INTENDED USE
The Hemagen DNA Kit (catalog # 6407) is intended for the qualitative and semi-quantitative determination of antibodies to double-stranded deoxyribonucleic acid (dDNA or dsDNA) in human serum. For in vitro diagnostic use.

SUMMARY AND EXPLANATION OF THE TEST
Antibodies that react with double-stranded DNA are indicative of systemic lupus erythematosus (SLE). Such antibodies often appear spontaneously in the serum of patients with SLE. Formation of dDNA/anti-dDNA immune complexes is thought to be of primary importance in the pathogenesis of SLE.1 The measurement of antibody to dDNA is used as a marker in the diagnosis and management of SLE. Some studies have suggested a correlation between high dDNA antibody titer and lupus renal disease.2 In addition, serum measurement of anti-dNA levels have been valuable in assessing disease activity and monitoring response to therapy.3

Many techniques for anti-dDNA detection are subject to interference. For example, DNA can bind to non-antibody serum proteins such as IgG under certain conditions. Systems that measure changes in turbidity, the viscosity of antigen-antibody complexes, or the absorbance of a color reaction when bound to anti-dDNA with no detectable interference from antibodies to single-stranded nucleic acids.4

A sensitive and specific assay for anti-dDNA is important for the proper diagnosis of SLE patients. Although a number of techniques are available, the sensitivity, specificity, and reproducibility of reagents, criteria and performance of these methods vary considerably.5 The Hemagen DNA Kit employs the extremely sensitive technique of passive hemagglutination together with advanced methods of cell fixation and antigen absorption. The result is a test kit which is easy to use and which gives rapid, reliable results at a high level of sensitivity and specificity.

TEST PRINCIPLE
In 1951 Boyden described a variety of protein antigens to the surfaces of mammalian red blood cells. He was able to demonstrate hemagglutination in the presence of the corresponding antibodies. Sharp et al. 1 first applied this technique to the detection and quantitation of antibody to a nuclear antigen, at Hemagen, the use of human erythrocytes and new preparative techniques has led to the production of a single standardised reagent with improved reproducibility of hemagglutination tests. Scott et al. 6 indicate that for certain applications, hemagglutination is a simpler, more rapid alternative to double antibody radioimmunoassay.7

This test is based on the agglutination of specifically sensitized human erythrocytes by patient serum containing antibodies to dDNA. In the presence of corresponding antibody, the red cells agglutinate and form a smooth mat on the bottom of the well. This hemagglutination activity of the antibody, if the erythrocytes form a distinct bottom in the bottom of the V-shaped test well.

REAGENTS
The following reagents are included in the Hemagen DNA Kit:

DNA Cells
Fixed and lyophilized human erythrocytes (O-negative) sensitized with dDNA antigen from calf thymus or deer testes, marked on label. Allow cells to rehydrate one hour at room temperature before use. Unused lyophilized DNA Cells must be refrigerated up to 7 days at 2-8 °C. Contains 0.1% sodium azide as a preservative.

Positive Control
Lyophilized human serum with 0.1% sodium azide as a preservative. Reconstitute with 0.3 mL Cell Diluent at least 30 minutes before use. Does not agglutinate DNA Cells. Stable at 2-8 °C for 30 days. Aliquots may be stored frozen at -20 °C for 6 months.

Negative Control
Lyophilized human serum with 0.1% sodium azide as a preservative. Reconstitute with 0.3 mL Cell Diluent at least 30 minutes before use. Does not agglutinate DNA Cells. Stable at 2-8 °C for 30 days. Aliquots may be stored frozen at -20 °C for 6 months.

TEST PROCEDURE
1. Mark off the wells of a V-well microplate in groups of two. Each two-well group will be used for one sample. The two-well groups will be used for each sample.
2. Deliver one free-falling drop of Serum Diluent to the second well of each two-well group.
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4. Deliver one free-falling drop of Serum Diluent to the second well of each two-well group.
5. Deliver one free-falling drop of Serum Diluent to the second well of each two-well group.
6. Repeat step 5 for each sample and control.
7. Reassess the DNA Cell reagent suspension by gently swirling the vial (do not vortex). Using the tuberculin syringe and its blunt dropping needle, add 50 µL of dDNA suspension to each well. Verify the free-falling drop of reagent to each well. Gently swirl vertically.
8. Mix the contents of the wells by gently tapping each side of the plate against a flat surface (do not vortex). Then, mix the contents of the wells by horizontal shaking for 90 minutes. Read the plate promptly, and transfer the results as indicated under INTERPRETATION OF RESULTS.

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Figures
1. See the statement of confirmation of antibody titer. The titer is the greatest dilution giving a positive reading.
2. Note some of the sera may occur in positive wells. In these cases, a comparison of the absorbance at A410 and the size of the negative control.
3. See EXPECTED VALUES for additional information.

LIMITATIONS OF THE TEST
1. The positive and negative control sera included with this kit should be used in each run to confirm the efficacy of the cell reagents.
2. The two dispensing syringes used to dispense Hemagen reagents should be held vertically, in order assure proper delivery to the test wells. Under no circumstances should the syringes be allowed to touch the bottom of the plate or other hard surface.
3. Use of the cell reagents during the first hour following reconstitution can result in agglutination and false positive readings.
4. Positivity for antibody to dDNA cannot be established if there are no detectable autoantibodies. The Hemagen DNA Kit uses a nDNA preparation which binds to anti-dDNA with no detectable interference from antibodies to single-stranded nuclei acids.

PRECAUTIONS
The procedure for performing titrations is similar to that for screening. In performing the serial dilutions of step 5 above, continue transferring 50 µL from well 2 to well 3 and so forth, on to well 8. Then discard 50 µL from well 8 along with the pipet tip.

INTERPRETATION OF RESULTS
Results are obtained by examining the settling pattern of the cell reagent. The following criteria should be used:

A. Negative: Reaction. In the absence of antibodies to dDNA, the red cells will settle to the bottom of the well, forming a compact button in the clear medium.
B. Positive: Reaction. Agglutination of cells by antibodies present in the patient or control sera results in the formation of a smooth mat covering the bottom of the well. In 2-well screen tests, a positive result at either of the two sera should give a reading at A410 of 0.75 or more. The titer is the greatest dilution giving a positive reading.
C. Titration

<table>
<thead>
<tr>
<th>Serum</th>
<th>Dilution</th>
<th>Titer</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos. Serum 1</td>
<td>30</td>
<td>1:80</td>
<td>7.8</td>
</tr>
<tr>
<td>Pos. Serum 2</td>
<td>27</td>
<td>1:320</td>
<td>5.8</td>
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Inter-run precision was assessed by assaying one lot of DNA positive control serum 46 times on 13 different days.

PERFORMANCE CHARACTERISTICS
A. Specificity
To demonstrate antigen specificity, the Hemagen DNA Cell reagent was tested against secondary reference sera (primary reference sera were obtained from the Centers for Disease Control, Atlanta, Georgia) known to contain autoantibodies associated with connective tissue diseases. Results were negative for each of the sera containing rheumatoid factor and antibodies to S-A (Ro), S-B (La), SSA/Ro, SIM, and centromeric antigen.

B. Precision
Intra-run precision was assessed by assaying two sera known to be positive for antibody to dDNA.

<table>
<thead>
<tr>
<th>Serum</th>
<th>CV (%)</th>
</tr>
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<tbody>
<tr>
<td>Pos. Control 1</td>
<td>8.4</td>
</tr>
</tbody>
</table>

C. Comparative Data
Sera from 105 randomly chosen normal human sera express negative results when tested with the Hemagen DNA Kit. Samples from 72 SLE patients were tested with the Hemagen DNA Kit and a C1q/Ro/SSA reactivity assay. Results are shown below in Table 1. Correlation with the C1q/Wolff and the Hemagen methods was 92%. Two of the three sera which were positive by passive hemagglutination and negative by immunofluorescence had low titers of 1:20.

Table 1 Comparison of the C1q/Wolff and the Hemagen methods

<table>
<thead>
<tr>
<th>Model</th>
<th>Number of Assays</th>
<th>Positive Results</th>
<th>Negative Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q/Wolff</td>
<td>34: 1032-1035</td>
<td>34</td>
<td>1032</td>
</tr>
<tr>
<td>Hemagen</td>
<td>34</td>
<td>1032</td>
<td>1035</td>
</tr>
</tbody>
</table>

REFERENCES


