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Anti-PCNA antibodies in samples with cell cycle dependent, PCNA-like indirect immunofluorescence staining pattern

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Background: Autoantibodies (aab) targeting proliferating cell nuclear antigen (PCNA) occur rarely in patients with systemic rheumatic autoimmune diseases (SARD) but are most commonly associated with systemic lupus erythematosus (SLE). The primary antigenic target is a 34 kDa auxiliary protein of DNA polymerase δ (ADPDPδ), which is part of a multi-protein complex. The objective was to evaluate two novel anti-PCNA assays that utilized purified recombinant ADPDδ as the analyte.

Methods: Sera (n=40) with a cell cycle dependent staining pattern in indirect immunofluorescence (IIF) on HEp-2 cells and controls (n=29) were tested for anti-PCNA aab by PCNA ELISA (Dr. Fooke Laboratorien), line immunoassay (LIA, Mikrogen) and an addressable laser bead assay (ALBIA) using commercially available recombinant PCNA (Diarect AG, Freiburg, Germany) coupled to laser reactive beads and reactivity determined in a Luminex 100 (Luminex Corp.).

Results and findings: Receiver operating characteristic (ROC) analysis showed good discrimination between samples with suspected anti-PCNA reactivity (n=40) and controls (n=29) for ELISA (AUC=0.78) and ALBIA (AUC=0.76). In addition, good qualitative agreement was found between all three methods (kappa 0.60 – 0.76). Pearson's correlation showed good quantitative agreement between ELISA and ALBIA (R=0.83; p<0.0001). At cut-off values defined after ROC analysis resulting in 100% specificity, 21 (52.5%) samples with PCNA like IIF staining pattern were positive by ELISA and 17 (42.5%) by ALBIA. 14 (35%) were positive by LIA.

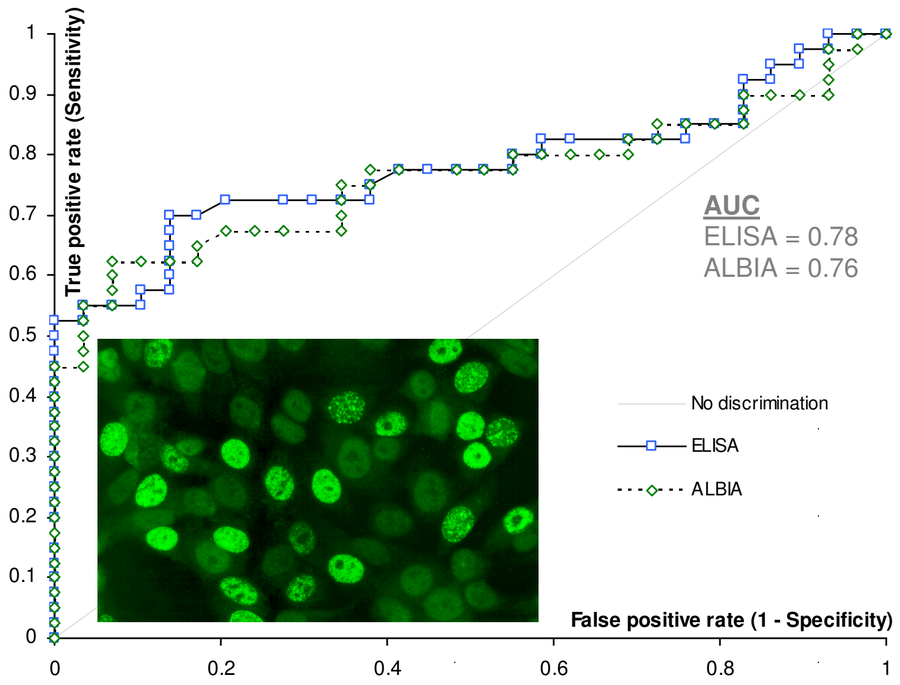

Figure 1 Receiver operating chararteristics (ROC). Comparative ROC analysis shows good discrimination between samples with PCNA-like staining pattern (n=40) and controls (n=29). Indirect immunofluorescence picture shows the typical PCNA-like staining pattern on HEp-2 cells.

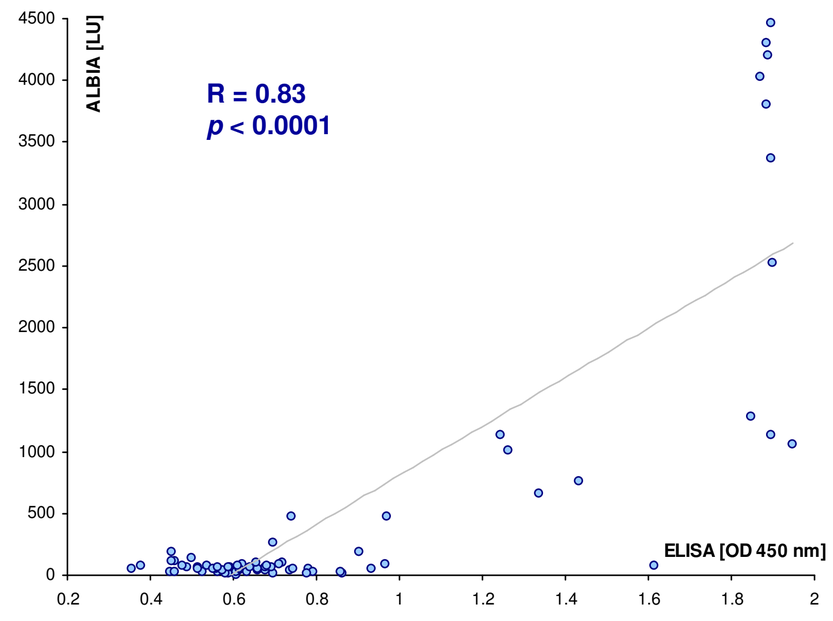

Figure 2 Pearson's correlation diagram ELISA vs. ALBIA. Good agreement between both methods was observed.

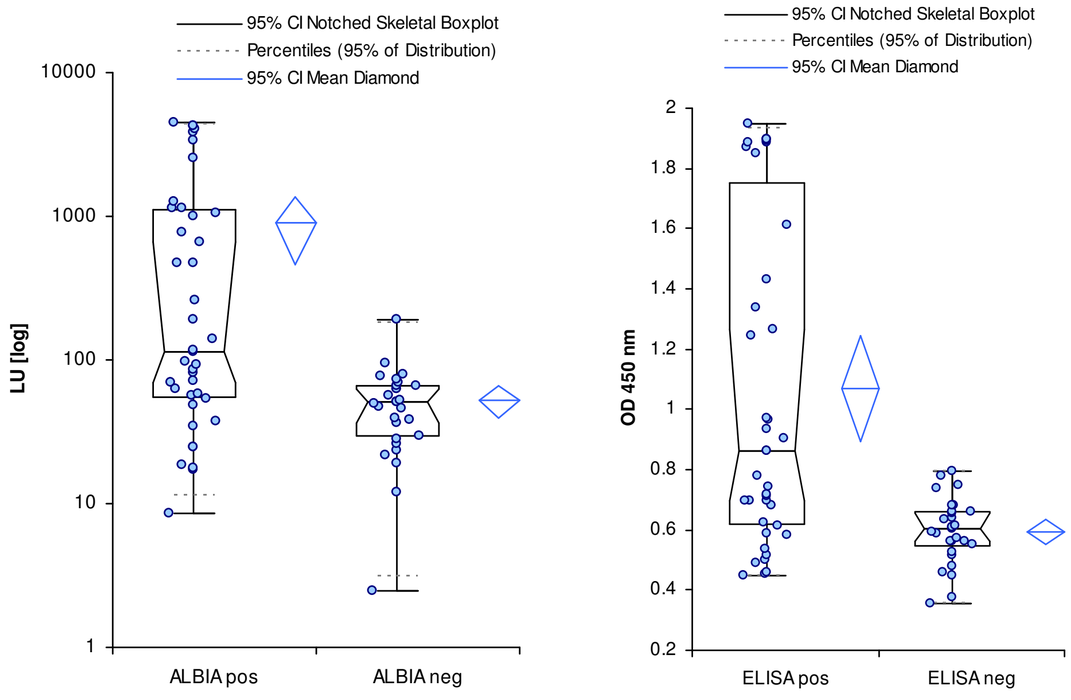

Figure 2 Comparative descriptive analysis of ELISA and ALBIA. Mean and 95% percentile with confidence (CI) values are shown (see legend).

Table 1 Qualitative agreement between three methods according to Chi-square and *kappa* test (including confidence interval, CI)

	ELISA	LIA
ALBIA	χ^2 = 15.14 *kappa* = 0.60 **(CI 0.36 – 0.84)**	χ^2 = 22.35 *kappa* = 0.74 **(CI 0.53 – 0.95)**
ELISA	/	χ^2 = 19.49 *kappa* = 0.66 **(CI 0.44 – 0.87)**

Conclusion: The three novel PCNA assays, namely ELISA, LIA and ALBIA show good agreement and represent promising tools for the detection of anti-PCNA aab. As 47.5 – 65% of our samples with anti-PCNA like staining pattern are negative by confirmation assays, other cell cycle dependent antigens or components of the PCNA multi-protein complex as previously reported, might be the target of aab in those sera.

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