Evaluation of the RheumaStrip ANA Profile Test: a Rapid Test Strip Procedure for Simultaneously Determining Antibodies to Autoantigens U1-Ribonucleoprotein (U1-RNP), Sm, SS-A/Ro, SS-B/La, and to Native DNA

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The "LipoGen RheumaStrip™ ANA Profile" test method (LipoGen, Inc.) is a new assay format for autoantibody detection in which recombinant autoantigens are used. This enzyme immunoassay, in test-strip format, detects antibodies to autoantigens U1-ribonucleoprotein (U1-RNP), Sm, SS-A/Ro, SS-B/La, and to native DNA (nDNA). We evaluated 200 antinuclear antibody (ANA)-positive and 100 ANA-negative sera for the presence of antibodies to U1-RNP, Sm, SS-A/Ro, SS-B/La, and nDNA by the new test-strip procedure. These data correlated well with those obtained with native or recombinant autoantigens. ANA sensitivity and specificity of the ANA Profile method as compared with those of established procedures were respectively as follows: 89.8% and 98.8% for U1-RNP, 86.4% and 95.3% for Sm, 97.9% and 97.3% for SS-A/Ro, 98.3% and 86.3% for SS-B/La, and 97.5% and 93.1% for nDNA. Agreement between the ANA Profile test and these other test methodologies ranged from 88.7% for the SS-B/La test to 97.2% for the U1-RNP test. This new test procedure substantially decreases the time and effort required to perform these assays. Total hands-on time and overall assay time were decreased by 72% and 97%, respectively.

Additional Keyphrases: antinuclear antibodies, connective tissue disease, immunodiffusion and immunofluorescence compared

The serological characterization of autoantibodies to nuclear antigens is of major importance in the diagnosis of connective tissue diseases. Distinct autoantibody profiles identified in patients with connective tissue diseases can have diagnostic as well as prognostic implications (1–3). Currently, antigen-specific antinuclear antibodies (ANA) are routinely identified by the Ouchterlony double immunodiffusion technique (4). This technique, although relatively simple to perform, has technical limitations with respect to assay sensitivity, interlaboratory standardization, and subjectivity of results. During the past several years, enzyme-linked immunosorbent assays (ELISAs), performed with antigens isolated from natural tissue sources, increasingly have been used to detect antinuclear antibodies (5–7). The sensitivity and specificity of these newer methods have been excellent, as demonstrated by correlation with existing methods and by the increased rate of autoantibody detection in patients with rheumatic diseases. Moreover, recent studies with autoantigens produced by recombinant DNA technology in microwell ELISA procedures have also shown excellent specificity and sensitivity (8–10).

The LipoGen RheumaStrip™ ANA Profile test (LipoGen, Inc., Knoxville, TN; also distributed as the r-CLONE® ANA Profile test by Access Medical Systems, Inc., Branford, CT) is a membrane enzyme immunoassay for the detection of ANA to U1-ribonucleoprotein (U1-RNP), Sm, SS-A/Ro, SS-B/La, and native DNA (nDNA). This test method (hereinafter called the "Profile" test) is based on detection of the reaction between ANA and the above-mentioned autoantigens on a nitrocellulose membrane. The U1-RNP, SS-A/Ro, and SS-B/La autoantigen substrates are purified proteins isolated from genetically engineered bacterial strains (11). The complementary DNA (cDNA) encoding each of these protein autoantigens were cloned from a human cDNA library. These cloned protein autoantigens are identical to the human autoantigens in their primary amino acid structure. The Sm and nDNA antigens used in the Profile test are derived from natural tissue sources.

In this clinical evaluation, we compared the performance of the Profile test-strip method with that of conventional diagnostic methods currently in use for ANA characterization. The comparison methods used in this study—Ouchterlony double immunodiffusion for U1-RNP, Sm, SS-A/Ro, and SS-B/La and indirect immunofluorescence (IIF) on Crithidia luciliae for nDNA—were in-house procedures largely developed and standardized by us.1

Materials and Methods

Serum Specimens

Patients' serum samples were obtained from the Immunology Laboratory at Maryland Medical Laboratory, Inc. The sera tested in this study were from selected patients on whom previous ANA tests had been performed as requested by physicians. We studied 200 ANA-positive and 100 ANA-negative serum specimens. The ANA-positive specimens were selected on the basis of a positive test result for autoantibodies to one or more of the following: U1-RNP, Sm, SS-A/Ro, SS-B/La, and nDNA. We selected 49 individuals positive for anti-U1-RNP, 22 positive for anti-Sm, 141 positive for anti-SS-A/Ro, 59 positive for anti-SS-B/La, and 40 positive for anti-nDNA. All specimens were initially screened by IIF on HEp-2 substrate (Biodes Enterprises, Ltd., Park Ridge, IL) and with cryostat sections of mouse kidney/stomach cells (SciMedx, Danville, NJ) fixed in acetone for 4 min at 4°C. Each of these specimens was also screened by either counterimmunoelectrophoresis (CIE) for the presence of precipitins to extractable nuclear antigens or by IIF for antibodies to nDNA. All

1 Maryland Medical Laboratory, Inc., 1901 Sulphur Springs Road, Baltimore, MD 21227.
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3 Nonstandard abbreviations: ANA, antinuclear antibodies; SS-A/Ro, Sjögren's syndrome antigen A; SS-B/La, Sjögren's syndrome antigen B; ELISA, enzyme-linked immunosorbent assay; RNP, ribonucleoprotein; nDNA, native DNA; IIF, indirect immunofluorescence; CIE, counterimmunoelectrophoresis; and IP, immunoprecipitation.
4 Received October 6, 1989; accepted February 26, 1990.
CIE-positive specimens were further characterized by the Ouchterlony technique (4).

To estimate the autoantibody prevalence with the Profile test in various connective tissue diseases, we requested diagnoses from the referring physicians, and obtained these for 117 of the 200 ANA-positive patients. Of these, 95 patients had a diagnosis of either a differentiated or an undifferentiated connective tissue disease. The differentiated connective tissue diseases with four or more patients per group included eight with rheumatoid arthritis, 54 with systemic lupus erythematosus, seven with Sjögren’s syndrome, and four with systemic lupus erythematosus overlap that involved mixed connective tissue disease or Sjögren’s syndrome. Because a total of only nine patients were diagnosed with drug-induced lupus, mixed connective tissue disease, vasculitis, progressive systemic sclerosis, thrombocytopenia, eosinophilic fasciitis, and dermatomyositis (one patient with each diagnosis except for two patients with mixed connective tissue disease), these were grouped together under the heading “Other CTD.” Twenty-two patients had nonconnective tissue disease diagnoses such as allergy, anemia, bacterial/viral infection, sinusitis, neoplasm, or peripheral neuropathy.

Procedures

Counterimmunoelectrophoresis screening procedure. CIE plates and antigen extracts were obtained from Integrated Diagnostics, Inc., Baltimore, MD. CIE plates were rehydrated in de-ionized water for 1 h, then soaked in pH 7.4 phosphate-buffered isotonic saline (per liter, 10 mmol of PO₄ and 150 mmol of NaCl) for at least 15 min but not longer than 30 min. Antigen solutions were loaded onto the cathode side and serum specimens were loaded on the anode side. The CIE plates were then run at 100 V for 10 min with Tris–barbital buffer (per liter, 50 mmol of Tris, 50 mmol of sodium barbital, and 14 mmol of barbital), pH 8.8. Reactions were read after an overnight incubation at room temperature. Controls for each ANA specificity were run with each plate. All sera were initially screened by CIE before Ouchterlony testing, giving the superior sensitivity of CIE.

Ouchterlony immunodiffusion test procedures for Sm, RNP, SS-A/Ro, and SS-B/La antibodies. We used an Ouchterlony double-immunodiffusion procedure to detect antibodies to U1-RNP, Sm, SS-A/Ro, and SS-B/La by rehydrating agarose gel sheets (Integrated Diagnostics) as above for CIE plates and then incubating them overnight at room temperature. Results on the gel plates were read after 24 and 48 h. After 48 h the plates were washed, dried, and then stained with Coomasie Brilliant Blue to enhance visualization of the precipitation reaction. Antigen extracts from rabbit thymus, calf thymus, and human spleen were obtained from Integrated Diagnostics. All in-house control sera were referenced to the ANA prototype sera obtained from the Centers for Disease Control, Atlanta, GA.

Crithidia lucilae indirect immunofluorescence procedure for anti-nDNA and for anti-histones. Antibodies to nDNA were detected by IIF on C. lucilae substrate (SciMedx). Sera were screened diluted 10-fold in phosphate-buffered isotonic saline. The procedure was performed by incubating the substrate slide with serum for 30 min, followed by a 15-min wash in phosphate-buffered isotonic saline. The slides were then incubated with polyvalent antibody to gamma-globulin conjugated to fluorescein isothiocyanate (F/P5.6; American Qualex, La Mirada, CA) for 30 min, followed by a 10-min wash, and then covered with coverslips. Fluorescence staining patterns were identified with a fluorescence microscope.

To test for histone autoantibodies, we modified the above procedure by extracting the C. lucilae substrate with acid. We incubated the substrate slide in 0.1 mol/L HCl for 20 min at room temperature, rinsed it in phosphate-buffered isotonic saline for 15 min and then performed the IIF procedure as above for the untreated substrate slide. Specimens positive for histone antibodies were reported as those that demonstrated positive staining on the untreated slide but negative staining on the acid-extracted slide. Likewise, specimens that gave positive staining on both the untreated as well as treated substrate were reported as positive for nDNA antibodies.

RheumaStrip ANA Profile test procedure. We performed this test procedure according to directions supplied in the manufacturer’s package insert (12). All materials required for performing the procedure—including plastic serum pipettes for the test specimen, measuring cups, and reagents—were supplied in the test kit. In addition, the diluent, conjugate, and substrate solutions were supplied ready-to-use in plastic dropper bottles; the wash was supplied as a 10-fold concentrate, which we diluted in de-ionized water before use. In brief, we diluted the test specimens by adding 1 drop (30 μL) of serum to a plastic measuring cup and then filling with diluent to the upper fill line to give approximately a 60-fold dilution. A membrane test strip enclosed in a plastic housing was then inserted into the measuring cup containing the sample and incubated for 45 min on a hematology rocker. We then rinsed the test strip with wash solution and incubated it for an additional 20 min with biotinylated Protein A/streptavidin–horseradish peroxidase detection reagent. After an additional wash step to remove unbound conjugate, we then developed the strip for 6 min in 4-chloro-1-naphthol/peroxide substrate. The color reaction on the test strip was scored by visual matching against a color reference card. Each Profile test strip contained an internal procedural control (labeled PC on the test strip) and a negative control (labeled NC on the test strip) spot to validate the assay procedure.

Immunoprecipitation procedure. Test samples, selected on the basis of interassay discrepancies for RNP, Sm, SS-A/Ro, and (or) SS-B/La, were further evaluated by an immunoprecipitation procedure as previously described by Forman et al. (13), in which immunoprecipitated RNA molecules were detected in polyacrylamide gels by using a silver-staining procedure. In brief, serum specimens were added to Protein A–Sepharose beads (Sigma Chemical Co., St. Louis, MO) to immobilize IgG in the test sample. The beads were then washed, incubated with an unlabeled HeLa cell lysate, and washed again to remove any unbound material. Bound autoantibodies were extracted in phenol/chloroform/isooamyl alcohol (50/50/1 by vol) containing 1 mL of hydroxyquinoline per liter. We combined the aqueous phase, containing the extracted nucleic acid, with cold ethanol to precipitate nucleic acids. The extracted RNA was then electrophoresed on a 7 mol/L urea–100 g/L polyacrylamide gel and made visible with a silver stain (BioRad, Richmond, CA). The banding pattern of immunoprecipitated RNAs on these gels was compared with reference reactions from known positive controls and with that from a total HeLa lysate run at the same time.
Results

Assay Procedure

The Profile test procedure was easily performed in batch runs of up to 20 test samples. The test procedure as detailed in the kit insert was quickly learned in one or two trial runs. In reading the developed test strip, we found it important to avoid over-scoring the color test spot reactions. This was most evident for those test reactions demonstrating very weak color development in the range of 0 to 1+. In general, after the initial learning period, there was excellent agreement among technologists in scoring the test-strip reactions.

The Profile test procedure could be performed much more quickly than our current gel-immunodiffusion methods because of its much shorter incubation periods. Positive reportable results by gel immunodiffusion usually require approximately 3.5–4 days of total assay time, although negative results can be reported in 24–48 h. (This period includes the initial screening for precipitins by CIE and follow-up testing by Ouchterlony immunodiffusion to determine antibody specificity.) The Profile test procedure, however, required about 2.5 h to perform batch runs of 10–20 specimens. In terms of total hands-on time needed to test between 10 and 20 samples, the gel immunodiffusion procedures required 90 min if positive and 45 min if negative. In comparison, the Profile procedure required 25 min for either positive or negative samples.

Assay Performance

The percent agreement between our Ouchterlony double-immunodiffusion method and the Profile test was 97.3% for U1-RNP, 94.7% for Sm, 88.7% for SS-B/La, and 93.3% for SS-A/Ro. Likewise, the percent agreement between the C. luciliae IIF procedure and the Profile test for nDNA was 93.6%. Assay sensitivity and specificity by autoantibody are summarized in Table 1. Calculated sensitivity for the five autoantibody tests ranged from 86.4% to 98.3%, with specificities ranging from 86.3% to 98.8%. The Profile assay for nDNA, for example, was 97.5% as sensitive and 93.1% as specific as the C. luciliae IIF method.

In our panel of 100 ANA-negative specimens, five samples tested weakly positive (scores ranged from 1+ to 2+) for SS-A/Ro by the Profile test method only; no other positive reactions were identified by either test method. Within the panel of 200 ANA-positive sera evaluated for each of the five test autoantibodies (1000 total autoantibody determinations), 97 autoantibody results (in 72 test specimens) differed between the two methodologies. This represents a discrepancy rate in ANA-positives of 9.7% and 6.8% for all test specimens in the study. The number of test results in disagreement between the Profile test and our currently used methods were as follows: U1-RNP = 8, Sm = 16, SS-A/Ro = 20; SS-B/La = 34; and nDNA = 19. Table 2 summarizes the reaction scores for both the discrepant test results and the test results in agreement. Thirteen test results (14% of those results in disagreement) were scored as positive by our current test methodologies, but negative by the Profile test. The greatest number of these discrepant reactions (five of 13) were for U1-RNP. Slightly more than 50% of the reactions in dispute were scored in the Profile test as weakly positive (1+); only 10% of the results not in agreement were strongly positive, with scores of 3+ or greater.

We retested by an enhanced Ouchterlony technique the samples for which the test methods gave different autoantibody reaction results for U1-RNP, Sm, SS-A/Ro, and SS-B/La. Antigen wells were doubly loaded to increase assay sensitivity. Test samples in which the results remained discrepant were then further evaluated by an immunoprecipitation (IP) procedure against a lysate of total HeLa cells. The IP data are presented in Table 3 (note that not all discrepant specimens were tested by IP, owing to insufficient sample volume). The IP analysis, performed on six of eight anti-U1-RNP sera, confirmed the Profile test result in three of six cases. Likewise, IP analysis confirmed Profile test results for eight of 13 discrepant Sm reactions, three of 17 SS-A/Ro reactions, and 14 of 31 SS-B/La reactions. The Profile result was confirmed for 28 of 65 discrepant specimens further analyzed by IP of RNA. In 24 of the 28 IP-confirmed test results, the Profile test detected additional antibodies not previously detected by the Ouchterlony procedure, whereas in the other four instances, the Profile test proved to be more specific than the Ouchterlony procedure.

In our initial testing for nDNA autoantibodies by C. luciliae IIF, 77 positive reactions were identified. However, 37 of these were subsequently found to be false-positive reactions when the substrate was extracted with acid to remove histone-like proteins. Only 40 of the original 77 nDNA reactions were therefore confirmed to be true-positives with the modified C. luciliae IIF assay. Eleven of the 37 sera giving false-positive reactions to nDNA in the C. luciliae IIF assay also tested positive for nDNA in the Profile test procedure. The Profile test scores for these apparently false-positive anti-nDNA reactions ranged from 1+ to 3+, although the majority (61%) were scored as weakly positive (1+).

Autoantibody Prevalence by Clinical Diagnosis

Although the number of patients in this study was

Table 1. Sensitivity and Specificity of RheumaStrip
ANA Profile Assay in Comparison with Ouchterlony
Gel-Diffusion* and C. luciliae IIFi Methods

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity, % (no. pos./total pos.)</th>
<th>Specificity, % (no. neg./total neg.)</th>
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</thead>
<tbody>
<tr>
<td>U1-RNP</td>
<td>89.8 (44/49)</td>
<td>96.8 (248/251)</td>
</tr>
<tr>
<td>Sm</td>
<td>86.4 (19/22)</td>
<td>95.3 (265/278)</td>
</tr>
<tr>
<td>SS-A/Ro</td>
<td>97.9 (136/141)</td>
<td>89.3 (142/159)</td>
</tr>
<tr>
<td>SS-B/La</td>
<td>98.3 (58/59)</td>
<td>86.3 (208/241)</td>
</tr>
<tr>
<td>nDNA</td>
<td>97.5 (38/40)</td>
<td>93.1 (241/259)</td>
</tr>
</tbody>
</table>

* Ouchterlony gel-diffusion method used to detect ANA specific for U1-RNP, Sm, SS-A/Ro, and SS-B/La. i C. luciliae IIF used to detect antibodies to nDNA.

Table 2. Distribution of RheumaStrip ANA Profile Test Scores

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>U1-RNP</td>
<td>5(248)</td>
<td>3(7)</td>
<td>0(3)</td>
<td>0(13)</td>
<td>0(21)</td>
</tr>
<tr>
<td>Sm</td>
<td>3(265)</td>
<td>9(0)</td>
<td>3(5)</td>
<td>1(6)</td>
<td>0(8)</td>
</tr>
<tr>
<td>SS-A/Ro</td>
<td>3(142)</td>
<td>15(31)</td>
<td>1(34)</td>
<td>1(45)</td>
<td>0(28)</td>
</tr>
<tr>
<td>SS-B/La</td>
<td>1(208)</td>
<td>15(5)</td>
<td>12(10)</td>
<td>4(12)</td>
<td>2(31)</td>
</tr>
<tr>
<td>nDNA</td>
<td>1(241)</td>
<td>11(9)</td>
<td>4(17)</td>
<td>3(12)</td>
<td>0(1)</td>
</tr>
</tbody>
</table>

Number of specimens with results not in agreement with comparison methods. o Number of specimens with results in agreement with comparison methods.
Table 3. Immunoprecipitation Analysis of Sera with Autoantibody Specificities Not In Agreement between Test Methods

<table>
<thead>
<tr>
<th>No. of reactions in disagreement</th>
<th>Test results confirmed by IP analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive*</td>
</tr>
<tr>
<td>U1-RNP</td>
<td>39 (2)</td>
</tr>
<tr>
<td>Sm</td>
<td>13 (11)</td>
</tr>
<tr>
<td>SS-A/Ro</td>
<td>17 (15)</td>
</tr>
<tr>
<td>SS-B/La</td>
<td>33 (30)</td>
</tr>
</tbody>
</table>

* Scored as positive or negative by the RheumaStrip ANA Profile test. Total number of reactions not in agreement. Actual number of specimens analyzed by IP. Two of four U1-RNP-negative reactions were confirmed negative by IP; one additional reaction was found to be Sm positive and therefore was indeterminate for U1-RNP by IP analysis. Twelve of 17 positive U1-RNP reactions were scored as weakly positive (1+) in the ANA Profile procedure.

Discussion

Gel-immunodiffusion analysis, used to detect and characterize autoantibodies in clinical specimens, is limited in both speed and assay sensitivity by the requirement for precipitin formation (14). Also, gel immunodiffusion is generally recognized to be relatively insensitive to IgM antibodies, which diffuse more slowly through the gel matrix than do IgG antibodies. Although CIE does have better sensitivity and specificity than Ouchterlony immunodiffusion, CIE too is limited by the fact that not all antibody–antigen systems will produce precipitin reactions. Furthermore, interpretation of results from these gel systems is often difficult in the presence of multiple precipitin reactions.

To overcome these technical limitations, investigators have developed over the past several years procedures involving hemaggutination (15–17), radioimmunoassay (18), and enzyme immunoassays (5–7). Although these assay methods have the potential for much greater sensitivity than procedures that rely upon formation of precipitin bands, they can in some instances suffer from poor discrimination (reduced specificity) because of low purity of the autoantigen substrate. As a means of circumventing this antigen substrate limitation, immunoblotting procedures with crude nuclear antigen or whole-cell lysates have been developed that show good specificity with excellent sensitivity (14, 19, 20). Immunoblotting has been shown to be up to 500 times more sensitive than immunodiffusion (19). Because of technical requirements, however, this method has remained for the most part a research tool in academic settings. Likewise, the analysis for autoantibodies with radiolabeled cell lysates (e.g., 32P-labeled HeLa cells) was shown to be 100 times more sensitive than immunodiffusion (18). The development of improved diagnostic procedures has recently been made possible by the availability of recombinant autoantigens. Since the first report in 1986 on the molecular cloning of SS-B/La (23), there have been several additional reports on the cloning of SS-A/Ro (24), U1-RNP (25, 26), Sm (27), Sc(70) (28), and the centromeric protein CENP-B (29). The use of these recombinant proteins in microwell ELISA and in dot-immunobinding procedures has shown enhanced performance with excellent sensitivity and specificity (8, 9, 29).

In light of these procedural limitations on autoantibody detection with gel immunodiffusion, a better method would be one that could utilize the strengths of enzyme immunoassay technology. This would also be a rapid test procedure, possessing excellent sensitivity and specificity, but without any additional requirement of crude antigen fractionation such as is required in immunoblotting techniques. The development of improved diagnostic procedures has recently been made possible by the availability of recombinant autoantigens. Since the first report in 1986 on the molecular cloning of SS-B/La (23), there have been several additional reports on the cloning of SS-A/Ro (24), U1-RNP (25, 26), Sm (27), Sc(70) (28), and the centromeric protein CENP-B (29). The use of these recombinant proteins in microwell ELISA and in dot-immunobinding procedures has shown enhanced performance with excellent sensitivity and specificity (8, 9, 29).

Our evaluation of the RheumaStrip ANA Profile test procedures demonstrated excellent performance characteristics, in comparison with our gel-immunodiffusion and C. luciliae IIF procedures currently in use. Furthermore, we found this autoantibody detection method to be easier to use than our current assay procedures. The amount of direct technologists’ time required to perform these tests as
well as the total elapsed assay time required to report a result was substantially shortened. Most importantly, the Profile test detected antibodies to U1-RNP, Sm, SS-A/Ro, and SS-B/La, which correlated well with results by our existing Ouchterlony double-immunodiffusion techniques, although some differences were noted. The Profile method in general appeared to be more sensitive in its ability to detect antibodies to Sm, SS-A/Ro, and SS-B/La.

We found the test strip immunodot technology to be user-friendly, and relatively easily learned. Although the Profile test kit was designed to be performed in batches of one to six tests per run, we found it possible in this study to easily accommodate 20 or more tests per batch. As for the visual interpretation of the color test spot reaction, we found it important for the user to accurately differentiate specific test spot color development from nonspecific membrane background. Failure to accurately differentiate background reactions from weakly positive reactions could lead to false-positive results. However, visual scoring was easily mastered after the first few runs.

Of those differences in antibody detection observed between the two test methodologies, 86% (Table 2) were due to additional autoantibodies detected by the Profile test but not by immunodiffusion. Confirmatory testing with the IP technique on seven of 13 specimens, which tested positive by immunodiffusion only, demonstrated that four were in fact negative for the particular autoantibody specificity. Immunoprecipitation analysis performed with silver staining to detect RNAs has significantly better sensitivity than does gel immunodiffusion (2). Even so, the silver-staining procedure used in this study to detect antibodies to U1-RNP, Sm, SS-A/Ro, and SS-B/La is less sensitive than IP with 32P-labeled cell lysates for detecting antibodies to SS-A/Ro and SS-B/La. Therefore, some fraction of discrepant reactions for SS-B/La not confirmed by the present technique might be detected by the more sensitive 32P method. Also, some of the weaker discrepant reactions detected by the Profile test might represent nonspecific background reactions.

Antibodies to nDNA are usually considered to be a specific diagnostic finding, limited to patients with systemic lupus erythematosus. However, differences in the diagnostic methodologies used to detect these antibodies have caused confusion on this issue. Determination of nDNA antibodies by the Farr procedure usually provides the greatest specificity but with less sensitivity than either ELISA or C. luciliae IIF procedures (30). Thus, the prevalence of nDNA antibodies in systemic lupus erythematosus is generally somewhat higher when detected by ELISA or C. luciliae IIF. However, several recent studies have demonstrated positive kinetoplasti binding activity on the C. luciliae IIF substrate by nDNA-negative sera (31, 32). In fact, these nDNA-negative specimens with positive C. luciliae binding were in some cases positive for histone antibodies. The false-positive binding was abolished by using the acid-extraction procedure detailed in this investigation. Likewise, our study casts some doubt on the specificity of even a positive C. luciliae assay result with systemic lupus erythematosus serum. The discrepancies noted between the Profile nDNA test and the C. luciliae IIF procedure used in this study most likely reflect some of these differences noted in previous method comparisons for nDNA antibodies. Additional studies with the Profile nDNA test will be needed to further address the nature of these differences in assay sensitivity and specificity.

Overall, the best agreement between the Profile test and our currently used test methods was for those results with higher Profile test scores (Table 2). Weaker reactions (i.e., 1+ to 2+ scores) were more frequently not detected by the less-sensitive comparison methods. The clinical significance of these differences remains to be elucidated with further studies. Correlation of these previously undetected ANAs with the clinical progression of connective tissue disease should provide further diagnostic insight into rheumatic disease states and associated conditions.

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References

Rapid and Simultaneous Determination of Lactulose and Mannitol in Urine, by HPLC with Pulsed Amperometric Detection, for Use in Studies of Intestinal Permeability

Simon C. Fleming, Moses S. Kapembwa, Michael F. Laker, Gerald E. Levin, and George E. Griffin

The lactulose/mannitol dual sugar absorption test is a non-invasive test of intestinal permeability. Its widespread use has been limited by the difficulties of analysis for carbohydrates in urine at low concentrations. We describe a “high-pressure” liquid-chromatographic method for determining lactulose and mannitol in urine, in which anion-exchange chromatography and pulsed amperometric detection are used. Sample preparation is simple and fast, and lactulose and mannitol and the internal standards arabinose and cellobiose are well resolved within 15 min. Analytical response of the method is linear to concentrations up to 3 g/L, and one can detect as little as 0.3 mg of lactulose per liter of urine. Analytical recovery was between 90% and 107% for all sugars analyzed, and there was good agreement with results by a gas-chromatographic method (r = 0.993 lactulose, 0.994 mannitol). The method may potentially be applied to the study of other carbohydrates present in biological fluids at low concentrations.

Additional Keyphrases: carbohydrates, chromatography, anion-exchange, arabinose and cellobiose as internal standards

Dual sugar absorption tests have been used to assess the permeability of the small bowel mucosa, in a variety of intestinal diseases (1–3). In these tests, the permeability to nonmetabolizable mono- and disaccharides such as mannitol/lactulose or rhamnose/cellobiose is compared and expressed as the ratio of the concentrations of these saccharides in a timed urine sample. Lactulose/mannitol excretion ratios have been proposed as a useful, noninvasive test for screening and monitoring celiac disease (4).

However, the difficulty in quantifying these urinary sugars has limited the widespread use of the tests. Current methods of carbohydrate determination include thin-layer chromatography, gas-liquid chromatography, and enzymatic analysis. These techniques can be time consuming (5), and samples may require prior derivatization (6, 7). “High-pressure” liquid chromatography (HPLC) with refractive index detection does not achieve the sensitivity or specificity required, and therefore both pre- and post-column derivatization methods have been developed in an attempt to overcome these problems (8, 9).

The introduction of anion-exchange chromatography coupled with pulsed amperometric detection (PAD) (10) has made it feasible to detect carbohydrates electrochemically. The detection system consists of a gold working electrode,