Bradford [3], with bovine serum albumin as a calibrator. Ghosts were diluted to protein contents of 0.5, 1, 1.5, 2, and 2.5 g/L. The samples with protein contents of 0.5 g/L (low activity), 1.5 g/L (medium activity), and 2.5 g/L (high activity) were divided into aliquots and stored at –80 °C until the measurements.

Samples (20 μL) were added to 380 μL of Reagent 1 (final concentration per liter: 100 mmol of NaCl, 20 mmol of KCl, 2.5 mmol of MgCl₂, 0.5 mmol of EGTA, 50 mmol of Tris-HCl, pH 7.4, 1.0 mmol of ATP, 1.0 mmol of phosphoenolpyruvate, 0.16 mmol of NADH, 5 KU of pyruvate kinase, 12 KU of lactate dehydrogenase; all from Sigma). After 300 s, 5 μL of 10 mmol/L ouabain (Reagent 2) was added to inhibit the ouabain-sensitive ATPase activity. The change in absorbance was monitored at 340 nm (reference wavelength 415 nm) by a twin test (i.e., combination of two assays in one cuvette); Rate A (i.e., slope of total ATPase activity), 80–280 s; Rate B (i.e., slope of ouabain-resistant ATPase activity), 400–600 s. The difference between the two slopes is proportional to the Na⁺/K⁺-ATPase activity.

For the estimation of total, between-day, between-run, within-day, and within-run CVs, two measurements per specimen per assay and two assays per day from the aliquots were done for 20 days [4]. In the range of 1.7–41.5 mU, the curve of NADH oxidation was linear during the measured intervals (r = 0.98). The activities changed proportionally with increasing protein concentrations (y = 50.6x; r = 0.99). The calculated CVs are presented in Table 1. The detection limit (mean ± 3 SD of spontaneous NADH oxidation) was 0.16 mU.

For the determination of healthy reference intervals, ghosts were prepared from 100 μL of heparinized blood samples taken from 53 neonates, 93 children of different ages (1–18 years), and 42 adults. The study was approved by the Institutional Ethical Committee.

The enzyme activities are lower in children (P < 0.05) [median (95% confidence interval) 5.30 (5.07–5.52) U/g of protein] than in neonates [7.15 (6.52–7.70)] or in adults [7.35 (5.63–8.22)]. No fluctuation of enzyme activity is present during childhood. Our results agree with the findings of others [5], who also reported decreased enzyme activities in children. Moreover, in spite of the difference of the methods used, our data are in the same range, as described [5].

Our automated method has several advantages compared with the manual ones (e.g., low blood requirement, high precision, speed), so it might be a valuable tool for gathering data for the clinical importance of Na⁺/K⁺-ATPase.

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References

Comparison of Antinuclear Antibody Testing Methods by ROC Analysis with Reference to Disease Diagnosis, Richard A. Gniewek,1* Carla Sandbulte,2 and Philip C. Fox3 (1 Bio-Rad Labs., 4000 Alfred Nobel Dr., Hercules, CA 94547; 2 Arthritis Center of Nebraska, Lincoln, NE 68506; 3 Natl. Inst. of Dental Res., NIH, Bethesda, MD 20892-1190; address for correspondence: fax 510-741-5824, e-mail rich_gniewek@bio-rad.com)

Assessing the concentration of antinuclear antibodies (ANA) in a patient’s serum is commonly done by the ANA-immunofluorescence assay (ANA-IFA) [1–6], a very tedious and subjective procedure. By contrast, ANA enzyme immunoassays (ANA-EIA) are easy to perform, and the results are not subjective. Although the performance of ANA-EIA was judged to be less than satisfactory [7], five different ANA-EIA kits were recently proven to be comparable with ANA-IFA [8]. Similarly, in a recent study involving one of us [9], a comparison of ROC curves for ANA-IFA and ANA-EIA showed equivalent performance. In this study we generated ROC curves with disease diagnosis as the gold standard and compared them with the ROC curves from the earlier study [10]. We also tested 98 serum samples from patients with primary Sjögren syndrome (SS).

The patients’ serum samples entered into this study from the Arthritis Center of Nebraska (ACN) were part of the routine medical care when ANA-IFA was ordered. A total of 283 serum samples were collected from April 4,
1995, to October 26, 1995. Diagnosis was made according to the criteria established by the American Rheumatism Association [11]. All of these samples were part of the daily laboratory routine, and none of the samples processed by the laboratory during this period was excluded. The 98 serum samples from patients diagnosed with primary SS had been stored frozen at the National Institute of Dental Research of the National Institutes of Health until needed for testing by ANA-EIA. Diagnosis of primary SS was made according to established criteria [12]. All information regarding ACN samples, which included ANA-IFA results, age, sex, diagnosis, and medications, was withheld until ANA-EIA results had been sent back to ACN. All information regarding primary SS samples was withheld until ANA-EIA results had been generated and sent back to NIH.

Samples from the ACN were tested by ANA-IFA (Kallestad Quantafluor; Sanofi Diagnostics Pasteur) according to the manufacturer’s instructions. Samples from NIH were tested by ANA-IFA (ANA-HEp-2; ImmunoConcepts) according to the manufacturer’s instructions. Samples were then stored frozen (−20 °C) and shipped to Bio-Rad Labs., where they were tested by ANA-EIA with the automated analyzer Raddias (Bio-Rad). The Raddias instrument was programmed by the manufacturer to interpret ANA-EIA results of <0.9 as negative, 0.9 to 1.1 as indeterminate, and >1.1 as positive. The ANA-EIA plates for the Raddias are coated with a HEp-2 cell extract containing ANA antigens, which include double-stranded DNA, SS antigen A (SS-A/Ro), SS antigen B (SS-B/La), Sm, ribonucleoprotein (RNP), Jo-1, and Scl-70.

Diagnostic sensitivity, specificity, positive predictive value, negative predictive value, and agreement were calculated with standard formulas [2]. These values were calculated after defining those diagnoses for which ANA is commonly used as a part of connective tissue disease (CTD) diagnosis as disease-positive. Hence, systemic lupus erythematosus, discoid lupus erythematosus, scleroderma/CREST (calcinosis, Raynaud phenomenon, esophageal motility abnormalities, sclerodactyly, and telangiectasia), Raynaud syndrome, SS, MCTD, overlap CTD syndromes, polymyositis, and dermatomyositis were considered disease-positive [11] and all other diagnoses were considered disease-negative. The exact McNeamar statistic [10] was used to compare sensitivities and specificities. The 95% confidence intervals (CI) and ROC curves were constructed, and the areas under the curves were compared [13].

Approximately 14% (39 of 283) of the ACN samples entered into this study were from patients diagnosed with a CTD. The most common diagnosis was systemic lupus erythematosus (n = 16), followed by Raynaud syndrome (n = 6), SS (n = 6), scleroderma/CREST (n = 5), MCTD (n = 3), discoid lupus erythematosus (n = 2), dermatomyositis/polymyositis (n = 2), and an undefined CTD (n = 9). Some of these patients were diagnosed with more than one CTD.

The area under the ROC curve for ANA-IFA (Fig. 1A) was 0.804 (CI 0.758–0.850) and did not differ significantly (P > 0.05) from the area under the ROC curve for ANA-EIA, which was 0.817 (CI 0.772–0.862). The ROC curve for the ANA-IFA from this study did not differ significantly (P > 0.05) from the ROC curves for the ANA-IFA in the previous study (Fig. 1B).

With a reference range of <1:80 for the ANA-IFA, the sensitivity, specificity, positive predictive value, and negative predictive value were 64.1%, 80.7%, 34.7%, and 93.4%, respectively. With a reference range of <1.2 for the ANA-EIA, the sensitivity, specificity, positive predictive value, and negative predictive value were 71.8%, 76.2%, 32.6%, and 94.4%, respectively. Comparison of the sensitivity and specificity between the ANA-IFA with a reference range of <1:80 and the ANA-EIA with a reference range of <1.2 showed no significant difference (P > 0.05).

When we tested the 98 serum samples from patients diagnosed with primary SS with a reference range for ANA-IFA of <1:80 and a reference range for ANA-EIA of <1.80 and a reference range of <0.9 for ANA-EIA, 6 samples were commonly called negative and...
ANA-IFA fluorescent patterns have been associated with ANA-EIA method, this may be a minor disadvantage. The because no definitive test for CTD exists.

reasonable to assume that this bias favored the ANA-IFA, not made available to the physicians. Although it may be to establish diagnoses whereas the ANA-EIA results were established with certain clinical states has been reported, the incidence of these patterns in non-CTD clinical states has not been investigated thoroughly.

We conclude that the ANA-EIA method is equivalent to the ANA-IFA in screening for ANA in serum from patients with CTD. Differences in performance between the ANA-EIA and ANA-IFA may be insignificant when unselected patients are used [14]. The earlier study [9] reported that only 3 of 8 patients with anticitromere antibody had a diagnosis of CREST syndrome, and none of 19 patients with nucleolar antibody had a diagnosis of scleroderma. In this study, 2 of 4 patients with anticitromere antibody had a diagnosis of CREST, and none of the 4 patients with nucleolar antibody had a diagnosis of scleroderma. Although the association of certain patterns with certain clinical states has been reported, the incidence of these patterns in non-CTD clinical states has not been investigated thoroughly.

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References