

A Novel Anti-Citrullinated Peptide Antibody Assay Using a Four-Analyte Multiplexed QuantiSpot™ Rheumatoid Arthritis Test System

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Abstract

Rheumatoid Arthritis (RA) occurs in about 1% of the population. Early detection and monitoring are beneficial in management of this disease. A sensitive anti-CCP antibody assay has been developed along with simultaneous measurement of all three rheumatoid factors (RF) -RF-IgA, IgG and IgM using SQI's QuantiSpot™ technology. Testing 10 microliter serum samples, SQI's multiplex microarray fluorescent immunoassay QuantiSpot™ Rheumatoid Arthritis Assay (the SQI test) offers quantitative/semi-quantitative and positive/negative results for anti-CCP IgG, and RF- IgA, IgG and IgM from each sample well on a 96-well microtiter-formatted microarray plate. Detection is achieved by using sensitive fluorescent-tagged markers captured on microarray spots and read in a microarray scanner. Each of the results comes with quality control of every sample well. Serum samples from 159 European and North American subjects (119 Rheumatoid Arthritis sufferers and 40 normal controls) were used to compare SQI's anti-CCP antibody test with conventional immunoassays. Based on clinical diagnosis extracted from patient charts, the study demonstrated that the SQI test achieved 81.5% sensitivity and 97.5% specificity. These performance characteristics compared favorably with single-analyte anti-CCP tests (Bizzaro et al., 2007). The sensitivity and the specificity were further compared with a leading predicate anti-CCP assay. At the fixed specificity of 95%, the SQI anti-CCP assay was 4.2% more sensitive relative to the compared method. Similar method concordance has also established full comparability between each of the RF tests in this multiplexed test with the corresponding predicate test. This novel test along with the three other tests in the SQI test system will permit the detection of rheumatoid arthritis with high sensitivity and specificity.

Introduction

The diagnosis of RA is based on clinical symptoms of the disease along with measurement of three rheumatoid factors and increasingly with the results from the anti CCP antibody marker. Auto antibodies to citrullinated proteins, due to their specificity and their presence in the early stages of the disease, have been reported to predict the development of RA in asymptomatic people. Several non-native synthetic peptides containing citrulline were tested for detection of anti-CCP antibodies. By using SQI's proprietary chemistry and peptide mix, high levels of performance of this CCP assay were achieved. SQI's automated multiplex microarray fluorescent immunoassay offers the quantitative/semi quantitative analyses of RF-IgA, IgG and IgM and anti-CCP IgG antibody, in a single test. SQI's multiplex automated assay screens 76 patient samples on a 96-well microtiter-formatted plate, all wells with quality control elements.

Methods

Several non-native peptides containing citrullines were synthesized. The peptides were polymerized and mixed to maximize their ability to detect the auto-antibodies in patient samples. An optimal mixture was printed onto microarray plates. Other proteins were also printed to capture IgA, IgG and IgM RF analytes. Fluorescence-labeled anti-human antibodies were used to detect the analytes captured on the microarray plates

Development of the assay

The printed CCP peptide and RF antigen microspots capture autoimmune antibodies present in RA patients. The control spots are also printed (see Figure 1). The assay was standardized with NIBSC rheumatoid arthritis reference serum and an FDA-approved predicate assay kit. All tests were performed on an automated SQIDworks™ Microarray System. 10 µl of serum each from 159 European and North American subjects (119 rheumatoid arthritis sufferers and 40 normal controls) was diluted and incubated for 45 minutes at RT in wells of the SQI test plates. Wells were washed and reacted with fluorescence-labeled reporter markers. After incubation, wells were washed again, dried and read in a microarray scanner. The responses were further analyzed. The final results were calculated against a standard curve.

Reference

Bizzaro N, Tonutti E, Tozzoli R, Villalta D. (2007) Analytical and diagnostic characteristics of 11 2nd- and 3rd-generation immunoenzymatic methods for the detection of antibodies to citrullinated proteins. Clin Chem.53, 1527-33.

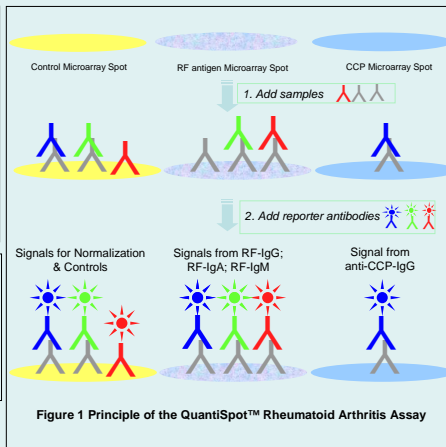


Figure 1 Principle of the QuantiSpot™ Rheumatoid Arthritis Assay

Results

Precision: The coefficients of variance (CVs) for the precision were found to be 0.6% to 7.0% (Table 1).

Reproducibility: The reproducibility was found to be 7.3 to 8.5% (Table 1).

Table 1 Precision and reproducibility of the CCP test of QuantiSpot™ RA Assay

U/mL	Precision-Within run				Reproducibility Between run (n=4)
	Run1	Run2	Run3	Run4	
75.0	1.4%	2.2%	5.3%	7.0%	8.1%
300.2	2.5%	0.6%	3.3%	5.0%	7.3%
600.4	3.4%	2.3%	0.5%	3.6%	8.5%

Study 1-Clinical Sensitivity and Specificity The clinical sensitivity and specificity were found to be 81.5% and 97.5% respectively (Table 2).

Study 2-Clinical Sensitivity and Specificity In a subsequent study, an independent set of samples was tested where sensitivity and specificity were found to be 81.1% and 97.2% confirming the results.

Table 2 Clinical Sensitivity and Specificity

	QuantiSpot™ RA Assay	
	+	-
N= 159		
RA patients (+)	97	22
Normal (-)	1	39

The study had 159 subjects, sera from 119 North American and European patients with confirmed Rheumatoid Arthritis diagnosis and 40 normal people.

Linearity: Linear between 10 to 2400 U/ml.

Relative sensitivity: 93.5%

Relative specificity: 83.3%.

Total agreement between the two methods was 88.8%.

Table 3 Relative Sensitivity and Specificity of the QuantiSpot™ RA Assay

	N= 159	Quanta Lite™ CCP3 (INOVA Diagnostics)	
		+	-
QuantiSpot™ RA Assay			
+		87	11
-		6	55

The same samples were also tested using the Quanta Lite™ CCP3 IgG ELISA test (INOVA Diagnostics, inc.). Method comparison showed high degree of agreement with a predicate method (INOVA Diagnostics' cp3 IgG ELISA test).

The performance characteristics of the three RF assays of the SQI test were also found to be comparable with those of single-test predicate tests (data not shown here).

Conclusions

1. The developed assay is capable of detecting the four analytes: IgA, IgG, IgM RF and IgG anti-CCP simultaneously.
2. The performance characteristics of the RA QuantiSpot™ CCP assay were comparable to those of an FDA cleared predicate assay.