Evaluation of recombinant Ro60 for diagnostic use in AI-Line ELISA

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Background: Detection of autoantibodies (aabs) directed against the hyRNP–associated Ro60 protein is the predominant diagnostic marker and a classification criterion for the diagnosis of the Sjögren syndrome (SjS). Anti-Ro60 aab are also associated with numerous systemic autoimmune rheumatic disorders (SARD) such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). Native (nRo60) has been reported to be superior to recombinant Ro60 (rRo60) for the detection of anti-Ro60 aab. A newly available, procaryotically produced rRo60 (DIARECT) was tested in two studies with 328 and 113 sera from different SARD and compared to purified bovine nRo60.

Methods: Both nRo60 and rRo60 were used to produce ELISA tests under production conditions of the newly available AI-Line ELISA series (Dr. Fooke Laboratories). In study 1, 113 sera of different SARD were tested to evaluate the correlation between rRo60 and nRo60.
In study 2, 328 sera from patients suffering from SjS (n=60), SLE (n=168), SSc (n=100) and Myositis (n=40) were tested with the new AI-Line Ro60 ELISA using rRo60 antigen and addressable laser based immunoassay (ALBIA) with nRo60 (FDA approved QUANTA Plex™ SLE Profile 8, INOVA Diagnostics, Inc.). Statistical evaluation of raw data was performed using ANALYSE-it 1.62 software.

Results : The correlation of rRo60 and nRo60 in an AI-Line ELISA format showed comparable results between rRo60 and nRo60 (see figure 1; Pearson´s r=0.98; 95% CI=0.98-0.99; p<0.0001). For the majority (47/53 ~89%) of positive sera, the signal intensities for the rRo60 assays did not differ more than 25% when compared to the assays using nRo60 (figure 2). The agreement of results between AI-Line rRo60 ELISA and ALBIA ranged from 88 to 90% dependent on the cut-off of ALBIA (figures 3 and 4).

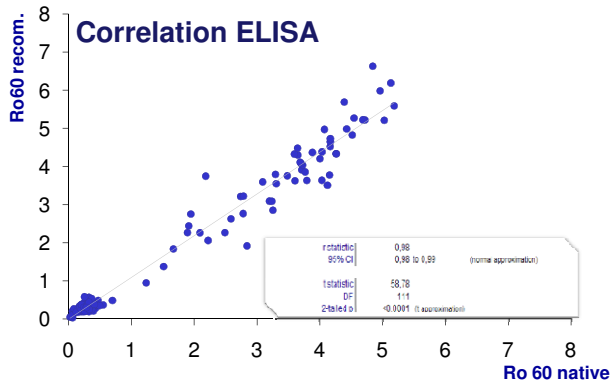

Figure 1 Correlation of rRo60 and nRo60 in AI-Line ELISA Setup. Values in relative units [RU/mL]; n=113, 53 anti-Ro60 positive and 60 anti-Ro60 negative sera.

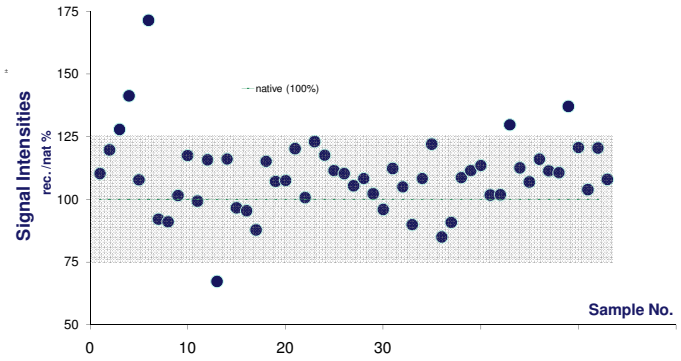

Figure 2 Signal ratio of anti-Ro60 positive sera measured using rRo60 relative to signal intensity of sera measured using nRo60. Values expressed in percent.

(ALBIA C/O set @ 85)	ELISA Ro60 +	ELISA Ro60 -	
ALBIA Ro60 +	128	19	**147**
ALBIA Ro60 -	15	166	**181**
	143	**185**	

Figure 3 Agreement between AI-Line Ro60 ELISA using rRo60 antigen and ALBIA using nRo60.

ALBIA cut off	Agreement [%]	Sensitivity [%]	Specificity [%]
55	87.2	93.7	78.3
60	87.8	93.0	80.6
70	88.4	91.6	83.6
80	89.0	90.2	86.5
85	89.6	89.5	88.6
90	89.3	88.8	88.6
95	89.9	88.1	90.5
100	89.9	87.4	91.2
120	90.2	86.7	92.4
200	89.6	83.9	93.7
300	89.6	82.5	94.9
522	88.1	79.0	94.9

Figure 4 Agreement, sensitivity and specificity between AI-Line Ro60 ELISA using rRo60 and ALBIA using nRo60 under different cut- off values.

Conclusion: We conclude that recombinant Ro60 protein expressed by a novel strategy is comparable to the homologous native bovine antigen. Since recombinant antigens are known to exhibit higher lot to lot consistencies, rRo60 may become the “golden standard” antigen for the detection of anti-Ro60 aab. Further, the new AI-Line Ro60 ELISA based on the new rRo60 antigen represents a reliable test with good qualitative agreement to ALBIA with nRo60 antigen.

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