Evaluation of a multiparametric spotimmunoassay (SIA) for the detection of autoantibodies in ANCA associated vasculitis

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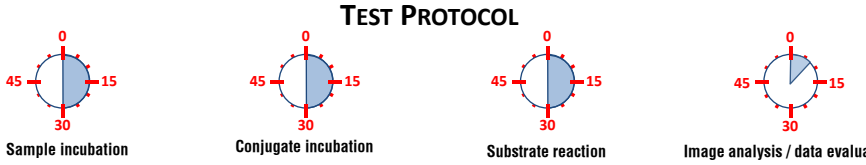
Introduction

Antineutrophil cytoplasmic antibodies (ANCA) directed against proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) are highly specific markers of ANCA associated vasculitis (AAV), an inflammatory and necrotizing disease affecting small blood vessels, leading to the damage of a single organ or multiple organ systems (e.g., skin, respiratory tract, kidneys). AAV has substantial morbidity and mortality, often presenting with aggressive renal failure and/or pulmonary haemorrhage. Therefore the reliable detection of PR3- and MPO-ANCA, as well as the parallel screening of glomerular basement membrane (GBM) antibodies as a marker of anti-GBM nephritis, is important for a rapid diagnosis and an early onset of adequate therapy. The simultaneous appearance of anti-GBM antibodies and ANCA indicates a progressive disease course.

Methods

An array-format based spot immunoassay (SIA) in 96well-microtitration plates (*SeraSpot*® Vaskulitis-3 IgG) was established for the detection of autoantibodies directed against PR3, MPO and GBM in human serum or plasma samples. Test specific controls are integrated in every array: a reference curve including a cut-off and negative control, and a serum control indicating an absence of sample. Results are calculated in relative units via the staining intensities of the reference curve (four parameter logistic nonlinear regression model).

Test Protocol



Bound autoantibodies from samples are detected by HRP-labeled anti-human IgG and chromogenic precipitating substrate solution. Immune complexes are visualized as pale blue to dark blue spots. Developed arrays are scanned with Seramun *SpotSight*®plate scanner and evaluated by software Seramun *SpotSight*®scan.

Materials

Samples:

(a) Samples of patients with clinically confirmed diagnosis and autoantibody results (ELISA, immunofluorescence (IIF)): ANCA associated vasculitis (n=25); Granulomatosis with polyangiitis (n=61); Microscopic polyangiitis (n=18); Anti-GBM nephritis (n=6)

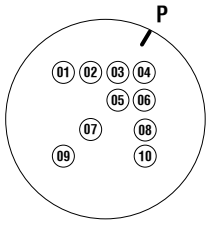
(b) Healthy blood donors (n=200)

Patient sera and previous findings were kindly provided by Dr. K. Conrad (Institute of Immunology at Technical University of Dresden).

Kits: *SeraSpot*® Vaskulitis-3 IgG (SP-003-3 G)

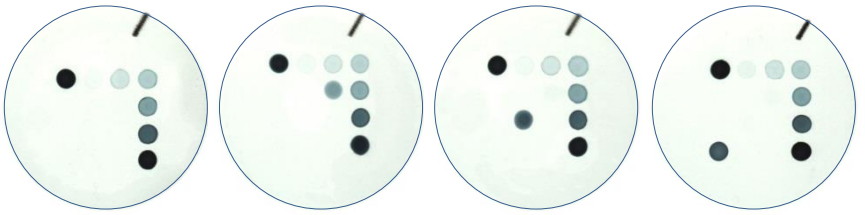
Tests were run under routine laboratory conditions using standard ELISA equipment (multichannel pipettes, microplate washers).

Array Layout

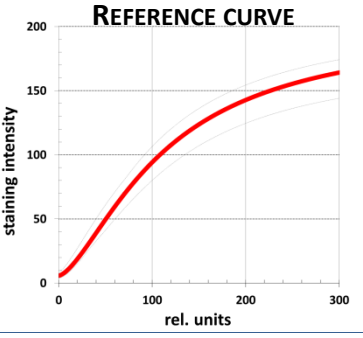


Parameter	
05	GBM
07	MPO
09	PR3
Controls	
01	Positive control (PC)
02	Negative control (NC)
03	Cut-off control (CO)
03	Reference 3 (R3)
06	Reference 2 (R2)
08	Reference 1 (R1)
10	Serum control (SC)
P	Well position marker

Samples



Reference Curve



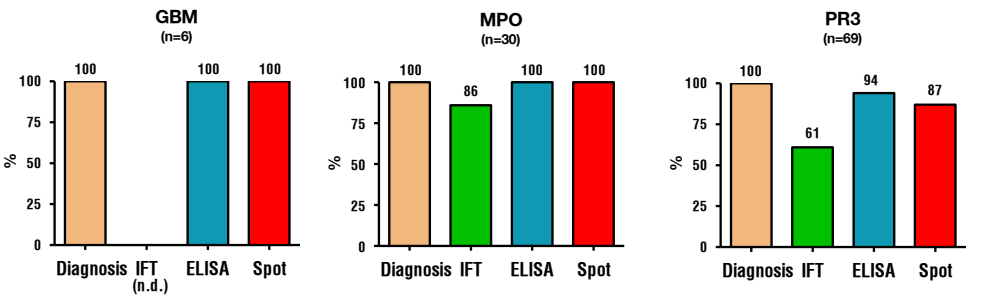
Results

1. Anti-GBM nephritis was correctly diagnosed in 100 % of the cases. The SIA shows 100 % total agreement with the commercial ELISA results. No IIF results were available.

2. Microscopic polyangiitis and AAV concerning MPO antibodies was correctly diagnosed in 100 % of the cases, The SIA shows 100 % total agreement with the referenced ELISA results. In comparison, only 86 % of these samples were determined positive by IIF.

3. The sensitivity for the detection of Granulomatosis with polyangiitis and AAV concerning PR3 antibodies was determined 87 % for the SIA in comparison to 94 % for the referenced ELISA and 61 % for the IIF. All *SeraSpot*® negative samples were confirmed anti-PR3 negative by IIF, as well as in an additional fluorescence enzyme immunoassay and a further high sensitive PR3 antibody ELISA.

4. The investigation of 200 healthy blood donor samples resulted in a specificity of 99.0 %, 99.5 % and 96.0 % for the determination of antibodies to GBM, MPO and PR3, resp. The anti-GBM and anti-MPO positive samples were confirmed positive by ELISA and by Line immunoassay, leading to amended specificities of 100 %.



GBM

n = 104		Diagnostic finding: positive	Diagnostic finding: negative
SeraSpot®	positive	6	0
	negative	0	98

MPO

n = 104		Diagnostic finding: positive	Diagnostic finding: negative
SeraSpot®	positive	30	4
	negative	0	70

PR3

n = 104		Diagnostic finding: positive	Diagnostic finding: negative
SeraSpot®	positive	60	0
	negative	9	35

Reference Panel Healthy Blood Donors

Parameter	GBM	MPO	PR3
SeraSpot® positive	0	0	8
Specificity	100 %	100 %	96 %

Conclusion

The *SeraSpot*® Vaskulitis IgG spot immunoassay is a reliable test system for the detection of autoantibodies in systemic vasculitis in routine laboratories. Both the assay procedure and data evaluation are easy to perform. In addition, the assay is very economical with regard to sample and reagent consumption.

However, the variability between assay results for the diagnosis of autoimmune diseases is a frequently mentioned fact. Possible reasons for these differences relate to variabilities in the origin, purity and coating conditions of the antigens used, and heterogeneities of samples as well. Appropriate standardization of material, techniques and methods is needed which are unfortunately currently not available.