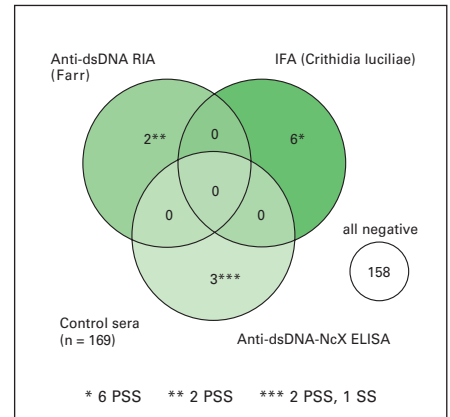
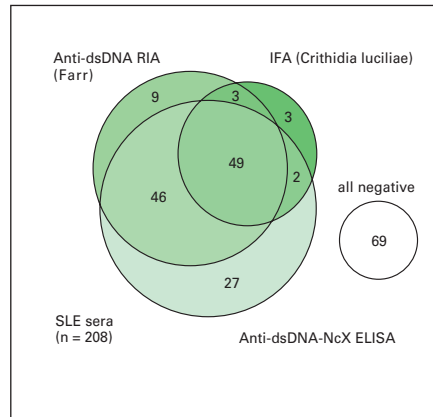
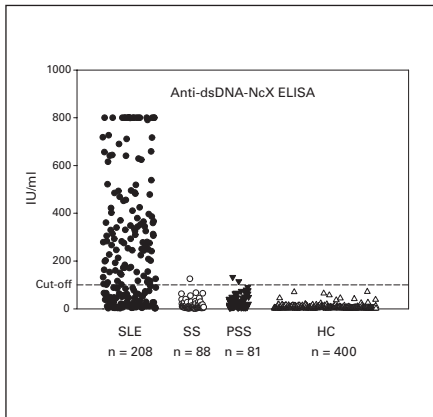


# Anti-dsDNA-NcX ELISA: Superior to Farr-RIA and IFA using *Crithidia luciliae* for SLE diagnosis

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## Introduction

Until now, antibodies against dsDNA have been the most important markers for the diagnosis of systemic lupus erythematosus (SLE) and for the determination of disease activity, especially in lupus nephritis. In many conventional ELISA systems, the target antigen dsDNA is linked to the solid phase by means of poly-L-lysine or protamine sulphate. However, these substances are prone to cause unspecific reactions.

As one result of our long-standing research on nucleosomes, we experienced their pronounced adhesiveness to plastic surfaces as well as their high affinity to pure dsDNA. The novel Anti-dsDNA-NcX ELISA takes advantage of the capability of nucleosomes to link DNA to microtiter plates.

## Methods

Purified nucleosomes, free of Scf70, histone H1 and other non-histone components<sup>1</sup>, were used as an ultra-thin adhesive layer for the immobilisation of dsDNA in the Anti-dsDNA-NcX ELISA (IgG). This test system was compared to a conventional Anti-dsDNA ELISA, the Farr-RIA (gold standard, IgA/G/M), an Anti-Nucleosomes ELISA (IgG) and indirect immunofluorescence using

*Crithidia luciliae* (IFA, IgG), all reagents from EUROIMMUN, Germany. The study comprised 208 patients with SLE, 88 with Sjögren's syndrome (SS) and 81 with progressive systemic sclerosis (PSS).

## Results

At a predefined specificity of 98%, the sensitivity of the novel Anti-dsDNA-NcX ELISA was increased to 60.8%, clearly exceeding the conventional Anti-dsDNA ELISA and the Anti-dsDNA RIA (data based on ROC analysis). With regard to SS and PSS, the new test system reached a maximum specificity of 98.2% for SLE. For comparison, the sensitivity of IFA was 27.4% at a specificity of 96.4%. However, all six control sera, which reacted exclusively in IFA, originated from patients with PSS, which frequently show

an overlap with SLE: possibly, IFA is more suitable to identify such cases than the other two test systems.

## Discussion

The special configuration of the Anti-dsDNA-NcX ELISA ensures clear presentation of the major dsDNA epitopes and minimizes false positive reactions. Of all the individual assays tested, the Anti-dsDNA-NcX ELISA revealed the highest sensitivity in the diagnosis of SLE. RIA and IFA might be used to identify some remaining SLE patients with negative ELISA results (7.2% in our study). In sum, our data demonstrate that the Anti-dsDNA-NcX ELISA is superior to the Farr-RIA and to the IFA using *Crithidia luciliae*, and has the potential to replace them as gold standards in the serological diagnosis of SLE.

	Anti-dsDNA-NcX ELISA	Anti-dsDNA ELISA	Anti-dsDNA RIA	Anti-Nucleosomes ELISA
AUC	0.85	0.82	0.85	0.83
CI	0.81-0.89	0.77-0.86	0.81-0.89	0.79-0.87
Sensitivity at a specificity of 95%	65.1%	49.3%	54.1%	54.1%
Sensitivity at a specificity of 98%	60.8%	35.4%	53.1%	52.6%
Sensitivity at a specificity of 99%	57.4%	34.9%	52.2%	52.6%
Maximal sum of sensitivity and specificity	161.3%	152.6%	156.0%	151.8%

Literature: <sup>1</sup>Suer W, Daehnrich C, Schlumberger W, Stoecker W. Autoantibodies in SLE but not in scleroderma react with protein-stripped nucleosomes. J Autoimmun 22 (2004) 325-334

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