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White Paper

Performance Evaluation of the IMMULITE 2000 Anti-CCP IgG Assay

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Abstract

Background: Rheumatoid arthritis is a debilitating disease causing cartilage and joint destruction. Detection of anti–cyclic citrullinated peptide (anti-CCP) IgG antibodies is useful in the diagnosis of rheumatoid arthritis and in the prognosis of disease severity. Serologic testing for anti-CCP antibodies has recently been integrated into the diagnostic algorithm for detection of rheumatoid arthritis and is an important tool in the evaluation of patients suspected of having the disease.

Objective: In this study, we evaluated the analytical and clinical performance of the newly developed automated chemiluminescent enzyme immunoassay* for measurement of CCP IgG levels in serum using the IMMULITE® 2000 analyzer. (Siemens Healthcare Diagnostics, Tarrytown, NY).

Materials and Methods: Precision was evaluated using a high control and two six-member precision panels (serum and lithium heparin) following CLSI guideline EP-5A. Positive and negative percent agreement as well as clinical sensitivity and specificity were determined using two-by-two tables comparing results to a commercially available method (DIASTAT ELISA, Axis-Shield Diagnostics) using 457 serum samples: 200 normal donor samples and 257 samples from patients diagnosed with rheumatoid arthritis. Additionally, endogenous interference was assessed using samples containing high concentrations of bilirubin (conjugated and unconjugated), human serum albumin, triglycerides, hemoglobin, and rheumatoid factor, while the cross-reactivity was evaluated using five antibody-reactive serum specimens from the following eight disease states: SLE, Sjögren's syndrome, scleroderma, polymyositis, osteoarthritis, autoimmune thyroiditis, Lyme disease, and infectious mononucleosis. All results were reported in U/mL. A result < 4.0 U/mL was considered nonreactive and a result ≥ 4.0 U/mL, reactive. The reportable range was <1.5 to 200 U/mL.

Results: Results were obtained in this study using IMMULITE 2000 Anti-CCP IgG reagents. Within-run and (CV) were <11% and <19%, respectively. The method comparison between IMMULITE 2000 and DIASTAT assays obtained overall percent agreement of 94.1%, positive percent agreement of 93.5%, and negative percent agreement of 94.6%. Clinical overall agreement was 87.5%, clinical sensitivity was 78.2%, and clinical specificity was 99.5%. Endogenous interference testing obtained a mean percent difference across all validation lots of ≤10% of the control sample for all interfering substances. To assess cross-reactivity, the mean doses of all disease state samples tested on the IMMULITE 2000 analyzer were compared to results generated on the DIASTAT assay and found to be in 100% agreement.

Conclusions: The IMMULITE 2000 Anti-CCP IgG assay performs well both analytically and clinically.

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*Not available for sale in the U.S. and its future availability in the U.S. cannot be guaranteed. Not all product offerings are available in all countries.

Introduction

Anti–citrullinated protein/peptide antibodies (ACPAs) are autoantibodies (antibodies directed against one or more of the individual's own proteins) frequently detected in RA patients. Recently, these antibodies have been found to be valuable biomarkers and are accepted as diagnostic tools for diagnosing RA in a very early stage of disease.

Cyclic citrullinated peptide, also known as CCP,[1] is a synthetic cyclic peptide incorporating the amino acid citrulline. During inflammation, the enzyme peptidylarginine deiminase incorporates citrulline into proteins.[2]

The presence of autoantibodies against citrullinated proteins in RA patients was first described in the mid-1970s when the biochemical basis of antibody reactivity against keratin and filaggrin was investigated.[3,4] Subsequent studies demonstrated that autoantibodies from RA patients react with a series of different citrullinated antigens, including fibrinogen, deiminated Epstein–Barr virus nuclear antigen 1, and vimentin,[5–7] which is a component of the intermediate filament. This white paper reports the analytical performance and clinical utility of the IMMULITE 2000 Anti-CCP IgG immunoassay. In these studies, the IMMULITE 2000 Anti-CCP IgG immunoassay was compared to the Axis-Shield DIASTAT Anti-CCP assay.

The intended use of the IMMULITE 2000 Anti-CCP IgG assay is for the semiquantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma on the IMMULITE 2000 analyzer. Detection of anti-CCP antibodies is used as an aid in the diagnosis of rheumatoid arthritis (RA) and should be used in conjunction with other clinical information. Autoantibody levels represent one parameter in a multicriterion diagnostic process encompassing both clinical and laboratory-based assessments.

Materials and Methods

Precision

CLSI protocol EP5-A2 was used as a guideline to evaluate the imprecision of the Anti-CCP assay using two IMMULITE 2000 analyzers. The study was conducted for 20 days with two runs per day and two replicates per sample. The samples consisted of the assay high control and two sets of six-member precision panels: one serum panel and one lithium heparin plasma panel.

Positive and negative percent agreement, and clinical sensitivity and specificity

The positive and negative percent agreement of the IMMULITE Anti-CCP assay was determined using 457 patient samples (257 patients diagnosed with rheumatoid arthritis and 200 apparently normal subjects) in a method comparison study against the DIASTAT Anti-CCP ELISA. Runs were evenly divided among five IMMULITE 2000 analyzers with an even distribution of normal and reactive samples in each run. All samples were also analyzed in the DIASTAT assay per the manufacturers' instructions. Clinical decision points of 4 U/mL for IMMULITE 2000 Anti-CCP IgG and of 5 U/mL for the DIASTAT assay were used to complete the analysis. In addition, the clinical sensitivity and specificity of the IMMULITE Anti-CCP assay were also determined.

Endogenous interference

To assess the effect of potentially interfering endogenous substances on test results with the IMMULITE 2000 Anti-CCP assay, the following substances at the stated concentrations were added to samples and tested:

Bilirubin, conjugated and unconjugated, at 40 mg/dL

Human serum albumin at 12 g/dL

Triglycerides at 1000 mg/dL

Hemoglobin at 500 mg/dL

Rheumatoid factor (RF) at 200 IU/mL

Serum pools were prepared with five levels of anti-CCP IgG:

Pool 1: 2.0–5.0 U/mL

Pool 2: 5.1–12.0 U/mL

Pool 3: 12.1–25 U/mL

Pool 4: 50.1–100 U/mL

Pool 5: 101–205 U/mL

Each potential interfering substance was then spiked separately into the sample pools. A control sample was prepared for each interfering substance by spiking the appropriate diluent at the same volume as the interfering substance. Interference was calculated as follows:

$$\% \text{ Interference} = 100 \times \frac{\text{Observed Dose Spiked Sample} - \text{Observed Dose Unspiked Sample}}{\text{Observed Dose Unspiked Sample}}$$

Cross-reactivity/specificity

The IMMULITE 2000 Anti-CCP assay was used in the assessment of the false-reactive or false-nonreactive results that may occur due to the presence of potentially cross-reactive viral/nonviral diseases. The following patient disease state samples were tested:

- Systemic lupus erythematosus (SLE)
- Sjögren's syndrome
- Scleroderma
- Polymyositis
- Osteoarthritis
- Autoimmune thyroiditis
- Lyme disease
- Infectious mononucleosis

Five antibody-reactive serum and plasma specimens were obtained for each of the eight different disease states. Patient specimens were tested with the IMMULITE 2000 Anti-CCP assay in triplicate. The mean dose was then compared to results generated with the DIASTAT assay.

Results

Precision

Precision was estimated using analysis of variance for nested model. Within-run and total imprecision in terms of standard deviation (SD) and coefficient of variation (CV) are shown in Table 1.

Table 1. Precision data for IMMULITE 2000 Anti-CCP IgG assay. (Total precision comprises between-day, between-run, and within-run precision.)

Sample	N	Mean Dose	Within-Run SD	Total SD	Within-Run CV	Total CV
LPIC2.101	160	47.03	0.63	2.31	4.7	4.9
PLASP2	160	2.30	0.13	0.28	9.2	12.4
PLASP3	160	4.35	0.18	0.37	5.9	8.5
PLASP4	160	8.33	0.28	0.45	4.2	5.4
PLASP5	160	38.59	0.78	2.00	4.7	5.2
PLASP6	160	140.25	3.48	7.08	4.3	5.1
SERP2	160	1.94	0.17	0.31	10.5	15.8
SERP3	160	4.11	0.22	0.37	6.3	9.0
SERP4	160	8.33	0.24	0.47	4.5	5.7
SERP5	160	37.00	1.28	2.23	4.3	6.0
SERP6	160	142.11	3.63	7.51	4.2	5.3

Positive and negative percent agreement, and clinical sensitivity and specificity

A total of 457 patient samples were tested for positive and negative percent agreement with the IMMULITE 2000 Anti-CCP IgG assay versus the Axis-Shield DIASTAT assay. The most conservative, representative data are shown in Table 2.

Table 2. Method comparison: IMMULITE 2000 assay vs. DIASTAT ELISA.

		IMMULITE 2000 Pos	IMMULITE 2000 Neg
DIASTAT ELISA	Pos	187	13
	Neg	14	243

N	457
Overall percent agreement	94.1%
Positive percent agreement	93.5%
Negative percent agreement	94.6%

The positive percent agreement of the IMMULITE 2000 assay for all reactive samples tested was 93.5%. The negative percent agreement for all nonreactive samples tested was 94.6%, and the overall percent agreement for the IMMULITE 2000 assay versus the DIASTAT assay was 94.1%.

Representative clinical performance of the IMMULITE 2000 Anti-CCP IgG assay is shown in Table 3.

Table 3. Method comparison: IMMULITE 2000 Anti-CCP IgG assay vs. rheumatoid arthritis status.

		IMMULITE 2000	
		Pos	Neg
Clinical Status	Pos	201	56
	Neg	1	199

N	457
Clinical overall agreement	87.5%
Clinical sensitivity	78.2%
Clinical specificity	99.5%

Endogenous interference

Mean percent difference between test and control samples was <10% for all interfering substances. Results are summarized in Table 4.

Table 4. Interference by endogenous substances.

Interferent (Spiked to)	Mean Interference
Human serum albumin (12 g/dL)	3.0%
Triglycerides (1.5 g/dL)	0.9%
Hemoglobin (500 mg/dL)	4.1%
Bilirubin, conjugated (0.2 mg/mL)	–1.8%
Bilirubin, unconjugated (0.2 mg/mL)	–2.7%
Rheumatoid factor (200 IU/mL)	0.1%

Cross-reactivity/Specificity

The data for all disease states tested are summarized in Table 5. All results for the IMMULITE 2000 Anti-CCP IgG assay cross-reactivity study demonstrated nonreactivity with all samples tested.

Table 5. IMMULITE 2000 Anti-CCP IgG assay cross-reactivity/specificity.

Disease State	Number Tested	Anti-CCP Positive by IMMULITE 2000 and DIASTAT	Number of Discordants by DIASTAT	Agreement
SLE	5	0	0	100%
Sjögren's synd.	5	1	0	100%
Lyme	5	1	0	100%
Osteoarthritis	5	0	0	100%
Scleroderma	5	0	0	100%
Polymyositis	5	1	0	100%
Thyroiditis	5	0	0	100%
Mononucleosis	5	0	0	100%

Conclusions

The performance data indicate that the IMMULITE 2000 Anti-CCP IgG assay is suitable to use as one parameter in the multicriterion classification of rheumatoid arthritis.

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