Publication of the Month

EliA™ Excellence in Autoimmunity

November 11/07: Detection of DNA antibodies – ELISA vs CLIFT

Antibodies to double-stranded DNA (dsDNA) are one of the 11 diagnostic criteria for the classification of SLE, which were established by the American Rheumatism Association (ARA) in 1982. In addition, dsDNA antibodies reflect disease activity, serve as a predictor of disease exacerbation and are suitable to monitor the response to therapy.

Sensitivity and specificity of dsDNA antibodies in SLE are highly dependent on the method used. The most frequently used methods in routine clinical laboratories are Farr assay (RIA), CLIFT (Crithidia luciliae immunofluorescence test), and ELISA. The Farr was the first quantitative anti-dsDNA assay used and remains the standard reference method. However, it is time-consuming, technically more difficult, and involves the use of radioactive dsDNA. CLIFT is highly specific though its sensitivity is recognised as being low. It also becomes cumbersome when large numbers of samples are involved, and is not quantitative unless the titre is determined by assaying serial sample dilutions. On the other hand, ELISA systems for anti-dsDNA are straightforward, rapid, quantitative, and reproducible but require highly purified antigens. Moreover, commercial anti-dsDNA ELISA kits are inconsistent in terms of performance which may relate to the antigens selected. To overcome these issues, many laboratories use a combination of methods for dsDNA antibody measurement – e.g. screen with ELISA and confirm with CLIFT. The problem then becomes what to do when there are discrepancies in the different method results.

In the following study the authors evaluate the clinical significance of ELISA positive but CLIFT negative results:

Kim KH, Han JY, Kim JM, Lee SW, Chung WT
Clinical significance of ELISA positive and immunofluorescence negative anti-dsDNA antibody
Clin Chim Acta 2007, 380:182-185

371 patients were tested with ELISA (Scimedx, USA) and CLIFT (MBL, Japan) according to the manufacturers' instructions. 93 blood donors were found negative by both assays. Of 131 ELISA positive samples, 60 were negative with the CLIFT.

Of the 60 discrepant samples, 44 were available in sufficient amounts for further analysis. These samples, as well as the 93 blood donors found negative in both the ELISA and the CLIFT, were further tested using Farr and another 3 commercial dsDNA ELISA tests. Full clinical review was performed on these patients to determine the clinical relevance of the results. 35 (79.5%) of the 44 discrepant samples had 3 or more SLE criteria, excluding the anti-dsDNA criterion. The performance of the kits and methods was as follows:

Kit	Antigen	Sens	Spec	Efficiency
QuantaLite	Calf thymus	54.29	95.10	84.67
Euroimmun	Salmon testis	11.43	97.06	75.18
MesaCUP	Bacteriophage	77.14	94.12	89.78
Scimedx	Plasmid	100.00	91.18	93.43
CLIFT		(0.00)	100.00	74.45
FARR	Recombinant	74.29	92.16	87.59

Table: Diagnostic efficiency of different anti-dsDNA tests with respect to the number of SLE criteria present for the 44 anti-dsDNA ELISA positive and CLIFT negative patients and for 93 healthy blood donors.

Although the study is limited by the fact that not all samples were tested by all methods, it still gives a likely indication as to the usefulness of the different methods in a clinical setting. The results of the study showed that some anti-dsDNA ELISA kits have diagnostic efficiencies that are similar to that of the Farr assay. The study identifies a group of patients that are ELISA positive but CLIFT negative for anti-dsDNA, and indicates that the majority of these patients have clinically relevant SLE.