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Multicenter Clinical Evaluation of the IMMULITE 2000 Anti-CCP IgG Immunoassay

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Abstract

Background: Anti–cyclic citrullinated peptide (anti-CCP) IgG, a specific marker for rheumatoid arthritis (RA), can be detected in early RA and is indicative of more progressive disease.[1] It has 41%–89% sensitivity and 89%–99% specificity for RA diagnosis.[2]

Methods: A multicenter clinical trial evaluated the IMMULITE® 2000 Anti-CCP IgG assay* performance for reproducibility and clinical equivalence to the Axis-Shield Diagnostics DIASTAT™ Anti-CCP assay using RA and non-RA diagnosed samples and control materials.

Results: The IMMULITE 2000 assay demonstrated high reproducibility: for samples ranging from 1.78 to 139.39 U/mL, within-run CVs were 10.8%–3.9%, respectively, and total within-device CVs (day-to-day, run-to-run, and within-run variation) were 13.1%–4.9% respectively. Method comparison results (n = 1515) showed good initial concordance, with positive agreement of 89.8%, negative agreement of 98.9%, and total agreement of 95.7%. Clinical specificity and sensitivity were 97.5% and 58.9%, respectively, which is consistent with the performance of other commercial anti-CCP assays.

Conclusion: The IMMULITE 2000 Anti-CCP IgG assay demonstrated good reproducibility as well as performance comparable to that of the Axis-Shield DIASTAT assay in terms of agreement, clinical sensitivity, and clinical specificity. Thus, the IMMULITE 2000 assay exhibited performance characteristics consistent with those of commercially available assays for the detection of anti-CCP IgG antibodies in clinically significant populations.

* Not available for sale in the U.S. The product is not commercially available in all countries. Please contact your local Siemens organization for further details.

Background

The 2010 Rheumatoid Arthritis Classification Criteria embody a scoring system that assigns point values to clinical signs and laboratory test results. These laboratory tests include either rheumatoid factor (RF) or anti–citrullinated protein antibody (ACPA) assays, the latter also known as anti–cyclic citrullinated peptide (anti-CCP) assays.[3]

The sensitivity and specificity of RF assays are moderate, at 69% and 85%, respectively.[4] Anti-CCP assays offer sensitivity at least comparable to that of RF assays in RA but with higher specificity.[5–7] Various studies report anti-CCP assay sensitivities of 41% to 92%[1,4,7–12] and specificities of 88% to 100%.[1,4,7,10–13] Assay performance, however, varies with the test population. When RF and anti-CCP assay results are both positive, the specificity for RA approaches 100 percent.[13]

Three sites participated in this multicenter clinical trial to evaluate the reproducibility of the IMMULITE 2000 Anti-CCP IgG assay and to compare its performance with that of the Axis-Shield Diagnostics DIASTAT Anti-CCP assay for detecting anti-CCP antibodies in clinical samples.

Materials and Methods

Assay principles

The Siemens IMMULITE 2000 Anti-CCP IgG assay is intended for the in vitro semiquantitative determination of IgG autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma. The assay is a two-cycle, sequential chemiluminescent immunometric assay. In the first reaction cycle, the patient sample (10 µL, prediluted) is incubated with a biotinylated CCP–coated bead for 30 minutes. A wash step removes unbound nonspecific antibodies. During the second reaction cycle, reagent containing anti–human IgG conjugated to alkaline phosphatase is added and incubated for 30 minutes. After washing to remove excess conjugate, the chemiluminescent substrate is added and incubated for 5 minutes. The assay provides a result expressed as reactive or nonreactive relative to a 4.0 U/mL cutoff.

Reproducibility testing procedure

Reproducibility testing was performed at three sites over 10 days, two runs per day, with four replicates per run for all sample pools and control materials.

Method comparison procedure

A method comparison study evaluated the IMMULITE 2000 Anti-CCP IgG assay performance against that of the Axis-Shield DIASTAT Anti-CCP assay. A total of 1515 samples were analyzed. Additionally, a subset of samples (n = 559) were retested to compare the IMMULITE 2000 system against the Abbott ARCHITECT system and the Phadia (now Thermo Fisher Scientific) ImmunoCAP 250 system.

Results

Reproducibility results

Total within-device CVs of results for six samples with mean index values ranging from 1.78 to 139.39 (n = 472 and 484, respectively) were 13.1% and 4.9%, respectively, as shown in Table 1.

Table 1. Reproducibility pooled across three sites and two lots.

Sample Material	Number of				Mean	Within-Run		Between-Run		Between-Day		Between-Lot		Between-Site		Total Within-Device	
	Sites	Lots	Days	Replicates	Index	SD Index	CV (%)	SD Index	CV (%)	SD Index	CV (%)	SD Index	CV (%)	SD Index	CV (%)	SD Index	CV (%)
P2	3	2	32	472	1.78	0.19	10.8	0.05	3.0	0.12	6.9	0.25	13.9	0.16	9.1	0.23	13.1
P3	3	2	32	484	3.85	0.24	6.2	0.10	2.7	0.16	4.2	0.18	4.8	0.15	4.0	0.31	7.9
P4	3	2	32	484	8.05	0.39	4.9	0.16	2.0	0.17	2.1	0	0	0.23	2.8	0.46	5.7
P5	3	2	32	483	36.43	1.42	3.9	0.71	1.9	0.81	2.2	0.49	1.3	0.92	2.5	1.78	4.9
P6	3	2	32	484	139.39	5.72	4.1	2.65	1.9	3.71	2.7	0	0	4.09	2.9	7.31	5.2
POS	3	2	32	486	44.62	2.24	5.0	0.41	0.9	1.24	2.8	1.49	3.3	1.63	3.7	2.59	5.8

Method comparison results

A total of 1515 samples were analyzed: 791 from patients clinically diagnosed with RA, the diagnosis having been made by a board-certified rheumatologist or internist whenever possible; 464 from patients clinically diagnosed without RA but diagnosed with potentially cross-reactive infections or clinical conditions ; and 260 from apparently healthy subjects. Samples from osteoarthritis, systemic lupus erythematosus, and psoriatic arthritis patients accounted for 47.6% of the non-RA samples. For the sensitivity and specificity analyses, unless otherwise stated, the data were split into two cohorts: RA and non-RA, the latter consisting of the non-RA disease state samples and the apparently healthy samples.

The cutoffs used for the method comparisons were as follows:
Siemens IMMULITE 2000: nonreactive, <4.0 U/mL; reactive, ≥4.0 U/mL
Axis-Shield DIASTAT: negative, <5.0 U/mL; positive, ≥5.0 U/mL.

Table 2 gives the method comparison results for the IMMULITE 2000 assay vs. the Axis-Shield DIASTAT assay. The positive and negative percent agreements were 89.8% and 98.9%, respectively, with an overall agreement of 95.7%.

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

		Axis-Shield DIASTAT		
		Positive	Negative	Total
IMMULITE 2000	Reactive	473	11	484
	Nonreactive	54	977	1031
	Total	527	988	1515

	Percent Agreement	95% LCL	95% UCL
Positive	89.8	86.8	92.2
Negative	98.9	98.0	99.4
Total	95.7	94.6	96.7

The clinical sensitivity of the IMMULITE 2000 assay was 58.9% (466/791), with a 95% confidence interval (CI) of 55.4% to 62.4%; the clinical specificity was 97.5% (706/724), with a 95% CI of 96.1% to 98.5% (Table 3). The clinical sensitivity of the DIASTAT assay was 63.8% (505/791), with a 95% confidence interval (CI) of 60.4% to 67.2%; the clinical specificity was 97.0% (702/724), with a 95% CI of 95.4% to 98.1% (Table 4).

Table 3. Method comparison: IMMULITE 2000 assay vs. RA diagnosis.

		RA Diagnosis		
		RA	Non-RA	Total
IMMULITE 2000	Reactive	466	18	484
	Nonreactive	325	706	1031
	Total	791	724	1515

	Percent	95% LCL	95% UCL
Sensitivity	58.9	55.4	62.4
Specificity	97.5	96.1	98.5

Table 4. Method comparison: DIASTAT assay vs. RA diagnosis.

		RA Diagnosis		
		RA	Non-RA	Total
Axis-Shield DIASTAT	Reactive	505	22	527
	Nonreactive	286	702	988
	Total	791	724	1515

	Percent	95% LCL	95% UCL
Sensitivity	63.8	60.4	67.2
Specificity	97.0	95.4	98.1

An ROC analysis of the IMMULITE 2000 and Axis-Shield DIASTAT assays vs. RA diagnosis is shown in Figure 1 and Table 5. The analysis indicates that the two assays are not statistically different (P = 0.0915).

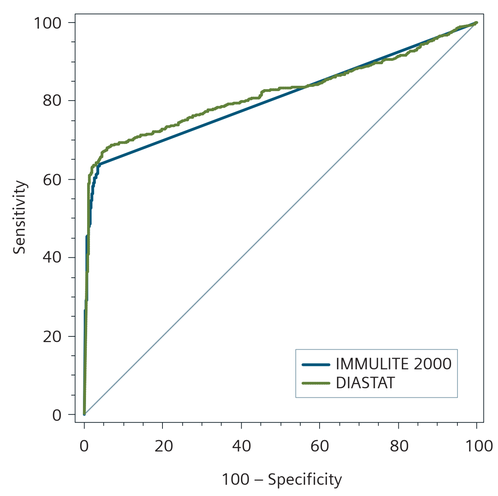

Figure 1. ROC analysis: IMMULITE 2000 assay vs. Axis-Shield DIASTAT assay.

Table 5. ROC analysis statistics.

	AUC	SE	95% CI
IMMULITE 2000	0.806	0.00914	0.785 to 0.826
DIASTAT	0.820	0.0114	0.800 to 0.839

IMMULITE 2000 vs. DIASTAT	
Difference between areas	0.0138
Standard error	0.00816
95% Confidence interval	–0.00222 to 0.0298
z Statistic	1.688
Significance level	*P* = 0.0915

In the additional method comparison performed on a subset of samples (n = 559), the ARCHITECT assay and the ImmunoCAP 250 assay were each compared to the IMMULITE 2000 assay and to RA diagnosis (Tables 6 and 7). Performance characteristics of the ARCHITECT and ImmunoCAP 250 assays were similar to those obtained in the comparison between the IMMULITE 2000 and DIASTAT assays.

Table 6. ARCHITECT assay vs. IMMULITE 2000 assay and vs. RA diagnosis.

		ARCHITECT		
		Positive	Negative	Total
IMMULITE 2000	Reactive	159	6	165
	Nonreactive	23	371	394
	Total	182	377	559

	Percent Agreement	95% LCL	95% UCL
Positive	87.4	81.6	91.8
Negative	98.4	96.6	99.4
Total	94.8	92.6	96.5

	Percent	95% LCL	95% UCL
Sensitivity	65.5	59.5	71.2
Specificity	97.6	95.1	99.0
Total	82.3	78.9	85.4

Table 7. ImmunoCAP 250 assay vs. IMMULITE 2000 assay and vs. RA diagnosis.

		Phadia			
		Positive	Negative	Equiv	Total
IMMULITE 2000	Reactive	159	6	0	165
	Nonreactive	16	376	2	394
	Total	175	382	2	559

	Percent Agreement	95% LCL	95% UCL
Positive	90.9	85.6	94.7
Negative	98.4	96.6	99.4
Total	96.1	94.1	97.5

	Percent	95% LCL	95% UCL
Sensitivity	64.5	58.4	70.3
Specificity	98.6	96.5	99.6
Total	82.4	79.0	85.5

Conclusions

The IMMULITE 2000 Anti-CCP IgG assay demonstrated good reproducibility, as well as performance comparable to that of the Axis-Shield DIASTAT assay in terms of agreement, clinical sensitivity, and clinical specificity. Thus, the IMMULITE 2000 assay exhibited performance characteristics consistent with those of commercially available assays, as further seen in this trial upon comparison against the Abbott ARCHITECT and Phadia ImmunoCAP EliA assays, for the detection of anti-CCP antibodies in clinically significant populations.

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