



New highly sensitive and specific Lateral Flow Tests for the detection of Proteinase 3 and Myeloperoxidase 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 Wegeners granulomatosis cases. 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 and MPO 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.

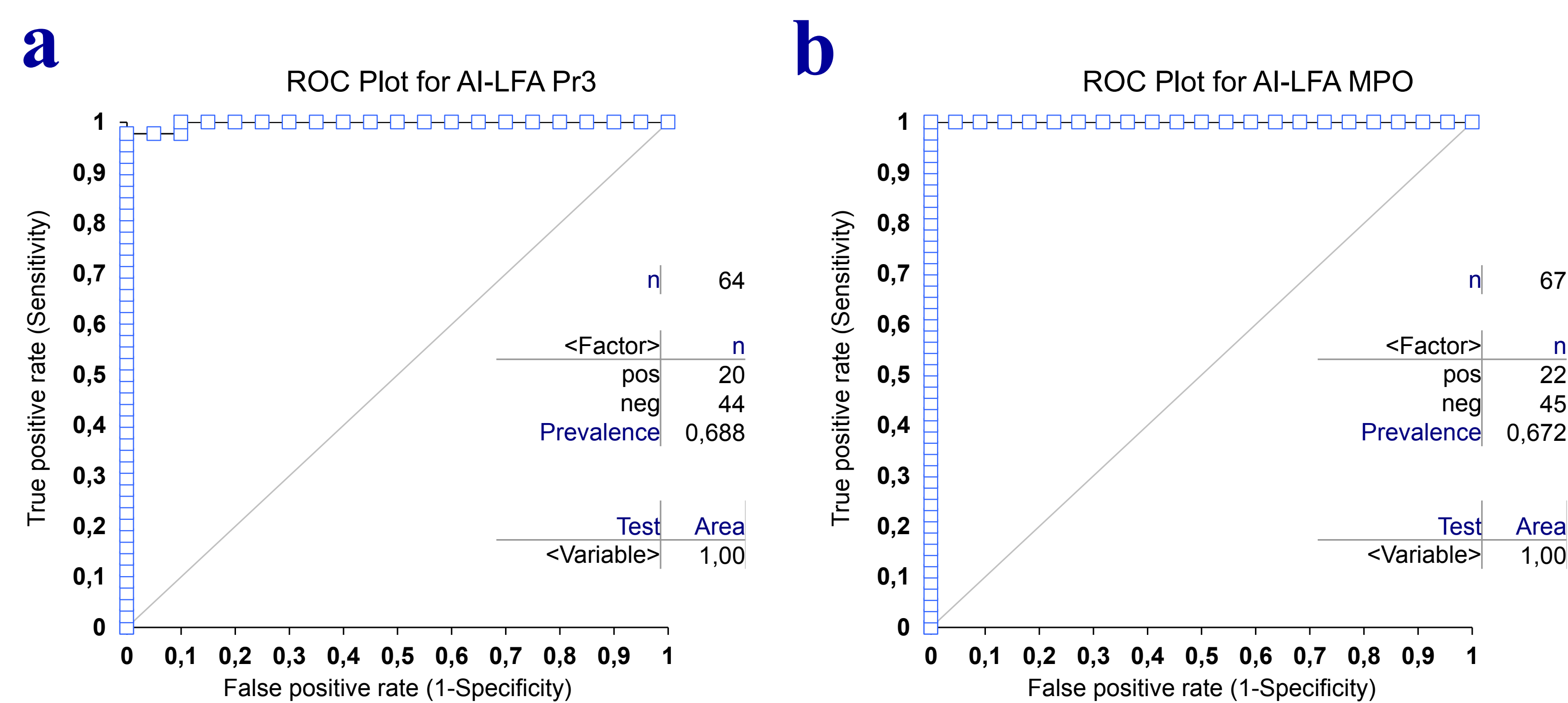


Figure 2 ROC Analysis of Pr3 (a) and MPO AI-LFA (b).

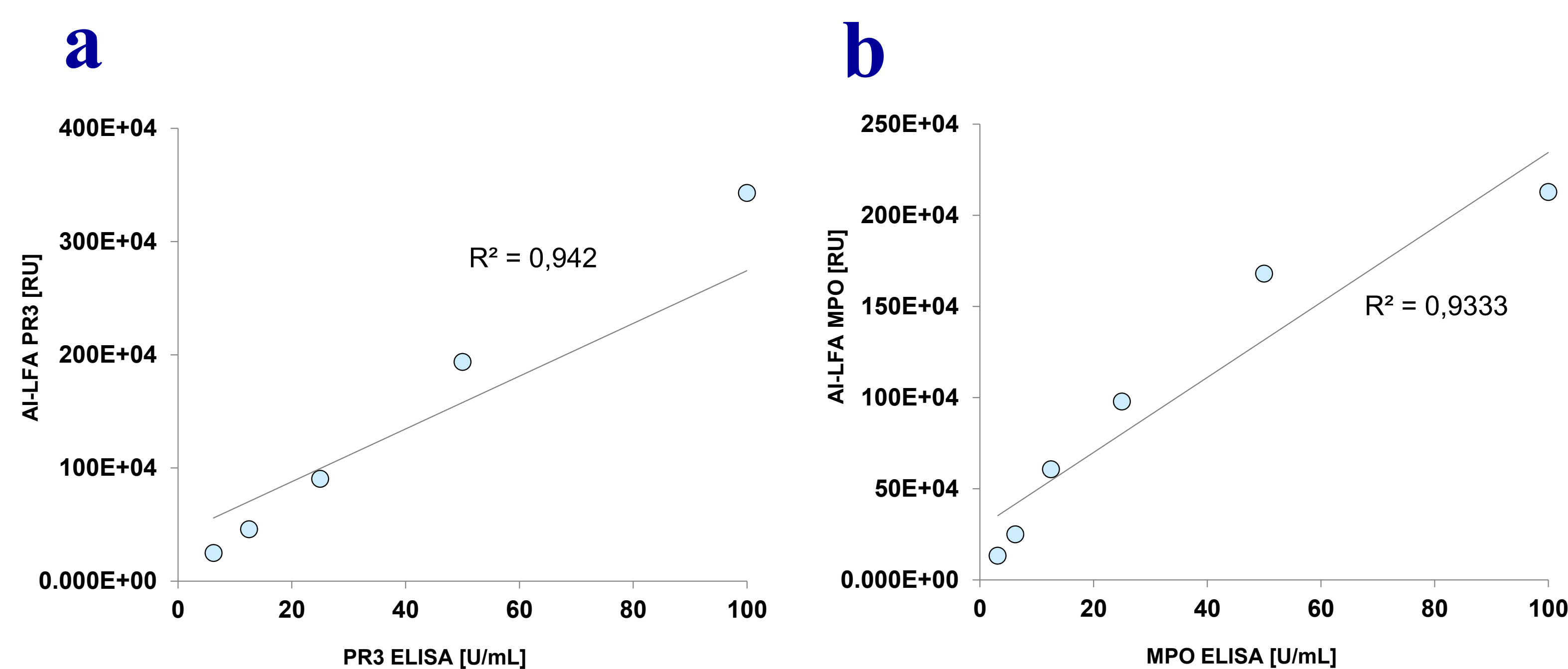


Figure 3 Linearity by Dilution of Pr3 (a) and MPO AI-LFA (b). Sera were diluted in negative serum.

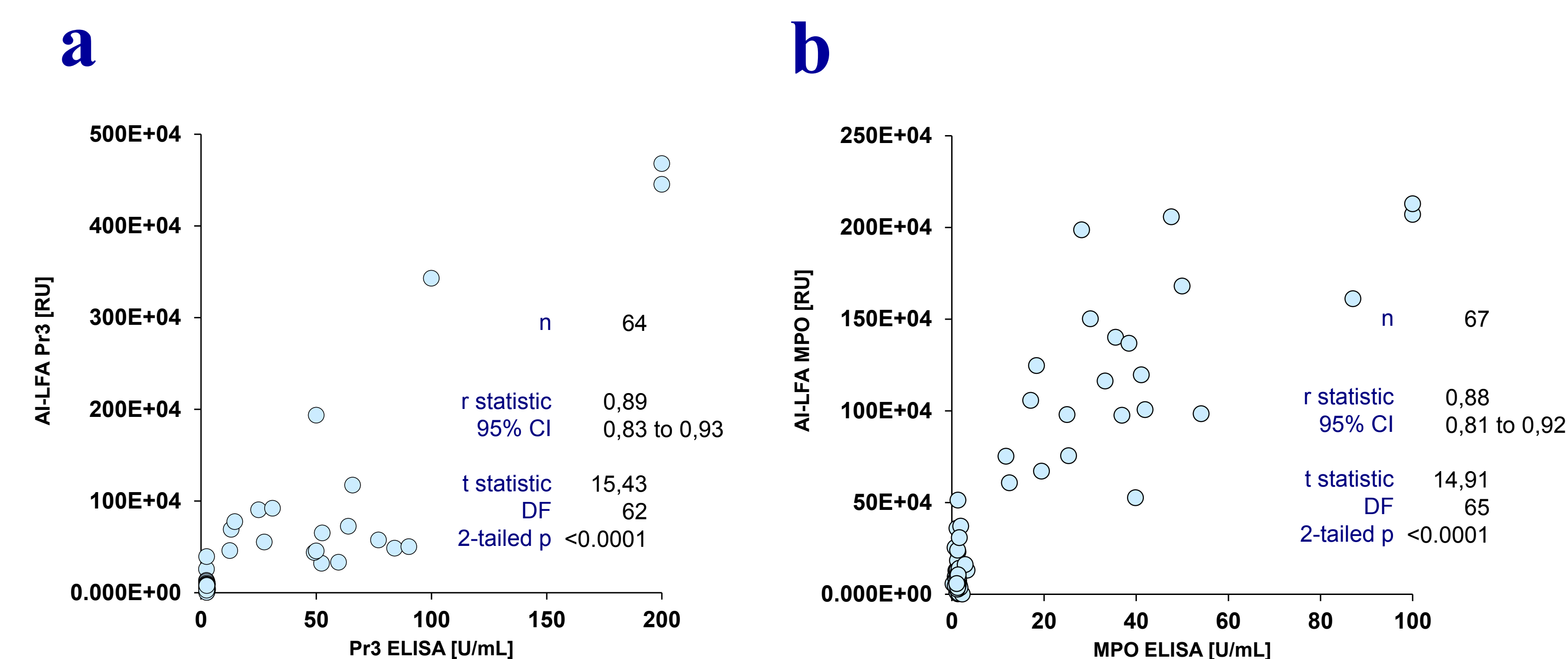


Figure 4 Pearson correlation of Pr3 (a) and MPO AI-LFA (b) to ELISA

Conclusion: The new Pr3/MPO 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, Schöbner W, Hiepe F, Fritzler M: **Myeloperoxidase Antibodies**. In *Autoantibodies in Systemic Autoimmune Diseases- A Diagnostic Reference*. Edited by Conrad K, Schöbner W, Hiepe F, Fritzler M. Pabst; 2007:111-113.
2. Conrad K, Schöbner W, Hiepe F, Fritzler M: **Proteinase 3 Antibodies**. In *Autoantibodies in Systemic Autoimmune Diseases- A Diagnostic Reference*. Edited by Conrad K, Schöbner 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.

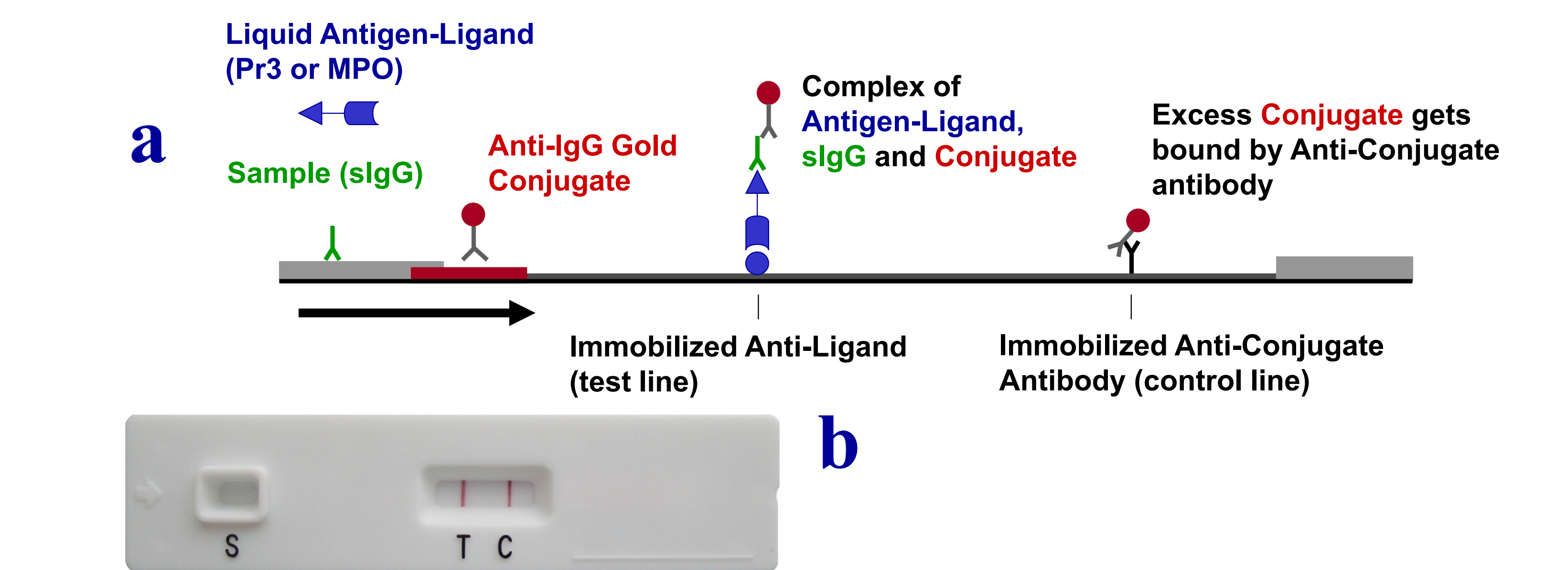


Figure 1 Principle of Pr3/MPO AI-LFA (a) and AI-LFA cassette (b)

Methods: All patient samples (n=20 Pr3; n=24 MPO) were tested positive by IFA and two commercial ELISA kits (Orgentec and The Binding Site). Control samples (n=44 Pr3; n=45 MPO) consisting of healthy donors and autoimmune disease controls were tested in Pr3 and MPO 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 and all MPO positive patient samples were also found positive in AI-LFA. 20/20 were found positive for Pr3 and 22/22 positive for MPO. Of the control panel 43/44 (cut-off 280000 RU) and 44/45 (cut-off 500000 RU) were found negative for Pr3 and MPO in AI-LFA. This corresponds to a technical sensitivity and specificity of 100%/98% for Pr3 and 100%/98% for MPO. 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 (Figure 2). Linearity by dilution was found at R²=0.94 for Pr3 and R²=0.93 for MPO (Figure 3). Pearson correlation coefficient of AI-LFA vs. ELISA was 0.89 for Pr3 and 0.88 for MPO (Figure 4).