A computer program that periodically monitors the ability to interpret the antinuclear antibody test

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Our laboratory has been developing computer programs that help medical technologists improve their performance of the microscope-based immunofluorescence assay for antinuclear antibodies (ANA). This image-based laboratory test has been associated with poor reproducibility. We have previously described our first program, ANA-Tutor™, which systematically teaches the ANA test by using ~150 processed digital images of ANA test results. The program we describe here, Pattern Plus Auditor™, is a logical extension to ANA-Tutor. Pattern Plus Auditor tests the ability of laboratory personnel to interpret the ANA test, and tracks individual and laboratory performance over time. The program consists of image-based questions that test a variety of ANA staining patterns, including homogeneous, speckled, centromere, nucleolar, mixed patterns, and rare patterns. For each question, the program provides correct answers with explanations and color overlays that highlight key image features. By entering the proper password, users gain access to exam results for individuals and for the laboratory as a whole. Results are available for the current exam, any previous exam, or cumulatively on all exams to date. Intra-laboratory testing with computer programs such as Pattern Plus Auditor might be a useful part of quality-assurance procedures for many image-based laboratory tests.

INDEXING TERMS: antinuclear antibodies • immunofluorescence assay • image analysis • computer-assisted instruction • quality assurance

The antinuclear antibody (ANA) test detects the presence of serum antibodies directed against a variety of antigens in the nucleus of cells [1–3]. These antibodies are found in systemic lupus erythematosus as well as several other systemic rheumatic diseases. The ANA test is ordered frequently because the systemic rheumatic diseases have diverse manifestations and are not uncommon. It has been estimated that ~1% of a large population will have an ANA test each year [4]. This testing is performed by thousands of hospital and commercial laboratories throughout the world.

The most common method of performing the test is a microscope-based indirect immunofluorescence assay (IFA) with cultured human epidermoid carcinoma (HEp-2) cells as the substrate for antibody binding [2]. More than 90% of laboratories in the US use this method for ANA testing. The cells are permeabilized and incubated with the patient's serum, and ANA, if present, will bind. The cells are washed and then incubated with fluorescein-conjugated antibodies against human immunoglobulins, which bind to the previously bound ANA. The cells are viewed with a microscope equipped for epifluorescence.

To interpret the test, technologists must analyze the intensity and pattern of fluorescence staining. The greater the intensity of nuclear staining, the more likely it is that a positive ANA test is clinically significant. ANA patterns are named after the pattern of nuclear staining; these patterns include homogeneous, speckled, centromere, nucleolar, and others. In addition, negative ANA tests can demonstrate a variety of weakly fluorescent, nonspecific staining patterns that must be distinguished from positive ANA test results. ANA staining patterns are loosely associated with particular autoantibodies and disease states. For example, the centromere pattern is caused by antibodies against various components of the kinetochore, and is

¹ Nonstandard abbreviations: ANA, antinuclear antibodies; IFA, indirect immunofluorescence assay; PC, personal computer; PBS, phosphate-buffered saline; and CAP, College of American Pathologists.
clinically observed in patients with the CREST variant (calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and relangiectasia) of scleroderma.

A significant problem with the IFA-ANA test is that it has poor intra- and interlaboratory reproducibility [5–7]. Poor reproducibility is caused by a number of factors, including: (a) lack of procedures for interpretation, (b) fading of the stained substrate because of fluorescence photobleaching, (c) lack of standard specifications for microscope equipment used to perform the test, (d) variability in the substrate, and (e) variability in technologist experience.

Processed digital images have several advantages over microscope images and photomicrographs [8], and it is now possible to capture digital images with imaging systems driven by personal computers (PCs). This has motivated our laboratory to use PCs and digital images to improve the standard IFA-ANA test. One component of our effort is a computer program called ANA-Tutor™ (9); Sanofi Diagnostics Pasteur, Chaska, MN), which is the ANA test by using ~150 processed digital images of ANA test results. The images, which were obtained with sera submitted to the clinical immunology laboratory for ANA testing, were collected by using a PC-based image analysis system. ANA-Tutor provides a uniform teaching platform that is aimed at reducing the technologist variability in the interpretation of the ANA test.

The work we present here is a logical extension of our previous work. We describe a computer program that can help laboratories identify problems in interpretation of the ANA test. The program, Pattern Plus Auditor™ (Sanofi Diagnostics Pasteur), tests a user’s ability to interpret images of ANA test results and monitors individual and total laboratory performance over time. We describe how the computer program was constructed and describe its features. We suggest that computer programs could be used as part of quality-assurance procedures for image-based laboratory tests in clinical and anatomic pathology.

**Materials and Methods**

The ANA test was performed by a standard indirect IFA [2] with a monolayer preparation of HEp-2 cells as the substrate (12-well slides; Sanofi Diagnostics Pasteur). Patients' samples were diluted between 1:40 and 1:640 with phosphate-buffered saline (PBS), pH 7.2. Glass wells containing HEp-2 cells were overlaid with the diluted sample and then incubated in a moist chamber at room temperature for 30 min. The wells were rinsed with a gentle stream of PBS, overlaid with fluorescein-conjugated anti-human IgG (Sigma Chemical Co., St. Louis, MO) diluted 1:250 in PBS, and incubated for 30 min at room temperature in a dark moist chamber. Finally, the wells were rinsed with PBS, counterstained with 0.05 g/L Evans Blue, rinsed again, and mounted with cover slips with glycerol/PBS (10:1 by vol, pH 7.2).

An image library of >1400 ANA test results, representing variations of nearly all previously described ANA staining patterns, was collected from June 1992 to October 1995. The images were collected with a PC-based digital image analysis system as follows: Slides of the stained HEp-2 cells were viewed through a light microscope equipped for epifluorescence (Carl Zeiss, San Leandro, CA). The microscope had an HBO-100 illumination system, and the intensity and duration of illumination were modulated by a computer-driven shutter/filter wheel (Filter Wheel 99A042 and MAC 2000 controller; Ludl Electronic Products, Hawthorne, NY) that contained neutral density filters with optical densities of 0.3, 0.6, 0.8, 1.0, and 1.2 (Omega Optical, Brattleboro, VT). Images were recorded by a mono-chrome CCD camera (CCD-725S series; Dage-MTI, Michigan City, IN) mounted on the microscope. The camera was interfaced to an 80486-based microcomputer (Dell Computer Corp., Austin, TX) through a video imaging board (MVP-AT; Matrox Electronic Systems, Dorval, PQ). The entire system was driven by the Optimas image analysis software package (Optimas Corp., Edmonds, WA), which runs under Microsoft Windows (Microsoft Corp., Redmond, WA). The images were colored in fluorescein-green with Optimas; Adobe Photoshop for Windows (Adobe Systems, Mountain View, CA) was used on some images to remove unwanted artifacts and adjust color intensity.

**Program Description**

Pattern Plus Auditor is designed to be distributed periodically to participating laboratories, and each distribution consists of a unique exam of questions that test a user’s ability to interpret the ANA test. The frequency of distribution and the number of questions per exam can vary. This year the program will be distributed four times with four questions per exam.

Pattern Plus Auditor is written in Microsoft Visual Basic for Windows and requires 1 Mbyte of hard-disk storage for images, an 80486 or Pentium-based microcomputer equipped with 4 Mbytes RAM, a mouse, and a supervideo graphics adapter capable of showing at least 256 colors at a display resolution of at least 640 × 480 pixels. The program requires minimal computer experience; it is operated primarily by positioning the mouse and pressing the left mouse button.

There are two types of users of the program. The first is the examinee, who in most cases will be a medical technologist who performs the ANA test in the laboratory. Medical technology students, medical students, and other trainees might also be appropriate examinees. The second type of user is the laboratory supervisor, who wants to monitor how individuals in the laboratory, including him- or herself, and the laboratory as a whole are performing on the exams. In laboratories preferring less formal evaluation, the supervisor can give the other examinees access to the results.

Figure 1 shows a schematic diagram of Pattern Plus Auditor. We describe the program features that are present when the program is operating in the default mode. In default mode, the names and responses of the examinees are recorded, and correct answers with explanations are given after each question is answered.

The instructions tell the examinee about the mechanics of taking the test. At the start of the test, the examinee is asked to type in his or her name. This is necessary for the program to tabulate results. After logging in, the examinee is presented with a series of image-based multiple choice questions; each question asks for an interpretation of an ANA staining pattern. Where appropriate, questions have a focusing feature, which simulates
focusing the microscope through a specimen. For some questions, arrows point to parts of the image to which the question refers. We have constructed a question library that tests the ability to identify most ANA patterns, including homogeneous, speckled, centromere, nucleolar, mixed, cytoplasmic, and rare patterns. In addition, there are questions related to the ability to distinguish positive from negative ANA test results.

The program provides correct answers with explanations by overlaying the image in the question with descriptive text. In addition, the explanations contain a "more information" button that, when activated, provides detailed information about the pattern and its autoantibody and disease associations. An example of a question and an associated answer is shown in Fig. 2. The question asks the examinee to identify a homogeneous pattern.

The control panel allows the program's default settings to be changed, and it allows the exam results to be viewed and analyzed. A password is given to the laboratory supervisor so that access to exam results can be restricted. Supervisors who desire open communication of results can make the password freely available.

The "Change Defaults" option in the control panel allows the supervisor to withhold correct answers and explanations from test takers, or to choose to have examinees complete the test without having names and responses recorded. In addition, the supervisor can change the password to the control panel.

Clicking on the "Exam Results" button allows the supervisor to view results for the current exam, any previous exam, or cumulatively on all exams to date. There are two basic classes of results, those for individuals and those for the laboratory as a whole. Results for the laboratory as a whole are expressed graphically; an example of this graphical display, derived from results from one examination of five examinees, is shown in Fig. 3. The program has a feature to export these results to a text file. This text file might eventually be useful for interlaboratory comparisons (see Discussion below).

For individual results, one can select a particular exam and view the questions in that exam—including images—and the responses of the individual compared with the correct responses. In addition, one can view a table showing an individual's cumulative performance to date.

A final significant feature of Pattern Plus Auditor is that the images from the program are added to the image index of the ANA-Tutor. Thus, the amount of reference material in the ANA-Tutor increases over time.

**Discussion**

The ANA Test

Inconsistent interpretation contributes significantly to the poor reproducibility of the ANA test [3, 6]. We have been developing image-based computer programs that try to overcome this problem. Our previously described program, ANA-Tutor [9], provides image standards and strategies for interpreting the
Fig. 3. A screen from the "Exam Results" section of the program showing a bar graph of the performance of five examinees on a four-question exam (exam name: September 1994).

The questions involved identifying four patterns: atypical speckled, centromere, homogeneous, and speckled. All five examinees (100%) correctly identified the homogeneous and speckled patterns, three of five (60%) correctly identified the atypical speckled pattern, and four of five (80%) correctly identified the centromere pattern.

ANA test. Pattern Plus Auditor, the program described here, helps laboratories periodically monitor their ability to interpret the test.

The alternatives to a computer program for monitoring intralaboratory performance for the ANA test are photomicrograph- or microscope-based exams. The computer program has several advantages over these conventional approaches. Unlike the microscope-based approach, the computer program requires no microscope or reagents, and the program's digital images do not fade from photobleaching. In addition, the computer program can give immediate and detailed feedback regarding key features of the ANA staining pattern. Unlike photomicrographs, the computer program can simulate focusing through a specimen. In addition, higher quality can be achieved consistently with digital images, since they can be edited to remove noise and correct color. Finally, the computer program requires much less time than the other methods because the program automatically keeps track of performance data. This is a significant advantage, given the labor constraints facing many laboratories [10, 11].

Pattern Plus Auditor could eventually be used for interlaboratory comparisons. Participating laboratories could periodically submit the computer file containing the laboratory's results, and these results could be tabulated. The interlaboratory comparison would be a useful complement to the College of American Pathologists (CAP) interlaboratory proficiency testing because the two types of testing fulfill different needs. CAP proficiency testing assesses all phases of test performance, including specimen handling, slide preparation, and image interpretation. In addition, CAP specimens must be handled like a typical clinical specimen, meaning that the test usually involves one or two technologists. In contrast, Pattern Plus Auditor tests all members of a laboratory, and restricts the test to the interpretation of fluorescence staining.

DEVELOPING PROGRAMS FOR OTHER IMAGE-BASED LABORATORY TESTS

In addition to ANA-Tutor, we have been developing computer tutorials that systematically teach the interpretation of other image-based laboratory tests. These programs include Gram-Stain-Tutor® ([12], Lippincott-Raven Publishers, Philadelphia, PA), which teaches the direct Gram stain; Urinalysis-Tutor® ([13], Lippincott-Raven), which teaches the microscopic examination of urine sediment; PeripheralBlood-Tutor® (Lippincott-Raven), which teaches the interpretation of stained peripheral blood smears; Electrophoresis-Tutor® ([14], Beckman Instruments, Brea, CA), which teaches the interpretation of agarose gel electrophoretic patterns; and others [15, 16].

Recently, we have adapted Pattern Plus Auditor to create programs that monitor Gram stains, microscopic urinalysis, and microscopic evaluation of peripheral blood smears. These three new monitoring programs are designed to complement Gram-Stain-Tutor, Urinalysis-Tutor, and PeripheralBlood-Tutor, respectively. Similar programs also could be helpful in selected areas of anatomic pathology, e.g., the interpretation of Papnicolaou smears.

In conclusion, the ability to perform sophisticated image presentation on PCs has allowed us to create a computer program that can help laboratories monitor their ability to interpret image-based laboratory tests. Computer programs such as Pattern Plus Auditor might be a useful part of quality-assurance procedures for many image-based laboratory tests in clinical and anatomic pathology.

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References

6. Beutner EH, Krasny S, Kumar V, Taylor R, Chorzelski TP. Prospects and problems in the definition and standardization of immunofluorescence: I. Present levels of reproducibility and disease speci-


