Three main immunological techniques have been developed for the measurement of anti-dsDNA antibodies: the enzyme linked immunosorbent assay (ELISA),\(^1\) the \textit{Crithidia luciliae} indirect immunofluorescence test (CLIF),\(^2\) and the ammonium sulphate precipitation method (Farr assay).\(^3\) We developed a rapid anti-dsDNA antibody test using the particle gel immunoassay (ID-PaGIA) format, and the standard methodology and equipment of the ID-Micro Typing System (DiaMed, Cressier sur Morat, Switzerland). The principle of the PaGIA immunoassay was derived from passive haemagglutination assays that have proved to be the fastest and most suitable methods of detecting human red blood cell antibodies.\(^4\)

**PATIENTS AND METHODS**

**Samples**

Serum samples were collected from 200 unselected patients with a positive antinuclear antibody (ANA) screen. Connective tissue diseases such as systemic lupus erythematosus (SLE; \(n=71\)), mixed connective tissue disease (\(n=4\)), undifferentiated connective tissue disease (\(n=6\)), systemic sclerosis (\(n=15\)), and primary Sjögren’s syndrome (\(n=20\)) were defined according to the established classification criteria. Other autoimmune diseases (not specified) were found in 76 of the patients, and no diagnosis could be established in eight patients. A total of 40 unselected healthy blood donors served as the negative control group.

**Methods**

**ID-anti-dsDNA antibody test**

**Preparation of dsDNA coated beads**

Human dsDNA obtained from Sigma-Aldrich GmbH (Deisenhofen, Germany) was coated onto red high density polystyrene beads as previously described.\(^5\)

**ID card**

The PaGIA assay format is adapted for the standard methodology and equipment of the ID-Micro Typing system using ID cards with buffer compositions specially adjusted to the requirements of the synthetic particles. The buffer used in the ID-dsDNA antibody test also contained rabbit antihuman antiglobulin serum.

**Test procedure**

Serial dilutions (with phosphate buffered saline) of serum samples (10 \(\mu\)l) and 50 \(\mu\)l volumes of the particle suspension were placed in the reaction chamber of an ID card. After incubation at room temperature for five minutes, the cards were centrifuged for 10 minutes at 85 \(g\) in a standard ID centrifuge and the results were read macroscopically. Positive reactions, as defined by a layer of beads on top of the gel or agglutinated particles dispersed through the gel matrix, indicated the presence of antibodies to dsDNA. In negative reactions, the non-agglutinated beads pass through the gel and form a pellet at the bottom of the gel tube after centrifugation.

**Crithidia luciliae immunofluorescence test (CLIF) and anti-dsDNA ELISA**

A standard indirect immunofluorescence technique was employed using slides prepared with \textit{Crithidia}.

**Statistical analysis**

The data were analysed using the SPSS software package (SPSS, Chicago, III, USA).

**RESULTS**

**Detection of anti-dsDNA antibodies with ID-PaGIA**

Fifty six of the 200 serum samples from ANA positive patients tested positive on ID-PaGIA analysis, while no positive reactions were seen in the 40 healthy blood donors. In the

**Abbreviations:** ANA, antinuclear antibodies; CLIF, \textit{Crithidia luciliae} immunofluorescence test; ELISA, enzyme linked immunosorbent assay; PaGIA, particle gel immunoassay; SLE, systemic lupus erythematosus.
patients with SLE, 45/71 samples tested positive for anti-dsDNA antibodies. Thus the ID-PaGIA had a sensitivity of 63% for SLE. Of the non-SLE patients, 11/129 (9%) tested positive in the ID-PaGIA. Eight of these patients had some type of connective tissue disease. Thus the specificity of the agglutination assay for SLE was 92% in our study group, considering the disease controls.

The ID-PaGIA positive sera showed a variable extent of agglutination, from weakly positive to strongly positive (fig 1). The antibody titres for SLE ranged from 1/1 to 1/1064 (median 1/8). The antibody titres in the non-SLE group were generally low and did not exceed 1/2.

Comparison of ID-PaGIA with CLIF and ELISA

For the 200 ANA positive patients, the number of positive test results was 45 by CLIF and 69 by ELISA. The three anti-dsDNA assays had 87% overall agreement. Table 1 shows that ID-PaGIA and ELISA had the highest rate of positive reactions, whereas ID-PaGIA and CLIF had the highest concordance. According to the $\chi^2$ test, the correlation between the three anti-dsDNA assays was significant ($p<0.0001$).

In the SLE group, the overall agreement of the three anti-dsDNA antibody detection tests increased slightly (to 89%) and the highest concordance (90%) was seen between ID-PaGIA and CLIF. Again, the statistical analysis showed a significant degree of correlation between all three anti-dsDNA tests ($p<0.0001$; table 1).

In our study the specificity of CLIF and ELISA for SLE was found to be 99% and 84%, respectively. The sensitivities of raised anti-dsDNA levels for SLE were 62% (CLIF) and 70% (ELISA).

Figure 2 shows the relation between the signals obtained by CLIF or ELISA and ID-PaGIA. Although a few samples showed discordant results, there was a highly significant correlation between the agglutination assay and the established anti-dsDNA antibody detection (ELISA: $r=0.73$, $p<0.0001$, Spearman’s correlation analysis; CLIF: $r=0.81$, $p<0.0001$, Spearman’s correlation analysis).

Table 1  Determination of anti-dsDNA antibodies in 200 ANA positive sera by three different methods: Crithidia luciliae immunofluorescence test (CLIF), anti-dsDNA ELISA (ELISA), and the particle gel immunoassay (ID-PaGIA)

<table>
<thead>
<tr>
<th>Test performed</th>
<th>Diagnosis</th>
<th>Number of samples</th>
<th>Overall agreement No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neg/neg</td>
<td>Pos/pos</td>
</tr>
<tr>
<td>ID-PaGIA/ELISA</td>
<td>Total</td>
<td>127</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>ID-PaGIA/CLIF</td>
<td>Total</td>
<td>141</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
<td>23</td>
<td>41</td>
</tr>
<tr>
<td>ELISA/CLIF</td>
<td>Total</td>
<td>130</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
<td>20</td>
<td>43</td>
</tr>
</tbody>
</table>

Neg, negative; pos, positive; SLE, systemic lupus erythematosus.

$^*$ $\chi^2=112$, $p<0.0001$; $^\dagger$ $\chi^2=39$, $p<0.0001$; $^\ddagger$ $\chi^2=123$, $p<0.0001$; $^\|$ $\chi^2=45$, $p<0.0001$; $^\|$ $\chi^2=103$, $p<0.0001$; $^\|$ $\chi^2=37$, $p<0.0001$.

Figure 1 Different aspects of agglutination reactions of dsDNA coated polystyrene beads with the gel matrix. Serum samples containing anti-dsDNA antibodies (1 to 4): strongly positive reactions (1 and 2) and weakly positive reactions (3 and 4); normal serum sample (5); in the absence of serum (6).
DISCUSSION

In this study, ID-PaGIA proved to be a sensitive and specific anti-dsDNA antibody detection method. There was a strong correlation between the positive results of the ID-PaGIA test and those of the two standard anti-dsDNA assays. The high concordance between ID-PaGIA and the other two anti-dsDNA tests indicates that the new agglutination assay detects dsDNA antibodies of high and low avidity. It must be emphasised that the exact sensitivity and specificity of the ID-PaGIA cannot be estimated from the present data because about one third of unselected patients with SLE can be expected to have a negative anti-dsDNA antibody test, and most of the patients with SLE in this study had undergone treatment before testing.

It is well known that various anti-dsDNA detection methods lead to partially discrepant results. The three assays compared in this study differed in their technical procedure, the source of antigen, as well as the subclass specificity of the secondary antibodies used. Therefore, it is difficult to determine which variables were responsible for the discrepancies between the ID-PaGIA and the other two tests. The high specificity of the ID-PaGIA may, in part, be due to the special care that was taken in preparation of the beads. The results of the new test system were reproducible using the same beads for at least three months.

The gel test in conjunction with the beads technology appears to be a sensitive and specific method for detecting antibodies against dsDNA. It provides clear and stable reactions that improve the interpretation of the results. In addition, the flexible and simple test procedure allows rapid testing of single serum samples as well as of larger numbers of serum samples. Therefore, this test might be useful as a rapid screening assay.

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Accepted 26 October 2002

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Rapid detection of autoantibodies to dsDNA with the particle gel immunoassay (ID-PaGIA)

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doi: 10.1136/ard.61.4.367