The *Crithidia luciliae* immunofluorescent test for the detection of antibodies to native DNA

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**Summary**

The *Crithidia luciliae* test was compared with the radio-immunoassay technique for n DNA antibodies on 93 randomly selected sera submitted for antibody testing, and was found to be a highly specific, reproducible and economical test. It appeared to be less sensitive than the radio-immunoassay technique in sera from some of the patients, but analysis of the clinical findings showed that the *C. luciliae* test correlated better with the clinical assessment of these patients.

Probably the most important auto-antibodies found in systemic lupus erythematosus (SLE) are those directed against double-stranded deoxyribonucleic acid (n DNA). Their importance in the diagnosis of SLE is well documented, in so far as high titres are considered virtually diagnostic of SLE.

In addition, several investigators have concluded that monitoring of n DNA antibodies in SLE patients is of value, since their levels correlate with the clinical course of the disease.

Radio-immunoassay (RIA) using the ammonium sulphate precipitation or Farr technique is probably the method most commonly used in routine tests for n DNA antibodies because of its sensitivity and reproducibility. However, this test has a number of disadvantages. Firstly, several investigators have reported the presence of n DNA antibodies in patients with diseases other than SLE and also in normal subjects. This is possibly due to contamination of the double-stranded DNA preparation with single-stranded DNA during the chemical purification of double-stranded DNA, and antibody to single-stranded DNA has been demonstrated in a number of diseases other than SLE. Secondly, there is a high cost factor for both reagents and equipment, and the RIA test is only suitable for large centres performing numerous assays.

Aarden et al. described an immunofluorescent test using the haemoflagellate *Crithidia luciliae* as substrate. The organism has an intracellular organelle, the kinetoplast, which contains only n DNA. They concluded that their test was of the same order of specificity and sensitivity as the Farr test. The economy and simplicity of this technique make it very attractive, particularly for use in smaller laboratories. This test has since been evaluated by several investigators. Some have reported the *Crithidia* fluorescence (CF) test as showing the same order of sensitivity as the Farr technique, whereas others showed decreased sensitivity. However, there is agreement that this test is highly specific for SLE.

In this study we compared the two tests, using routine sera submitted for the detection of n DNA antibodies with a view to determining whether the CF test can adequately replace the more elaborate and expensive RIA procedure.

**Materials and methods**

A total of 93 sera with known RIA values was randomly selected to give a range of positive and negative values.

**Radio-immunoassay**. The Farr ammonium sulphate precipitation technique using Amersham reagents was employed. Values of less than 25% are considered normal, values greater than 35% abnormal and the 25-35% range doubtful.

**Crithidia immunofluorescence**. The detailed methodology used is as described. Briefly, the organisms were inoculated into approximately 10 ml growth medium, incubated at room temperature for 72 hours and then washed 3 times in phosphate-buffered saline, pH 7.4. A suspension containing about 20 x 107 organisms per ml was prepared in a solution of 0.1% bovine serum albumin in distilled water. The resulting distension facilitated recognition of the kinetoplast, and the addition of the bovine albumin to the distilled water improved the morphology of the organisms.

Multiphot slides were prepared, air-dried and fixed in methanol for 10 minutes at room temperature. Slides were stored at -20°C for 3-4 weeks. The standard indirect immunofluorescence staining technique was used with sera tested both undiluted and diluted 1:10. Polyvalent antihuman immunoglobulin conjugate (Wellcome) was used for the initial tests. The positively staining kinetoplast appears as a discreetly outlined fluorescent ring or solid circle lying against the cytoplasmic membrane approximately equidistant from the opposing nuclear and flagellar ends of the organism.

**Results**

Of the 93 sera examined, 30 were positive by both RIA and CF, 49 negative by both tests, 1 positive by CF and negative by RIA and 13 negative by CF and positive by RIA. The latter 13 sera were re-tested using both techniques and the presence of antinuclear antibody was determined by immunofluorescence using rat liver sections. Three patients had negative antinuclear factor tests, their repeat RIA values were normal and clinically they did not fulfil the necessary criteria for a diagnosis of SLE. We therefore considered their original RIA values to be false-positive results.

Of the remaining 10 sera, 3 were excluded due to inadequate information, leaving 7 sera from 5 patients.
These 5 patients were confirmed cases of SLE on long-term steroid therapy but only 1 was considered to be clinically active. The other 4 patients showed persistently elevated n DNA antibody values over a long period in spite of apparent good health, and their negative CF tests seemed to give a better correlation with their clinical status than the RIA.

Screening sera undiluted and diluted 1:10 confirmed the increased sensitivity of undiluted serum (5 sera with RIA values ranging from 40% to 100% were positive only when diluted) (Fig. 1). There was no loss of specificity as false-positive values were not obtained with undiluted serum. Testing with undiluted serum alone is not recommended as some samples show a negative or doubtful reaction but a clear positive reaction at 1:10 or higher. The reason for this prozone-like effect is not clear but may lead to false-negative reports.

Fig. 1. Comparison of n DNA antibody levels in 30 sera positive by both tests.

Discussion

Our findings confirm that CF is a specific, reproducible and economical test which should be undertaken on all antinuclear factor-positive sera. The results also show a reasonable correlation between RIA and CF levels (Fig. 1). Titres of 1:5 and 1:10 by CF had RIA values of 35-70%, titles of 1:20 ranged between 70% and 100% and CF titles greater than 1:80 had RIA values greater than 100%. These tests have been used to follow response to therapy in SLE but CF values fall much more rapidly than RIA values. 

The comparative sensitivity of the two tests was more difficult to evaluate. Although greater numbers of RIA-positive results were obtained in the SLE cases, their clinical significance was doubtful. Raised n DNA antibodies may occur during long periods without major symptoms of disease and it is not known whether these patients have persistent active disease suppressed by medication or are actually in remission. A negative CF test in the clinically inactive cases with persistently high RIA values may be due to high levels of antibodies to single-stranded DNA, detectable only by RIA.

CF is an attractive test for smaller laboratories, particularly those already employing the immunofluorescence test for antinuclear factor but not having the necessary equipment or the specimen volume to justify the more elaborate RIA test. Owing to its greater specificity for active SLE this test should at least complement the RIA test in larger centres.

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References


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Intrathecal human tetanus immune globulin in early tetanus

Now that human tetanus immune globulin is available, there is no reason why it should not be injected intrathecally. However, an earlier trial in Delhi suggested that so used it did not improve the case fatality rate in patients already having severe spasms. A second report (Gupta et al., Lancet, 1980 2, 439) carried out on 97 patients with symptoms of early tetanus at the time of admission, produced strong evidence that intrathecal tetanus immune globulin used early in the course does lower mortality. In their trial the condition of only 3 out of 49 patients given 250 IU of the immune globulin worsened and only 1 patient died; in a matched group of 48 patients in whom the globulin was administered intramuscularly in doses of 1 000 IU, the clinical features of the disease became more severe in 15 and 10 patients died. There were no side-effects from intrathecal administration.