

PERFORMANCE OF THE AxSYM[®] ANTI-CCP (ANTI-CYCLIC CITRULLINATED PEPTIDE) ASSAY AS A DIAGNOSTIC TEST FOR RHEUMATOID ARTHRITIS (RA)

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ABSTRACT

Antibodies to cyclic citrullinated peptides have been widely reported as an excellent diagnostic marker for Rheumatoid Arthritis (RA) with a sensitivity of 65-80% and a specificity of 95-97%. The antibody has been shown to be present early in the disease and an independent predictor of joint damage and disease progression. We report on the latest results from our development of an automated assay for anti-CCP antibodies (second generation) on the Abbott AxSYM analyser. Sensitivity and specificity were determined using 125 clinically confirmed RA patients, 45 asymptomatic individuals and 43 samples from non-RA rheumatic diseases and infectious diseases. Using a cut off of 5 U/ml (arbitrary units), clinical sensitivity, specificity and concordance figures obtained for the AxSYM assay are shown below:

Disease Group	Number	Positive (n)	Positive (%)
RA/Early RA	125	101	81
Asymptomatic	45	0	0
Non-RA	43	2	5

Clinical Sensitivity = 81% Clinical Specificity = 98%
Overall Concordance with confirmed RA disease = 88%

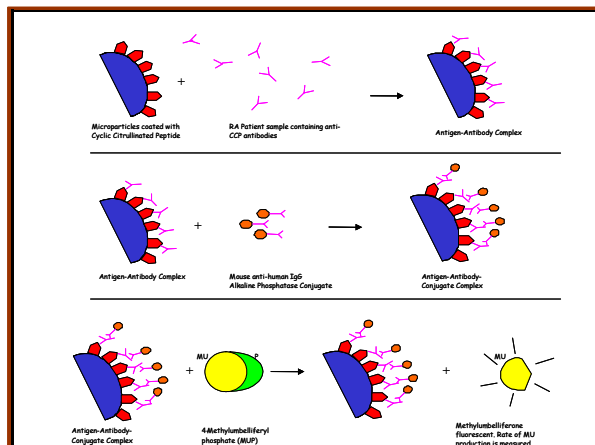
Precision was evaluated following a CLSI 20-day study. From this, total imprecision for both within and between run was calculated to be $\leq 10\%$ across the calibration curve (0-200 U/ml). Analytical sensitivity i.e. limit of detection (LOD), was determined to be < 1 U/ml. Based on our evaluation, we conclude that the AxSYM Anti-CCP assay is a highly sensitive and specific method for the detection of anti-CCP antibodies and our results are in line with published data using the manual ELISA systems. The full automation, high throughput, low imprecision and inbuilt autodilution facility of the AxSYM make it an ideal platform for the routine testing of this important parameter.

INTRODUCTION

Measurement of antibodies to cyclic citrullinated peptides has been reported to be a reliable and accurate diagnostic marker for RA. Currently, the testing for anti-CCP is performed almost exclusively using the manual ELISA test formats. The manual methods typically have limited through-put, relatively long assay times, poor precision at the high end of the calibration curve and lack of an autodilution facility for samples falling out with the calibration curve. We compare the preliminary assay performance of the fully automated AxSYM anti-CCP to the manual DIASTAT anti-CCP ELISA. Both methods employ the so-called CCP2 second generation peptides.

MATERIALS AND METHODS

The automated assay under development utilises microparticle enzyme immunoassay (MEIA) technology in a two-step sandwich assay format as depicted below:



Precision was estimated over 20 days for the AxSYM assay, using the CLSI protocol. Using the AxSYM and ELISA methods, anti-CCP titres were measured in a patient group consisting of asymptomatic individuals (n=45), non-RA (n=43) and clinically confirmed RA (n=125).

RESULTS

Precision over 20 days for the AxSYM assay was as follows:

Pool	Mean U/ml	Within-run CV%	Total CV%
1	13.2	7.8	8.9
2	29.8	5.9	7.8
3	157.5	6.8	9.3

The AxSYM and DIASTAT anti-CCP data from the population studied are tabulated below:

Sample Type	n	AxSYM Positive	DIASTAT Positive
Asymptomatics	45	0	0
Viraemia	12	0	0
Infectious disease	5	0	0
Osteoarthritis	3	0	0
Ankylosing spondylitis	3	0	0
Inflammatory polyarthritis	4	1	2
Psoriatic arthritis	3	0	0
SLE	7	1	1
Sjögren's Syndrome	3	0	0
Dermatomyositis	2	0	0
Scleroderma	1	0	0
Rheumatoid Arthritis	125	101	115

AxSYM Clinical Sensitivity = 81%
 AxSYM Clinical Specificity = 98%

CONCLUSIONS

In this sample cohort, the clinical performance of the new automated assay is similar to the manual ELISA and with other studies reported in the literature. The automated assay benefits from a shorter time to first result (18 minutes) and higher throughput (55 tests per hour) when compared with the manual ELISA. Furthermore, the precision across the dynamic range of the AxSYM assay was $< 10\%$ and the analytical sensitivity of < 1 U/ml is well below the cut-off value currently set at 5 U/ml. These features support the utility of the AxSYM Anti-CCP assay in a routine clinical setting.

REFERENCES

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