun (ELISA), GA Generic Assays (ELISA), Orgentec (ELISA) and Thermo Fisher Scientific (EliA (FEIA)).

The results are shown in [figure 1](#). Overall, results obtained with the different assays were in the same order of magnitude. For PR3-ANCA, the best correlations were found between the ELISAs from Euroimmun, Inova Diagnostics and GA Generic Assays (Spearman's $r_s > 0.89$). For other comparisons, such as between automated FEIA and automated CLIA, the correlation was poor (0.44). For MPO-ANCA, the best correlations were found between the ELISAs from Inova Diagnostics and GA Generic Assays (0.84) and between the ELISA from Euroimmun and FEIA from Thermo Fisher Scientific (0.82). Differences in analytical measuring ranges (AMRs) as well as significant portions of specimens outside the AMR for some assays likely contribute to the low agreement between assays. Concordance between the different assays is shown in online supplementary figure 1. For PR3-ANCA, 39/98 and 46/98 samples were concordant positive and negative, respectively, with all assays. For MPO-ANCA, 21/98 and 74/98 samples were concordant positive and negative, respectively, with all assays.

Taken together, using the certified reference material for PR3-ANCA and MPO-ANCA squeezes test results within the same order of magnitude. However, depending on the assays compared, differences and poor correlation between individual results remain. This is in agreement with a previous study reporting that the majority of assays do not give comparable ANCA values, including assays calibrated on the IUIS reference standards.⁵

Reporting results in mass units might give the false impression of a correct and conclusive result that is interchangeable between methods. We, however, found that ANCA results are only partly exchangeable. ANCA from different patients may differ from each other and from the reference material in terms of avidity, glycosylation status, subclass distribution and epitope specificity and many factors may affect quantitation of autoantibodies (the autoantigen, the way the autoantigen is immobilised to a solid phase, the coupling medium, the incubation medium, the conjugate and the detection method). The reference material might not be commutable across all methods. Consequently, assigning mass units to autoantibodies with clinical assays is intricate. Therefore, we propose that values should not be reported as mass units but rather as mass unit equivalents or arbitrary units. Using of terminology other than mass units does not resolve the poor correlation for some assay comparisons, but it will not give the impression that results are fully interchangeable. Using a common calibrator does not guarantee a common clinical interpretation, as has been demonstrated for rheumatoid factor assays.⁶

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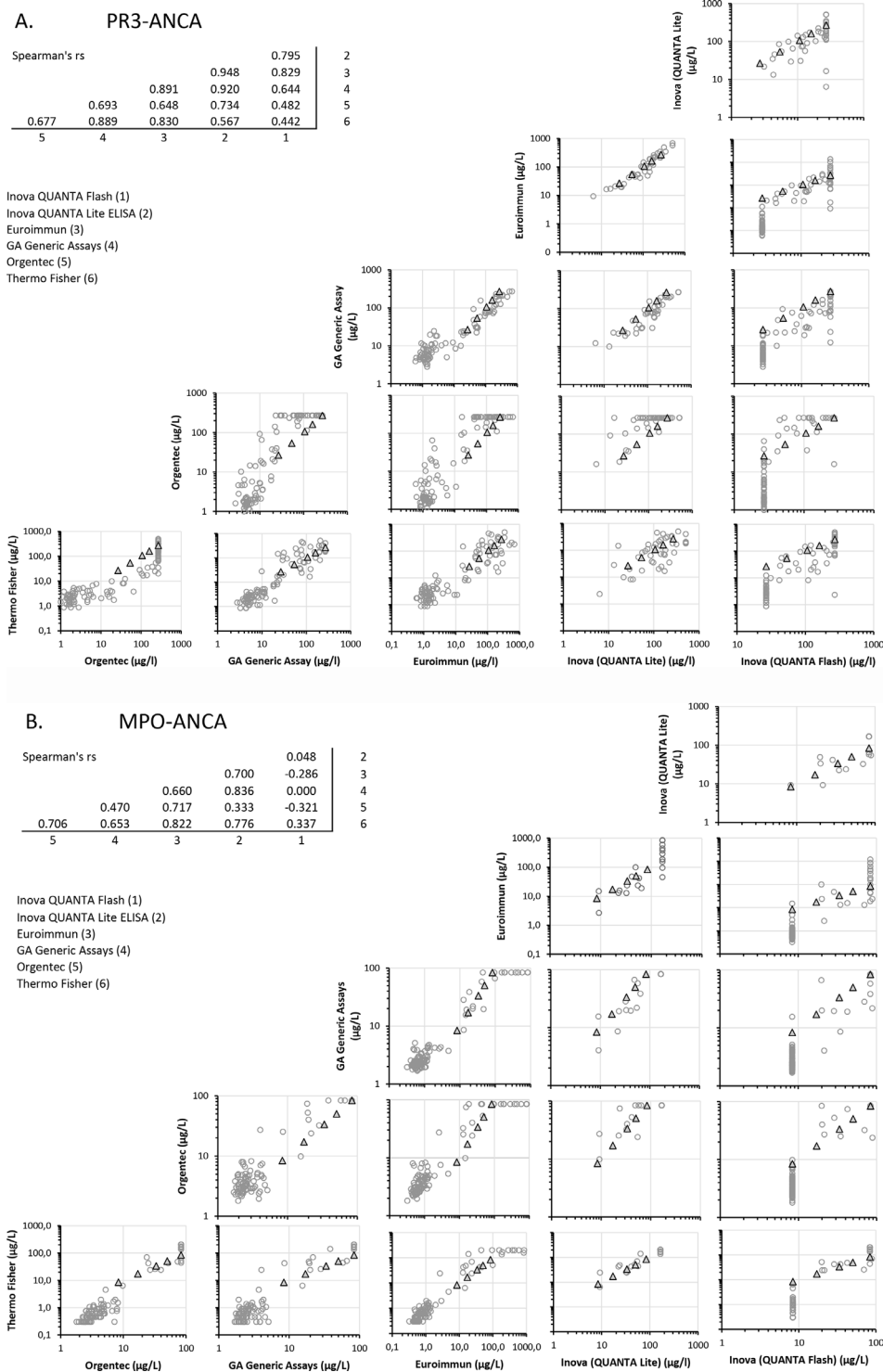


Figure 1 Correlations of individual values of PR3-ANCA (panel A) and MPO-ANCA (panel B) measured by six different immunoassays calibrated with the PR3-ANCA and MPO-ANCA certified reference material from the Institute for Reference Materials and Measurements. Spearman's rank correlation coefficients (rs) are shown in the insert on the graph. The immunoassays included were Inova QUANTAFlash CLIA (1) and QUANTALite ELISA (2), Euroimmun ELISA (3), GA Generic Assays ELISA (4), Orgentec ELISA (5) and Thermo Fisher FEIA (EliA) (6). The samples (n=98, open circles) were from the Leuven and Maastricht cohort included in the EUVAS study⁴ and selected such that low, medium as well as high antibody levels for PR3-ANCA and MPO-ANCA were represented. Panel A: the PR3-ANCA certified reference material (269.9 µg/L) and dilutions thereof (161.1, 106.7, 53.7 and 26.8 µg/L) are represented by triangles. Panel B: the certified MPO-ANCA reference material (83.7 µg/L) and dilutions thereof (50.2, 33.5, 17.1 and 8.4 µg/L) are represented by triangles. The analyses were performed by the manufacturers and results were reported in µg/L based on the certified reference material. Detailed statistical data on Spearman's correlation and passing Bablok fit are given in the online supplementary table 1. ANCA, antineutrophil cytoplasmic antibodies; CLIA, chemiluminescence assay; FEIA, fluoroenzyme immunoassay.

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