New highly sensitive and specific Lateral Flow Tests for the detection of Proteinase 3, Myeloperoxidase and Glomerular Basement Membrane antibodies

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Background: Vasculitis occurs due to inflammation of blood vessel walls and exhibits many different clinical pictures, of which antineutrophil cytoplasmic antibodies (ANCA) associated small vessel vasculitis is one of the most common causes. ANCA associated vasculitis includes microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome and drug induced vasculitis. The sensitive and specific detection of antibodies to Proteinase 3 (Pr3) and/or Myeloperoxidase (MPO) is highly recommended even at the slightest suspicion of renal vasculitis occurring in about 80% of all Wegners granulomatosis cases. Anti-Glomerular basement membrane (GBM) autoantibodies, also refferred to as Good-pasture- antibodies, are highly specific for the anti-GBM-Nephritis. In around 75% of all cases these autoantibodies are associated with the Goodpasture syndrome, characterized by glomerulonephritis and lung hemorraghe.
Only fast and adequate treatment can avoid the development of renal failure [1,2]. For this purpose we developed two highly sensitive and specific 15 minute lateral flow assays (see figure 1), for the detection of Pr3, MPO and GBM specific IgG antibodies, to shorten the time between diagnosis and patient treatment. Furthermore, these tests make single patient testing economical.
The new Autoimmune Lateral Flow Assays (AI-LFA) take use of liquid phase antigens in a most native form, thereby offering highest sensitivity and specificity.

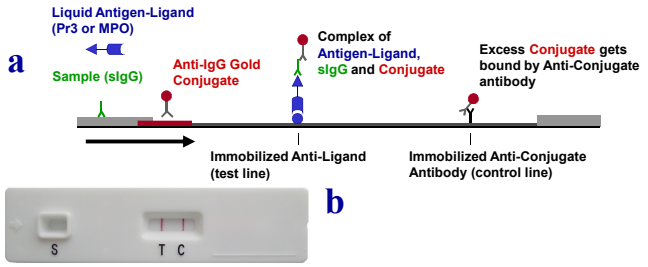

Figure 1 Principle of Pr3/MPO/GBM AI-LFA (a) and AI-LFA cassette (b)

Methods: All patient samples (n=20 Pr3; n=24 MPO, n=7 GBM) were tested positive by IFA and two commercial ELISA kits (Orgentec and The Binding Site). Control samples (n=44 Pr3; n=45 MPO, n=32 GBM) consisting of healthy donors and autoimmune disease controls were tested in Pr3, MPO and GBM ELISA (Orgentec). All samples were assayed by AI-LFA and read-out was done by a lateral flow reader system. Statistics were done using Analyse-it for Microsoft-Excel.

Results and findings: All Pr3, MPO and GBM positive patient samples were also found positive in AI-LFA. 20/20 were found positive for Pr3, 22/22 positive for MPO and 7 of 7 positive for GBM. Of the control panel 43/44 (cut-off 280000 RU), 44/45 (cut-off 500000 RU) and 32/32 (cut off 200000 RU) were found negative for Pr3, MPO and GBM in AI-LFA. This corresponds to a technical sensitivity and specificity of 100%/98% for Pr3, 100%/98% for MPO and 100%/100% for GBM. ROC (Receiver Operating Characteristic) Analysis comparison to ELISA results revealed Area under the Curve (AUC) values of 1.0 for Pr3 AI-LFA and for MPO AI-LFA respectively and AUC of 1.0 for GBM (Figure 2). Linearity by dilution was found at R^2=0.94 for Pr3 and R^2=0.93 for MPO Data not shown. Pearson correlation coefficient of AI-LFA vs. ELISA was 0.89 for Pr3, 0.88 for MPO and 0,89 for GBM (Figure 4).

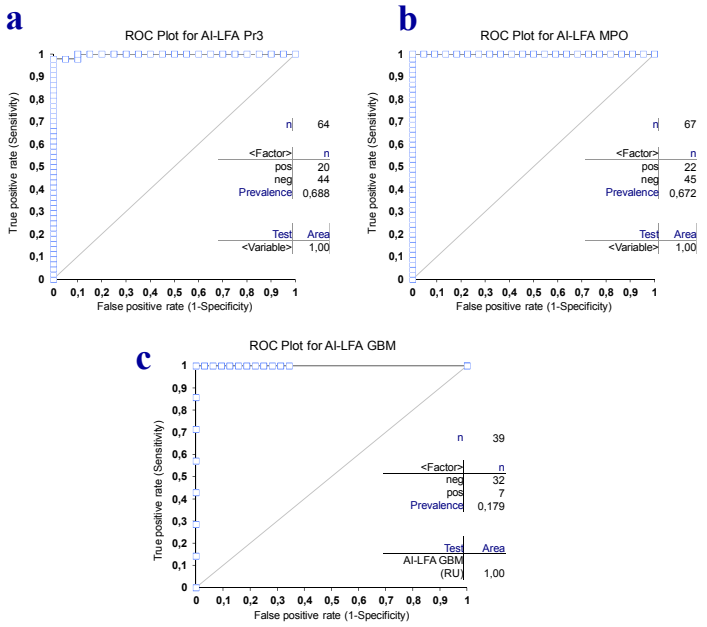

Figure 2 ROC Analysis of Pr3 (a), MPO AI-LFA (b) and GMB AI-LFA (c)

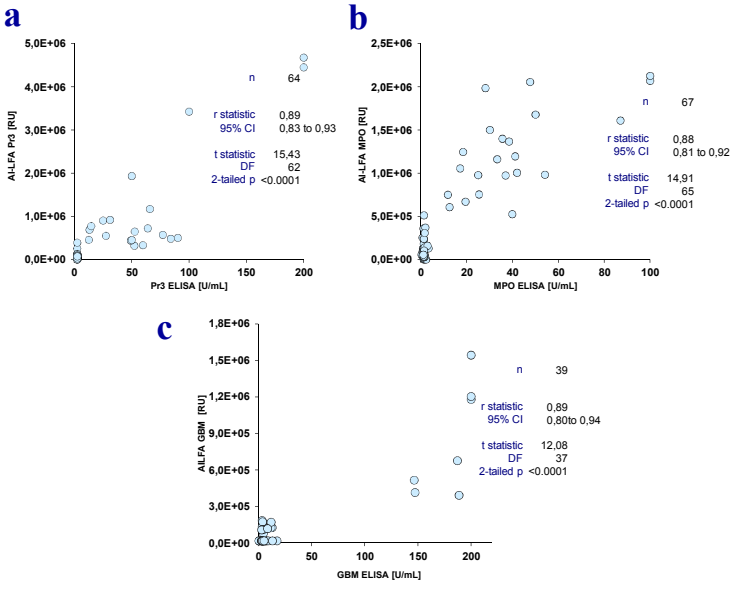

Figure 3 Pearson correlation of Pr3 (a), MPO (b) and GBM (c) AI-LFA to ELISA

Conclusion: The new Pr3/MPO/GBM AI-LFAs give results equal to the latest third generation ANCA ELISAs [3] and IFA in 15 min, without the need for expensive laboratory equipment. AI-LFA shortens the time between first diagnosis and adequate treatment and makes single patient testing economical.

References:
1. Conrad K, Schößler W, Hiepe F, Fritzler M: **Myeloperoxidase Antibodies.** In *Autoantibodies in Systemic Autoimmune Diseases- A Diagnostic Reference*. Edited by Conrad K, Schößler W, Hiepe F, Fritzler M. Pabst; 2007:111-113.
2. Conrad K, Schößler W, Hiepe F, Fritzler M: **Proteinase 3 Antibodies.** In *Autoantibodies in Systemic Autoimmune Diseases- A Diagnostic Reference*. Edited by Conrad K, Schößler W, Hiepe F, Fritzler M. Pabst; 2007:147-149.
3. Roggenbuck D, Buettner T, Hoffmann L, Schmechta H, Reinhold D, Conrad K: **High-sensitivity detection of autoantibodies against proteinase-3 by a novel third-generation enzyme-linked immunosorbent assay.** *Ann N Y Acad Sci* 2009, 1173: 41-46.

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