Method Comparison of Multiplex Planar Microarray and Enzyme-linked Immunoassay for Detection of ANCA-associated Vasculitis PR3 and MPO Autoantibodies and Glomerulonephritis-associated GBM and C1q Autoantibodies

Terryberry J[1]; Damoiseaux J[2]; Jennette C[3]; Berg E[3]; Kahama A[1]; Carmichael S[1]; McCabe S[1]; Sawyer J;[1] Lea P[1]; Falk R[3].

1. SQI Diagnostics Toronto, ON Canada 2. Laboratory of Clinical Immunology, University Hospital Maastricht, Netherlands 3. Kidney Center, University of North Carolina, USA

Introduction:

Simultaneous measurement of a number of correlated analytes is useful for reasons of reduced specimen volume and reagent costs, and enhanced clinical utility wherein a combination of markers facilitates disease- or organ-oriented profiling, prognostic studies of disease activity and severity and therapeutic monitoring.[1] SQI Diagnostics' IgX PLEX™ planar microarrays allow multiplexing of both antigen and antibody class in an automation compatible 96 well format. Assays which facilitate the detection of multiple biomarkers from multiple patients in a fully automatable platform better reflect the complexity of autoimmune disease processes.[2] Use of specifically labelled reporter molecules permit simultaneous measurement of autoantibody immunoglobulin classes.[3]

Objective:

To evaluate diagnostic accuracy of a novel multiplex planar microarray assay, samples with blinded MPO and PR3 test results (n=81) were supplied to SQI by Dr R. Falk at UNC Kidney Center. To improve statistical confidence in the performance of the IgX PLEX™ Vasculitis and Nephritis assay, additional lab-defined samples with predicate ELISA results were obtained from Dr. J. Damoiseaux at University Hospital Maastricht and a commercial supplier, SLR Research, for further method comparisons.

Materials and Methods:

For initial method comparison studies, 81 blinded samples were received from Dr. R. Falk (UNC Kidney Center). Autoantibodies to MPO, PR3 and GBM in patient samples were detected with a research use only version of the IgX PLEX™ Vasculitis and Nephritis assay on a manual adaptation of the fully automated SQiDworks™ Diagnostic Platform. Assay results were sent to UNC Kidney Center, the blind removed, and agreement to ELISA predicate results calculated. Of the 81 samples, 21 were predicate positive anti-MPO, 32 predicate positive anti-PR3 and 25 systemic lupus erythematosus (SLE) disease controls. Additional samples positive to MPO, PR3, GBM and C1q were received from Dr. J. Damoiseaux (University Hospital Maastricht) and a commercial source (SLR Research). Specimens included 44 ELISA predicate tested anti-MPO, 42 anti-PR3, 28 anti-GBM, 62 anti-C1q IgG tested samples (including 39 clinically defined lupus nephritis), and 132 control samples.

Commercially available ELISAs for measuring antibodies to MPO (INOVA Diagnostics), PR3 (INOVA Diagnostics), GBM (Genesis Diagnostics, INOVA Diagnostics) and C1q (Buhlmann Laboratories) were performed according to manufacturer's instructions and results compared to relative intensity units measured for each analyte on the IgX PLEX™ planar microarray. For comparison, the CIC-C1q binding assay was also performed per manufacturer's (Buhlmann) instructions and results compared to the ELISA and microarray data.

For MPO and PR3, positive, negative and overall agreement and kappa coefficient, κ, for concordance[4] were calculated from the results for the predicate ELISAs and the multiplex assay on the blind-removed samples. For C1q and GBM method comparison data, positive agreement and Spearman rank correlation were computed.[5]

Figure 1. IgX PLEX™ Multiplex Planar Microarray Assay for Vasculitis and Nephritis

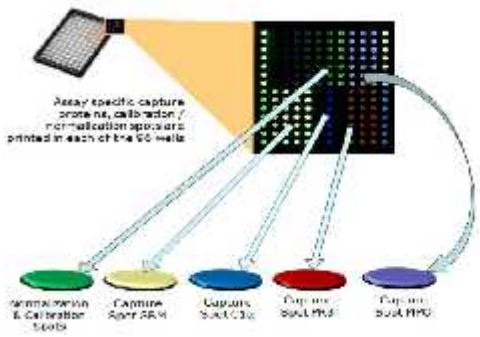

Table 1. Method Comparison Data for IgX PLEX™ Vasculitis and Nephritis Assay Compared to ELISA

Microarray Antibody	Statistic	Result
MPO IgG	Positive Agreement	95.5% (42/44)
	Negative Agreement	93.0% (119/128)
	Overall Agreement	93.6%
	Concordance (Kappa)	0.840
PR3 IgG	Positive Agreement	95.2% (40/42)
	Negative Agreement	90.9% (120/132)
	Overall Agreement	92.0%
	Concordance (Kappa)	0.797
GBM IgG	Positive Agreement	92.9% (26/28)
	Spearman Rank Correlation	0.890
C1q IgG	Positive Agreement	85.5% (53/62)
	Spearman Rank Correlation	0.747

References:

1. Plebani M, Pittoni M, Celadin M, et al. Autoimmun Rev 2009; 8:238-43
2. Gibson DS, Banha J, Penque D, et al. J Proteomics 2010; 73:1045-60
3. Lea P, Keystone E, Mudumba S, et al. Clin Rev Allergy Immunol 2009; Dec 9.
4. Streiner DL. J Personality Assessment 2003; 81:209-19
5. http://www.wessa.net/

Results:

Results for positive, negative and overall agreement with appropriate method comparison measures of Kappa concordance coefficient or Spearman rank correlation coefficient are presented in Table 1. Correlation of the multiplex anti-C1q response and ELISA results is shown in Figure 2.

Discussion:

The method comparison results demonstrate good agreement between the two assay systems of microarray and ELISA. The agreement data for MPO and PR3 ELISA results and SQI's IgX PLEX™ Vasculitis and Nephritis assay are especially robust since the ELISAs were performed on a multisite collaborative basis. A high level of agreement and correlation was also demonstrated between the Vasculitis and Nephritis multiplex assay and anti-GBM ELISA results.

Measurement of C1q antibodies in the multiplex assay was compared to anti-C1q ELISA results, and also to the more common CIC-C1q binding assay. Good agreement and correlation was seen between the multiplex assay and the anti-C1q ELISA. Relative sensitivity of C1q antibody detection in lupus nephritis patients was 80.3% and was similar to that obtained with the CIC-C1q binding assay (79.5%). However, the CIC-C1q binding assay, which does not measure circulating autoantibodies, did not show correlation to results obtained for the C1q antibody microarray or ELISA.

Conclusion:

SQI's IgX PLEX™ Vasculitis and Nephritis assay provided a reliable measure of MPO, PR3, GBM and C1q antibodies compared to traditional ELISA performed on FDA-approved kits. The utilization of this multiplex assay will aid physicians in characterizing and monitoring vasculitis and nephritis patients with reported results concordant with predicate single-plex assays.

Figure 2. Anti-C1q Spearman Rank Correlation Graph for Comparison of IgX PLEX™ Vasculitis and Nephritis Assay (y) vs. Predicate ELISA (x). Rs = 0.747