


Values of the U.S. National Reference Serum for Human Antibodies to Native DNA Obtained with Commercial Immunoassays for Anti-DNA in Systemic Lupus Erythematosus

Bernard H. Berne, Kathryn T. Galland, and Richard C. Welton

The Arthritis Foundation and the Centers for Disease Control have recently prepared a “U.S. National Reference Serum” for human antibodies to native DNA. We tested this serum with 13 commercial assays for antibodies to native DNA, to permit comparisons of the values obtained in each test. Titers ranged from 10 to 2560 in Crithidia luciliae immunofluorescence assays. The serum produced 794 int. units/mL in the Cordis ELISA assay, 136 Amersham units/mL in a radioimmunoassay, and 88 FIAAX units in a fluorometric immunoassay. These results can be used for interlaboratory comparisons of differing methodologies for measuring anti-DNA.

The Arthritis Foundation and the Centers for Disease Control have recently developed a "U.S. National Reference Serum" for human antibodies to native DNA and for fluorescent antinuclear antibody (homogeneous/rim pattern.) This preparation is intended to permit workers to standardize their assays with a single reagent (1, 2).

Many commercial assays for antibodies to native DNA are available for the diagnosis and management of systemic lupus erythematosus (3). However, results obtained with these have never been systematically inter-compared with use of a single reference serum.

We therefore tested the U.S. National Reference Serum, using all of the quantitative commercial assays that we knew of. Our values should allow laboratories to evaluate anti-DNA concentrations in their own assays by reference to others.

Materials and Methods

Reference sera. A vial containing a mean of 35.9 mg (dry weight) of the above-mentioned lyophilized U.S. National Reference Serum ANA Human Serum no. 1, cat. no. IS2072, lot 82-007, was obtained from the Arthritis Foundation—Centers for Disease Control Anti-Nuclear Antibody Reference Laboratory, Immunology Branch, CID, Centers for Disease Control, Atlanta, GA 30333 (1, 2). The contents of the vial were reconstituted with 0.5 mL of water, aliquoted, and stored at −70 °C for up to four months.

A positive control serum (human), lot 90842, was obtained from Cordis Laboratories, Inc., Miami, FL 33152. The value assigned to this serum (760 int. units/mL) is traceable to the World Health Organization’s (WHO) 1st International Reference Preparation of Anti-Nuclear-Factor Serum (Homogeneous), 1970, formerly termed the "Proposed Research Stan-

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Cordis has assigned a value of 100 int. units per vial for anti-DNA to the WHO reference serum, to equal the value given by WHO for the preparation's anti-nuclear-factor (8, 9). Each int. unit of the WHO preparation contains 0.186 mg ± 5.8% (dry weight) of the freeze-dried residue of 2.13 mg (2 µL) of pooled human serum (100 int. units per 0.2-mL ampule) (4–7). We made three two-fold dilutions of the Cordis positive control serum to construct standard curves.

We obtained FlAX® calibrator solutions from International Diagnostics Technology (IDT), Santa Clara, CA, 95050. These calibrators were: no. 1, lots 2264037 (151 FlAX anti-DNA units) and 224097 (140 units); no. 2, lot 2273027 (81 units); no. 3, lot 2264047 (29 units); and no. 4, lot 2273037 (0 units). We used the FlAX calibrators to standardize all assays in this study.

Calibrators and positive control sera from other commercial test kits were quantified in their respective assays. In most instances, positive controls for immunofluorescence assays had been prediluted 10-fold by their vendors.

Test kits. All kits were designed for the clinical management of systemic lupus erythematosus and were claimed to detect antibodies to native DNA. We obtained Crithidia luciliae IFA kits from Antibodies Incorporated, Davis, CA 95617; Calbiochem-Behring Corp., La Jolla, CA 92121; BioDx, Denville, NJ 07834; Electro-Nucleo-Logics, Inc., Bethesda, MD 20814; Kallestad Laboratories, Austin, TX 78701; M.A. Bioproducts, Walkerville, MD 21793; FlAX Laboratories, Inc., Springfield, VA 22151; Sigma Chemical Co., St. Louis, MO 63178; and Zeus Scientific, Inc., Ranitan, NJ 08615.

Farr-type radioimmunoassay (RIA) kits were supplied by Amersham Corp., Arlington Heights, IL 60005, and Wampole Laboratories, Cranbury, NJ 08512. We also tested a Farr-type RIA used in our laboratory (10).

Cordis Laboratories provided an enzyme-linked immunosorbent assay (ELISA). IDT supplied a solid-phase FlAX fluorometric immunoassay.

We performed all commercial assays in accordance with the vendors' instructions, using reagents supplied in their kits. Counterstain was used in all immunofluorescence assay tests where provided. Titters in immunofluorescence assay were found by first diluting sera 10- to 640-fold and then two-fold serially.

Calculations. Statistics involving titers found in Crithidia assays were performed by the methods used for antinuclear antibody tests by the Proficiency Testing Laboratory of the Centers for Disease Control and by the WHO (6, 11). The geometric mean titer (XG) was determined by log XG = (Σ log x)/n. The geometric standard deviation (SDG) was found by

\[ \log SD_G = \sqrt{\frac{\Sigma (\log x - \log X_G)^2}{n - 1}} \]

We calculated FlAX units of Crithidia assays by entering the assigned units of at least two FlAX calibrators and the logarithms of calibrator or test titters into a linear regression program on a Hewlett-Packard 9815A calculator. Other calculations were performed as described by vendors' instructions or by standard statistical techniques. Reference curves for Farr assays were drawn manually.

Results

The values for the U.S. National Reference Serum varied widely among the methods tested. The serum was assayed four to nine times by each Crithidia test (Table 1); titers varied by over two orders of magnitude, from 10 to 2560. The SDG of the products ranged from 1.7 to 2.2.

Kits from two different lots were tested in the Antibodies Inc., Bio-Dx, and Electro-Nucleo-Logics Crithidia assays. There were no significant lot-to-lot differences in these tests.

When results of Crithidia assays were expressed as FlAX units after standardization with FlAX calibrator solutions, variations were still apparent. Mean values ranged from 12 to 62 units, with SDs of 9 to 37 and CVs of 21 to 80%.

Although not always reflected in the actual precisions obtained, some Crithidia slides were easier to read than others. The Meloy, Zeus, and Calbiochem-Behring products gave the clearest definitions of stained kinetoplasts.

The range of FlAX units produced by the U.S. National Reference Serum in Crithidia assays was large, but all means fell below the means produced by this serum in the RIA, ELISA, and FlAX assays (Table 2). These more quantitative tests showed less variability than did the Crithidia assays. Tested two to four times by each method, the U.S. National Reference Serum gave mean values ranging from 73 to 122 FlAX units, with SDs of 1 to 15 and CVs of 1 to 15%.

The values of the U.S. National Reference Serum slightly exceeded those of the highest calibrators included in the kits for the Cordis FlAX (760 int. units/ml) and the Amersharm RIA (107 anti-DNA units/ml). They fell well within the working ranges of the Walter Reed, Wampole, and FlAX assays, which seem more satisfactory for use with sera of high antibody activity. Although titers of the U.S. National Reference Serum exceeded those of the positive controls in Crithidia assays, it should be noted that vendors had prediluted their positive controls 10-fold.

Discussion

It is often difficult to compare results obtained by different workers testing for antibodies because of the different standards and techniques used in each assay. Widely recognized reference sera will simplify these comparisons where they are used.

Tables 1 and 2 permit workers to compare the results that they obtain with the U.S. National Reference Serum with those obtained with various commercial products. Each vendor attempts to standardize its assay by restricting the results of its own calibrators or positive controls to a narrow range of values. If vendors are able to achieve good lot-to-lot reproducibility over a prolonged period of time, users of the RIA, ELISA, and FlAX assays can refer to Table 2 to directly compare their results with others, even where other workers

<table>
<thead>
<tr>
<th>Table 1. Values of the U.S. National Reference Serum for Antibodies to Native DNA in Crithidia luciliae Immunofluorescence Assays</th>
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<tbody>
<tr>
<td><strong>U.S. National Reference Serum</strong></td>
</tr>
<tr>
<td><strong>Titters</strong></td>
</tr>
<tr>
<td><strong>Vendor</strong></td>
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<tr>
<td>Antibodies Inc.</td>
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<tr>
<td>BioDx</td>
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*Positive control provided in each kit; **XG = geometric mean; ^R/P = XG of U.S. National Reference Serum + SDG of positive control serum.
Table 2. Values of the U.S. National Reference Serum for Antibodies to Native DNA in RIA, ELISA, and FIAx assays

<table>
<thead>
<tr>
<th>Lot no.</th>
<th>% DNA binding</th>
<th>Vendor units*</th>
<th>FIAx units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amersham RIA</td>
<td>320</td>
<td>76%</td>
<td>136</td>
</tr>
<tr>
<td>Wampole RIA</td>
<td>2J723N-1</td>
<td>42%</td>
<td>260</td>
</tr>
<tr>
<td>Walter Reed RIA</td>
<td>—</td>
<td>34%</td>
<td>434</td>
</tr>
<tr>
<td>Cordis ELISA</td>
<td>90602</td>
<td>—</td>
<td>794</td>
</tr>
<tr>
<td>IDT FIAx</td>
<td>2274379</td>
<td>—</td>
<td>88</td>
</tr>
</tbody>
</table>

*Each vendor provides units referable to its own calibrator sera. The Amersham RIA uses "anti-DNA binding activity units/mL", the Cordis ELISA uses "IU/mL" and the IDT FIAx uses "FIAx anti-DNA units". **Micrograms of DNA bound per deciliter of serum. In the Wampole assay, the value was calculated by comparison with a calibrated positive control serum included in the kit.

The U.S. National Reference Serum appears to be more reactive with native DNA than does the WHO 1st International Reference Preparation of Anti-Nuclear-Factor Serum (Homogenous), 1970. The Cordis ELISA gave a value of 794 int. units/mL for the U.S. Reference Serum in the present study. The WHO reference preparation can be calculated to have a value of 500 int. units/mL based on the 100 int. units per vial assigned to the WHO serum by anti-DNA by Cordis Laboratories (but not yet so assigned by WHO).

Expressed in terms of dry weight, the anti-DNA activity of the WHO serum is 1 int. unit per 186 μg, and the activity of the U.S. National Reference Serum in the Cordis ELISA assay is 1 int. unit per 90.4 μg. Each 0.5-mL vial of the U.S. Reference Serum contains approximately 400 int. units in this assay.

Both the U.S. and the WHO reference sera were obtained from SLE patients, react with native DNA, and give homogeneous patterns in fluorescent antinuclear antibody (FANA) tests. The preparers of the U.S. Reference Serum compared its FANA activity to that of the WHO preparation, but made no comparison of anti-DNA activity (2). Amersham (12) and IDT have developed independent reference standards with unique anti-DNA values for use in their assays.

In this study, we used IDT FIAx calibrators to produce a common numerical system to translate data between different methodologies. There remains a need for an internationally recognized primary reference preparation with an assigned unit value for anti-DNA activity. This primary standard would greatly aid interlaboratory comparisons. If accepted by individual workers and by manufacturers, it would simplify a complex situation that was only partly resolved by the U.S. National Reference Serum, which was assigned no unitage by its preparers.

Assays for antibodies to native DNA often lack good reproducibility (10). For this reason, the numerical values given in this report must be regarded as approximations. They should usually suffice, however, to allow workers to compare values for anti-native DNA obtained with different commercial products.

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References