# Comparative analysis of different commercial ELISA systems for the detection of anti-neutrophil cytoplasm antibodies in ANCA-associated vasculitides

J.U. Holle, K. Herrmann, W.L. Gross, E. Csernok

*University Hospital Schleswig Holstein and Klinikum Bad Bramstedt, Bad Bramstedt, Germany.*

*Julia U. Holle, MD*
*Kristine Herrmann, MD*
*Wolfgang L. Gross, MD*
*Elena Csernok, MD*

*Please address correspondence to:*
*Dr Julia Holle,*
*Dept. of Rheumatology and Immunology,*
*University Hospital Schleswig-Holstein,*
*Campus Lübeck and Klinikum*
*Bad Bramstedt,*
*Oskar-Alexander Straße 26,*
*24576 Bad Bramstedt, Germany.*
*E-mail: j.holle@klinikumbb.de*

*Received on February 24, 2012; accepted in revised form on March 2, 2012.*

*Clin Exp Rheumatol 2012; 30 (Suppl. 70): S66-S69.*

*© Copyright Clinical and Experimental Rheumatology 2012.*

**Key words:** granulomatosis with polyangiitis, microscopic polyangiitis, anti-neutrophil cytoplasm antibodies, ELISA

*Competing interests: none declared.*

## ABSTRACT

**Objective.** *To assess the diagnostic performance of 11 commercial PR3- and MPO-ANCA ELISA systems (direct, capture and high sensitive [hs] ELISA).*

**Methods.** *Sera from 90 patients with AAV (GPA, MPA and CSS) and 20 disease controls (SLE; RA) and healthy individuals were tested for the presence of ANCA by IFT and by different ELISAs for the presence of PR3-and MPO-ANCA, respectively. Furthermore, the binding capacity of the IUIS-CDC reference sera for PR3-/MPO-ANCA in different commercial assays was analysed.*

**Results.** *Commercial ELISA kits for PR3-ANCA differed moderately in their sensitivity (from 45% to 62.5%). The highest sensitivity for PR3-ANCA was obtained with hs ELISA (kit A) and capture ELISA (kit N). Testing for MPO-ANCA the highest sensitivity (85%) was obtained with direct ELISA (kit D and I). Specificity was high in all kits. Only three PR3-ANCA commercial kits and three MPO-ANCA kits produced binding at the expected value for the IUIS-CDC reference sera (100 U/ml). In all of the kits, serial dilutions of the reference sera did not yield linearity.*

**Conclusion.** *Second (capture) and third (high sensitivity) generation PR3-ANCA ELISA kits are superior to conventional ELISAs. Direct and capture MPO-ANCA ELISAs showed a good overall performance in all kits. Most of the kits have not been standardised to allow their results to be compared.*

## Introduction

The detection of antineutrophil cytoplasmatic antibodies (ANCA) directed against proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) is an important finding support the diagnosis of of granulomatosis with polyangiitis (GPA Wegener's) (1), microscopic polyangiitis (MPA) and Churg-Strauss-Syndrome (CSS) (2) in the correct clinical context. Consensus guidelines currently recommend an immunofluorescence test (IFT) together with an ELISA to detect the ANCA-pattern and the target antigen (3).

Recently, several studies suggested that the sensitivity of capture and high sensitive (hs) ELISA (such as anchor ELISAs and other third generation ELISAs) are superior to conventional ELISA and even to IFT (4-6). Conventional ELISAs using PR3 directly immobilised to the surface of the ELISA plate, show great variations in performance and often lack sensitivity (4). To reduce the covering of possible epitopes by the plastic plate, capture ELISA has been developed and is superior in overall diagnostic performance compared to direct ELISA, however, the sensitivity of capture ELISA may also be reduced by the capturing antibodies, which may hide relevant epitopes (4). In the ongoing search to optimise the detection of PR3-ANCA, novel assays such as high sensitivity ELISAs have been developed with promising results (5-7). Furthermore, all commercial kits apply arbitrary units and show a great variability in ANCA binding (8). Therefore, it has been demanded to standardise assays in order to allow ANCA kits results to be directly compared (8).

This study evaluates the diagnostic performance of 11 commercial PR3- and MPO-ANCA ELISA systems (direct, capture and high sensitivity ELISA) with respect to performance (sensitivity/specificity) and standardisation.

## Patients and methods

*Patients*

Generalised GPA patients and all CSS patients fulfilled the American College

of Rheumatology classification criteria (9, 10). Patients with localised GPA were defined as previously described (11, 12) and all of them had at least one biopsy specimen compatible with GPA. MPA patients fulfilled the Chapel Hill Consensus Definitions (13). AAV patients were stratified according to disease activity (active disease vs. remission) as previously described (14) and assessed by BVAS, version3 (15). Sera from 90 from patients with AAV (GPA: n=40, n= 20 with localised GPA, n=20 with generalised GPA, ten of each disease group with active disease [BVAS ≥3, median BVAS: 10 in generalised GPA, 6.5 in localised GPA], and ten of each group in remission/response [BVAS:<3, median BVAS: 0 in generalised GPA, 0.5 in localised GPA]; MPA: n=40, twenty of whom with active disease, median BVAS:10, twenty with inactive disease, median BVAS:0; CSS: n=10, five with active disease, median BVAS: 9, five with inactive disease, median BVAS: 0) and 20 disease controls (n=10 with systemic lupus erythematosus (SLE), n=5 with rheumatoid arthritis (RA), with additional rheumatoid vasculitis and five healthy controls were analysed. This study was carried out according to the 1997 Declaration of Helsinki of the World Medical Association and has been approved by the ethics committee of the University of Schleswig-Holstein, Campus Lübeck.

*ANCA-detection*

Ten manufacturers of ANCA kits agreed to participate and kits were provided by manufactures without charge and without restriction on their use. Assays were performed as recommended by the manufacturer's instructions. Indirect IFT on ethanol- and formalin-fixed leukocytes was performed as previously described (4, 5). To assess binding of ANCA, the International Union of Immunologic Societies-Centres for Disease Control (IUIS-CDC) reference sera for PR3-ANCA and MPO-ANCA were used. The reference sera were diluted as recommend by the IUIS-CDC (to produce a value equivalent to 100 U/l) and serial dilutions were tested. The test results were expressed in the individual kit units.

**Table I.** Sensitivity (%) of in house IFT and individual PR3-ANCA and MPO-ANCA ELISA kits.

| | IFT | A | B | G | L | N | D | F | I | J | K | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PR3-ANCA | 62.5 | 62.5* | 60* | 60* | 60** | 62.5** | 52.5 | 55 | 45 | 60* | 52.5 | 55 |
| MPO-ANCA | 82.5 | 70 | 82.5 | 75 | 80** | 80** | 85 | 82.5 | 85 | 75* | 75 | 62.5 |

*PR3-ANCA kits A, B, G and J and MPO-ANCA kits J are high sensitivity ELISAs. **PR3-ANCA and MPO-ANCA ELISA kits L and N are capture ELISAs.

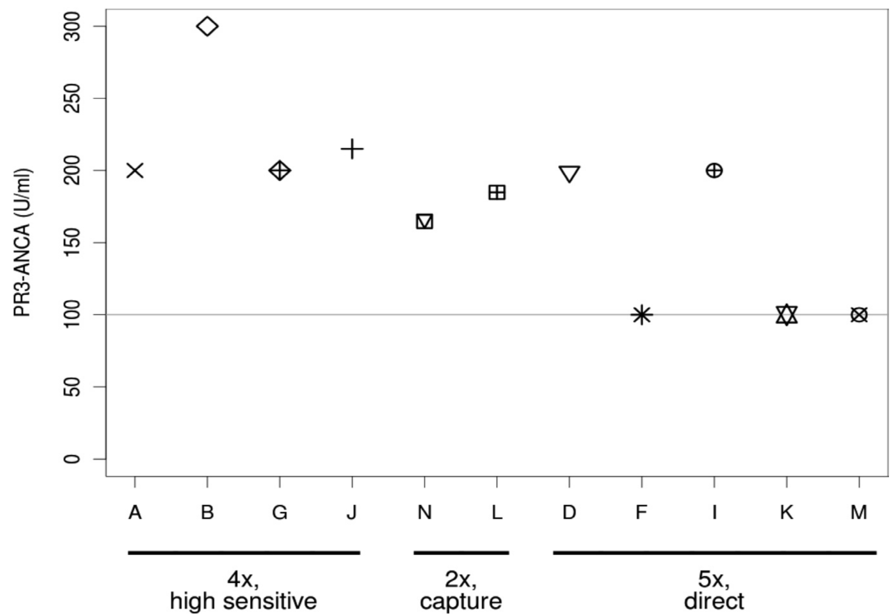


**Fig. 1.** Binding of the IUIS-CDC reference serum for PR3-ANCA by commercial PR3-ANCA ELISA kits. Only kits F, K and M bound at the expected value of 100 U/l.

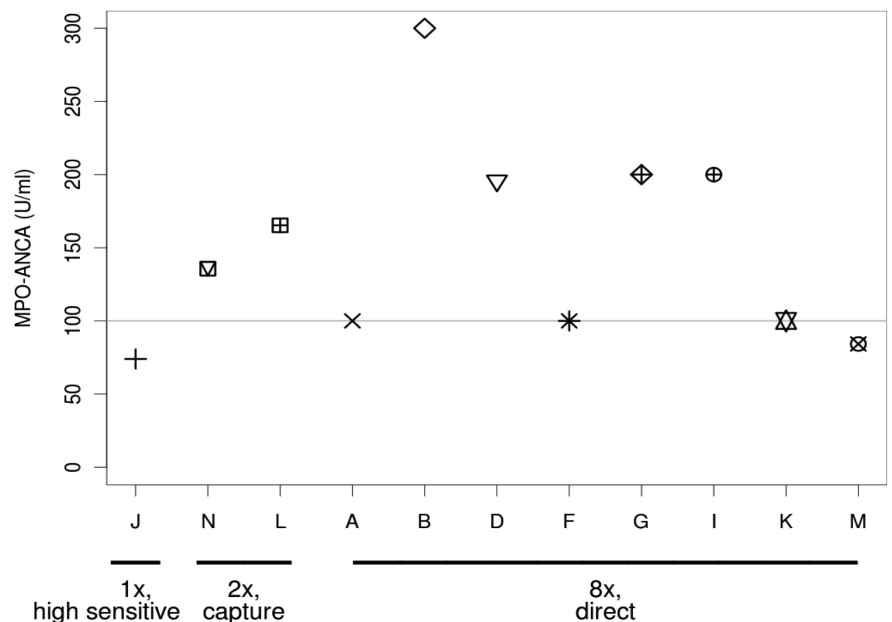


**Fig. 2.** Binding of the IUIS-CDC reference serum for MPO-ANCA by commercial MPO-ANCA ELISA kits. Kits A, F and K bound at the expected value of 100 U/l. Kit M produced binding close to the expected value.

*Statistical analysis*

Sensitivities and specificities were calculated by 2x2 tables. For specificities, all SLE, RA and healthy control samples were analysed together.

## Results

In the disease control group, PR3-ANCA was detected in one of the sera by kits D, I and F and no MPO-ANCA was detected in these sera. Additionally, five healthy controls were analysed. In all tests, these controls yielded negative test results when tested for PR3-ANCA and MPO-ANCA, respectively. PR3-ANCA ELISA kits displayed a specificity of 100% in kits A, B, D, G, L and N. PR3-ANCA ELISA kits F, I, J, K and M showed a specificity of 95%. MPO-ELISA kits yielded a specificity of 100% for all kits except for kit B (95%). Specificity of the IFT was 100%.

Sensitivity varied among the different PR3-ANCA commercial kits and was dependent on the ELISA type. The conventional PR3-ANCA displayed lower sensitivities compared to IFT (62.5%) capture ELISA (kits L, N) and high-sensitivity (hs) ELISAs (kits A, B, G, J). IFT and the hs ELISAs kit A and capture kit N performed best with respect to sensitivity (62.5%) followed by hs ELISA kits B, G and J, and capture ELISA kit L (60%) (Table I).

MPO-ANCA ELISA tests yielded sensitivities between 62.5% (kit M) and 85% (kits D and I), both of the latter were conventional direct ELISAs. IFT and kits B and F (direct MPO-ANCA-ELISAs) also displayed a high sensitivity (82.5%) followed by the two capture MPO-ELISAs (kits L and N) which had sensitivities of 80%. The hs MPO-ANCA ELISA kit J had a sensitivity of 75%.

With respect to ANCA binding, only three of the PR3-ANCA ELISAs (kits F, K and M, all direct ELISAs) and three of the MPO-ANCA ELISAs (kits A, F and K) bound at the expected value of the international standard (100 U/l). Additionally, one of the MPO-ANCA ELISA kits (kit M) produced binding close to the expected value (Figs. 1-2). Serial dilutions showed poor linearity in all PR3-ANCA ELISAs and MPO-ANCA ELISAs (data not shown).

## Discussion

Previous studies suggest that conventional ELISAs have a great variation in their overall performance and often lack sensitivity. Performance was improved by second generation (capture) ELISAs (4) and third generation (high sensitivity) PR3-ANCA ELISAs which showed even more promising results with a substantial increase in sensitivity and specificity in AAV patients (5-7).

In this study, the performance of high sensitivity (anchor and other third generation assay), capture and direct ANCA ELISAs was directly compared for the first time. We confirm high sensitivity rates for high sensitivity PR3-ANCA ELISA kits and capture PR3-ANCA ELISA kits but there was no superiority of high sensitivity kits compared to capture kits. Furthermore, in this study the overall performance of the high sensitivity PR3-ANCA ELISA kits was lower compared to previous studies with high-sensitivity ELISAs (5-7) which may be due to the smaller number of samples tested in this study. IFT yielded comparable results, whereas the sensitivity of conventional PR3-ANCA ELISAs was lower. With respect to MPO-ANCA ELISAs, we could not demonstrate a superiority of capture and hs ELISA kits compared to conventional ELISA kits. In general, all MPO-ANCA ELISA kits and IFT had an excellent performance regarding sensitivity.

Moreover, we assessed the commercial ANCA ELISA kits with respect to standardisation. Only three kits each of the PR3-ANCA and MPO-ANCA ELISAs bound on the expected value of the international standard. Serial dilutions performed according to the instructions of the manufacturers did not display linearity. Therefore, in spite of the demand for standardisation (5) the results of the different test kits are still not comparable which results in persistent problems in data interpretation of different test kits/laboratories.

To conclude, we demonstrate that second and third generation PR3-ANCA ELISA kits are superior to conventional ELISAs. Further studies need to evaluate whether third generation (high sensitivity) PR3-ANCA ELISAs are superior to second generation (capture) PR3-ANCA ELISAs. MPO-ANCA ELISAs showed a good overall performance in all kits. There is urgent need for the standardisation of commercial assays in order to make test results directly comparable.

## Acknowledgements

We thank Menarini Diagnostics, Euro-Diagnostica AB, Orgentec, Euroimmun, Generic assays GmbH, Inova Diagnostics, Human GmbH, IBL International GmbH, and Dr. Fenning BioMed GmbH, Phadia. Furthermore, we thank Monika Backes for her excellent technical assistance.

## References

1. DE LEEUW K, BIJZET J, VEN DER GRAAF AM *et al.*: Patients with Wegener's granulomatosis: a long-term follow-up study. *Clin Exp Rheumatol* 2010; 28 (Suppl. 57): 18-23.
2. MARITSI D, CHAVASSE R, PILKINGTON CA *et al.*: Churg-Strauss syndrome in childhood: a rare form of systemic vasculitis posing a great diagnostic challenge. *Clin Exp Rheumatol* 2011; 29 (Suppl. 64): S135.
3. SAVIGE J, DIMECH W, FRITZLER M *et al.*: Addendum to the International Consensus Statement on testing and reporting of anti-neutrophil cytoplasmatic antibodies. Quality control guidelines, comments and recommendations for testing in other autoimmune diseases. *Am J Clin Pathol* 2003; 120: 312-4.
4. CSERNOK E, HOLLE J, HELLMICH B *et al.*: Evaluation of capture ELISA for detection of antineutrophil cytoplasmic antibodies against proteinase-3 in Wegener's granulomatosis: first results from a multicenter study. *Rheumatology* 2004; 43: 174-180.
5. HOLLE JU, CSERNOK E, FREDENHAGEN G *et al.*: Clinical evaluation of hsPR3-ANCA ELISA for detection of antineutrophil cytoplasmic antibodies directed against proteinase 3. *Ann Rheum Dis* 2010; 69: 468-469.
6. DAMOISEAUX J, DÄHNRICH C, ROSEMANN A *et al.*: A novel enzyme-linked immunosorbent assay using a mixture of human native and recombinant proteinase-3 significantly improves the diagnostic potential fo antineutrophil cytoplasmic antibody-associated vasculitis. *Ann Rheum Dis* 2009; 68: 228-33.
7. ROGGENBUCK D, BUETTNER T, HOFFMANN L *et al.*: High sensitivity detection of autoantibodies against proteinase 3 by a novel third-generation enzyme-linked immunoabsorbent assay. *Ann N Y Acad Sci* 2009; 1173: 41-6.
8. TREVISIN M, NEESON P, SAVIGE J: The binding of proteinase 3 antineutrophil cytoplasmic antibodies (PR3-ANCA) varies on different ELISAs. *J Clin Pathol* 2004; 57: 303-8.
9. LEAVITT RY, FAUCI AS, BLOCH DA *et al.*: The American College of Rheumatology 1990 Criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum* 1990; 22: 1101-7.

10. MASI AT, HUNDER GG, LIE JT *et al*.: The American College of Rheumatology 1990 Criteria for the classification of Churg-Strauss syndrome (Allergic granulomatosis and angiitis). *Arthritis Rheum* 1990; 33: 1094-100.
11. HOLLE JU, GROSS WL, HOLL-ULRICH K *et al*.: Prospective longterm follow-up of localized Wegener's granulomatosis: Does it occur as persistent disease stage? *Ann Rheum Dis* 2012; 69: 907-14.
12. HOLLE JU, WU QJ, MOOSIG F *et al*.: Membrane proteinase 3 (mPR3) expression on neutrophils is not increased in localized Wegener`s granulomatosis (WG) and Churg-Strauss syndrome (CSS). *Clin Exp Rheumatol* 2010; 28 (Suppl. 57): 46-50.
13. JENNETTE JC, FALK RJ, ANDRASSY K *et al*.: Nomenclature of systemic vasculitides. Proposal of an International Consensus Conference. *Arthritis Rheum* 1994; 37: 187-92.
14. FAGIN U, CSERNOK E, MÜLLER A *et al*.: Distinct proteinase 3-induced cytokine patterns in Wegener's granulomatosis, Churg-Strauss syndrome, and healthy controls. *Clin Exp Rheumatol* 2011; 29 (Suppl. 64): S57-62.
15. MUKHTYAR C, LEE R, BROWN D *et al*.: Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis* 2009; 68: 1827-32.