A practical guide for serologic evaluation of autoimmune connective tissue diseases

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Serologic testing is important in the evaluation of patients with autoimmune connective tissue diseases (CTD). There are many techniques. Each of the tests has different sensitivity and specificity with varying diagnostic value. These serologic tests detect antibodies to numerous cellular components. The diagnostic significance and specificity of each antibody vary. Choosing the appropriate test and understanding its clinical utility is an important aspect in the diagnostic evaluation of patients with CTD. (J Am Acad Dermatol 2000;42:159-74.)

Learning objective: At the conclusion of this learning activity, participants should be familiar with the various serologic tests for CTD, should understand the associations of specific antibodies with individual CTD, and should identify the factors that influence the predictive value of these serologic tests.

Connective tissue diseases (CTDs) are a group of autoimmune disorders that have overlapping clinical features (Table I). The accurate diagnosis of a patient with one of these disorders depends on the evaluation of 4 parameters, namely clinical findings, histopathology, tissue immunofluorescence, and serologic testing. This article is limited to the serologic evaluation. Serologic testing does not substitute for evaluation of the other criteria. Serologic testing does help to confirm a clinical diagnosis and classify subsets of a CTD and thus help predict prognosis. For example, a patient who presents with cutaneous lupus erythematosus and who is found to have significantly high antibody titer to native DNA (nDNA) (or double-stranded DNA [dsDNA]) likely has systemic lupus erythematosus (SLE) with cutaneous involvement.[1-4] In addition, a patient who has cutaneous sclerosis, calcinosis, and esophageal dysmotility and who is found to have anticentromere antibodies is much more likely to have a benign course associated with the CREST syndrome (*c*alcinosis, *R*aynaud's phenomenon, *e*sophageal dysmotility, *s*clerodactyly, and *t*elangiec-

tasia)[5,6] rather than the usually severe course associated with systemic sclerosis (SSc).

BIOLOGY OF THE ANTIBODY SPECIFICITY

Patients with CTD have an autoimmune phenomenon that results in the production of antibodies against several self-antigens. These autoantibodies are directed against all cellular components, that is, nuclear, cytoplasmic, and cell membrane molecules. The binding of these antibodies to commercially available tissue extracts is the basis for serologic testing. Whether these antibodies play a role in the pathogenesis of the clinical manifestations of the disease is suspected but not confirmed with certainty. The most common antibodies that are of diagnostic value are shown in Table II.

In evaluating the results of these tests, it is important to be aware of two findings. First, some of the antibodies are not unique to patients with CTD and may be present in the sera of normal persons or persons with other conditions.[7-14] Therefore the mere detection of these antibodies does not always indicate a CTD. In general, however, the total amount of antibodies to a certain antigen is much larger in patients with CTD.[9,10] The total amount of antibodies is usually indicated by the titer or the absolute value given to the test. Second, the specificity of each of the antibodies for the various CTD varies. For example, some antibodies, such as Sm antibodies and dsDNA antibodies, are highly specific (for SLE).[11-21] Other antibodies (eg, single-stranded DNA

Table I. Autoimmune CTDs

1. LE
   A. Systemic LE
   B. Discoid LE
   C. Subacute cutaneous LE
   D. Neonatal LE
   E. Overlap of two or more LE subsets
   F. Overlap of LE with other CTDs
2. Scleroderma
   A. Cutaneous scleroderma (morphea)
   B. Systemic scleroderma
      1. Limited disease (acrosclerosis, CREST syndrome)
      2. Diffuse disease (SSc)
3. Dermatomyositis
4. Sjögren's syndrome (primary and secondary)
5. MCTD
6. Overlap and undifferentiated CTD

*CREST, C*alcinosis, *R*aynaud's phenomenon, *e*sophageal dysmotility, *s*clerodactyly, and *t*elangiectasia; *CTD,* connective tissue disease; *LE,* lupus erythematosus; *MCTD,* mixed connective tissue disease; *SSc,* systemic sclerosis.

Table II. Antibodies in autoimmune CTDs

1. Antibodies to DNA
   A. Antibodies to nDNA (dsDNA)
   B. Antibodies to ssDNA
2. Antibodies to small ribonucleoproteins
   A. Antibodies to Ro(SS-A)
   B. Antibodies to La(SS-B)
   C. Antibodies to U_1RNP
   D. Antibodies to Sm
3. Antibodies to histones
4. Antibodies to centromere
5. Antibodies to phospholipid (cardiolipin)
6. Antibodies to other cellular components

dsDNA, Double-stranded DNA; *nDNA,* native DNA; *ssDNA,* single-stranded DNA; *U_1RNP,* uridine-rich ribonucleoprotein.

[ssDNA] antibodies) are of low diagnostic value because of their high nonspecificity[17,22]; they may be present in the sera of patients with most CTDs.

The type of antibodies present and the frequency of their occurrence vary among the various CTDs. For example, patients with mixed CTD (MCTD) have antibodies to nuclear ribonucleoprotein (also known as uridine-rich ribonucleoprotein [U_1RNP]),[8,12,13,17,22-27] and patients with CREST syndrome have antibodies that are almost limited to the centromere.[13,19,28,29] In contrast, patients with SLE may have antibodies to several cellular antigens.[7,8,11,12,17,19,21,22,26] Fig 1 reveals the frequency of the various autoantibodies in 6 selected CTDs. Each CTD has a unique profile of antibodies.

TECHNIQUES FOR SEROLOGIC TESTING

The methods for the detection of the various antibodies have changed over the past few decades. Immunologic techniques that are commonly used during each time period have been utilized for the detection of these antibodies. For example, radioimmunoassay and immunoelectrophoresis were commonly used in the past.[30-36] Radial immunodiffusion and immunofluorescence[8,10,30,32,37-42] remain of important value although both (especially the former) are being slowly replaced by newer techniques such as the enzyme-linked immunosorbent assay (ELISA).[8,18,32,38,39,43-46] Several types of antibodies may be detected by multiple techniques. The principles of immunofluorescence and ELISA are similar. An antigen (in the substrate) is placed on a glass slide or plastic plate (for immunofluorescence and ELISA, respectively). The substrate is incubated with the patient's serum. If the serum has antibodies, they will bind to the substrate. This binding is detected by a series of amplification steps that produce visible fluorescence (immunofluorescence) or a colored dye that will be detected and quantified by a machine's photometer (ELISA). Both tests may be quantitative by diluting the serum to various titers until the test is negative. The larger the titer, the higher the amount of antibody in the serum. ELISA has many advantages. It is cheaper, less labor intensive, may be used to screen a large number of sera together, is less subjective (does not need human technical interpretation), and is more sensitive.[44,46] ELISA, however, is less specific and results need to be interpreted with caution (see later).[8,44]

Radial immunodiffusion takes advantage of the ability of molecules (both antigen and antibody) to migrate through agarose gel, bind together, precipitate, and produce a visible line that indicates the presence of antibodies.[47] This test is less sensitive than immunofluorescence and ELISA but is more specific. For a serum to be positive by radial immunodiffusion, the serum must contain a relatively higher amount of antibodies (compared with more sensitive techniques).[10,11] Accordingly, the diagnostic value of a positive test by radial immunodiffusion is higher than that by ELISA because the diagnostic value of the antibodies does not solely rely on their presence, but also on their total amount.

The frequency of positivity for each of the various antibodies in the various CTDs varies among different reports. Some of the figures quoted in this article are an estimated average of the various percentages.
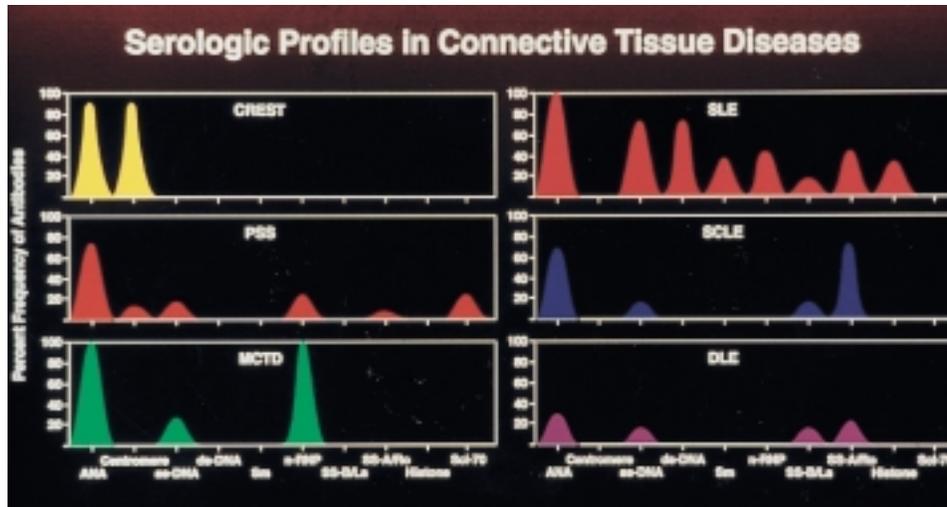
Fig 1. Frequency of various antinuclear antibodies. Each CTD has a unique profile.

ANTIBODIES TO DNA

Serum DNA antibodies may recognize nDNA (double-stranded) or denatured ssDNA by testing, depending on the type of epitope within the DNA molecule that they recognize. The diagnostic significance of each of the two antibodies is different. The two types of antibodies will be discussed separately.

nDNA antibodies

Testing technique. nDNA antibodies have been determined by several techniques, including radio-immunoassay.[1,48-51] Presently, ELISA is used more frequently than immunofluorescence. Some laboratories use indirect immunofluorescence in place of, or in addition to, ELISA. The immunofluorescence test is performed on *Crithidiae luciliae*. *Crithidia* is a hemoflagellate organism that possesses a giant mitochondrion. Concentrated mitochondrial DNA is found within the mitochondrion and is called the kinetoplast. The kinetoplast contains primarily nDNA (and histone) with no ssDNA. This organism's unique structure makes it an ideal substrate for determining the presence of antibodies to nDNA.[3,4,52-55] The ELISA test for nDNA uses calf thymus extract and is more sensitive than immunofluorescence.[1,3] The result of the immunofluorescence test is reported as positive or negative. A titer level may be determined but is usually unnecessary for diagnosis because the detection of nDNA antibodies by immunofluorescence at any titer has significant diagnostic value. The result of the ELISA is reported as a value with a range for normal values.

Disease association. nDNA antibodies are highly characteristic of SLE.[1,2,4,12,13,17,19,48,51,56] Their presence is usually associated with positive direct immunofluorescence in the patient's normal skin (the lupus band), low circulating complement levels, renal disease, and generally poor prognosis.[11,21]

Interpretation of results. Significant levels of nDNA antibodies (positive immunofluorescence test or ELISA value higher than 2-3 standard deviations above the mean) confirm a clinical diagnosis of SLE. Low levels of nDNA antibodies may be detected in rheumatoid arthritis, Hashimoto's disease, Graves' disease,[48] Waldenström's macroglobulinemia,[53] MCTD, SSc,[54] autoimmune liver disease,[13] and Sjögren's syndrome.[55]

Indications to order nDNA antibody testing. The most practical indication to obtain nDNA antibody testing is in the setting of a patient with a clinical suspicion of SLE. Although a significantly positive test confirms the diagnosis, a negative test does not exclude SLE because nDNA antibodies are positive in only 50% to 83% of patients with SLE.[40,46]

ssDNA antibodies

Testing technique. ssDNA antibodies are detected by ELISA.[2,57-59] Unlike the extracts used for nDNA antibodies, extracted nDNA molecules are further denatured to produce ssDNA molecules. The most common source of DNA used for both nDNA antibody and ssDNA antibody determination is calf thymus.[2,59-61]

Disease association. ssDNA antibodies have a very low diagnostic value. They have been detected in the sera of patients with various forms of lupus erythematosus as well as other CTDs, including dermatomyositis,[62] morphea,[63] and Sjögren's syndrome.[64] ssDNA antibodies are especially prevalent in linear morphea in children.[65] The role that the

Table III. Drugs reported with drug-induced SLE

Allopurinol[76]
Captopril[83]
Chlorpromazine[69,76,77,83]
Clonidine[83]
Danazol[83]
Diphenylhydantoin[69]
Ethosuximide[69,72,76,83]
Griseofulvin[76,83]
Hydralazine[69-71,76-79,83]
Isoniazid[74,76,79,83]
Lithium[79,83]
Lovastatin[83]
Mephenytoin[76]
Mesalazine[75]
Methyldopa[79,83]
Minocycline[82]
Oral contraceptives[76,83]
para-Amino salicylic acid[74]
Penicillamine[76,78,79,83]
Penicillin[83]
Phenothiazines[79]
Phenylbutazone[76]
Piroxicam[80]
Practolol[76]
Primidone[76]
Procainamide[69-71,76,77,79,83]
Propylthiouracil[73,76,83]
Quinidine[76,79,83]
Streptomycin[76]
Sulfasalazine[78,79]
Sulfonamides[76]
Tetracycline[76,79]
Thiamazole[73]
Trimethadione[76]
Valproate[81]

antibodies play in the pathogenesis of morphea, if any, is unknown. ssDNA antibodies as well as nDNA antibodies may play a role in some of the systemic manifestations of SLE.[11,36]

Interpretation of results. Because low levels of ssDNA antibodies may be detected in persons without CTD, a patient's level of antibodies should be much higher than the normal range (>3 standard deviations above the mean) to be of value in the diagnosis of CTD.[66]

Indications to order ssDNA antibody testing. Because ssDNA antibodies are nonspecific, their diagnostic value in the work-up of patients with CTD is low.

Histone antibodies

Histones are basic proteins that bind the DNA helical structure to contribute to the supercoil for-

mation.[67,68] Histone antibodies are characteristic of drug-induced SLE. Drugs that have been reported with drug-induced SLE are shown in Table III.[69-83]

Testing technique. Histone antibodies may be detected by various assays including immunofluorescence,[68,69,84-87] complement fixation,[68,86] radioimmunoassay,[68,70] and ELISA.[68,87-89] Quantitative assays such as ELISA use commercially available histones. Immunofluorescence assay utilizes animal substrates such as rat liver.[70,84,86,87]

Disease association. Histone antibodies are characteristic of SLE. The majority (approximately 90%) of patients with drug-induced SLE[69,90,91] have antihistone antibodies to the exclusion of other antibodies. Approximately 30% of patients with idiopathic SLE also have antihistone antibodies.[11] Most of these patients, however, have other antinuclear antibodies.[11,86,89]

Interpretation of results and indications to order histone antibody testing. Histone antibody testing is indicated in patients suspected of having drug-induced SLE. Their presence strongly supports the diagnosis. Idiopathic SLE, however, cannot be excluded on the basis of the presence of antihistone antibodies.

RNP ANTIBODIES

Of all the types of cellular RNA, autoantibodies in patients with CTD are directed to the small ribonucleoproteins (sRNP). This type constitutes the smallest portion of cellular RNA (<1% of the total RNA). sRNP consists of several molecules that contain RNA and an associated protein, thus the term *ribonucleoprotein.*[26,92] The protein component has enzymatic activity and plays a role in the processing of the RNA molecule.[92] Antibodies to sRNP are directed against epitopes within the protein component of the molecules.[23,26,92-94] Antibodies to various sRNP molecules are named after the name of the sRNP molecule, for example, Ro(SS-A),[95-101] La(SS-B),[97,102] U_1RNP,[92,94,103] and Sm.[104-106] The exact role that these antibodies play in the pathogenesis of the associated CTD is not clear. The detection of these antibodies, however, is of value in the diagnosis of the various CTDs. The diagnostic specificity of each of these antibodies is variable. For example, Sm antibodies are characteristic of SLE,[16,107] whereas Ro(SS-A) antibodies have been reported in various subsets of lupus erythematosus and other CTDs.[95-97,101,108-111]

There are two major techniques for the detection of sRNP antibodies. The first is radial immunodiffusion, which has high specificity and low sensitivity; the other is ELISA, which has higher sensitivity and less specificity.[112] Most large laboratories (usually

Table IV. Incidence of anti-Ro(SS-A) antibodies in autoimmune CTDs (by radial immunodiffusion)[11]

Diagnosis	%
Antinuclear antibody negative SLE	70
Subacute cutaneous LE	70
Homozygous C2 or C4 deficiency	70
Late onset SLE	80
Neonatal LE	95
Mothers of infants with neonatal LE	95
Discoid LE	0-20
Sjögren's syndrome	50
SSc, dermatomyositis	Rare
Healthy persons	< 1

SSc, Systemic sclerosis.

Table V. Indications for anti-Ro(SS-A) and anti-La(SS-B) antibody testing*

Work-up for photosensitivity
Screening for certain patients with LE
Suspicion of subacute cutaneous LE
Suspicion of neonatal LE
Suspicion of Sjögren's syndrome
Work-up for idiopathic chronic vasculitis
Patients with systemic or subacute cutaneous LE with negative screening fluorescent ANA test

*References 95-97, 102, 108-110, 115-117, 121.

national) utilize ELISA because of the advantages discussed earlier.

The interpretation of sRNP antibody testing is technique specific. As mentioned earlier, the mere presence of antibodies is of less diagnostic value than the total amount as detected by the quantitative test. Because of the lower sensitivity of radial immunodiffusion, a patient's serum needs to contain large amounts of antibody for the test to be positive. Accordingly, a positive test by radial immunodiffusion has a high diagnostic value. On the other hand, because ELISA is highly sensitive, a positive test by ELISA is of low diagnostic value. The inherent low specificity of ELISA is made up for by the ability of the test to provide a quantitative assessment of the antibodies that is provided as a value and compared with the normal range. For an ELISA result to be of high diagnostic value, the level of antibodies must be more than 2 to 3 standard deviations above the mean of the normal range.

Anti-Ro(SS-A) and anti-La(SS-B) antibodies

Disease associations. Anti-Ro(SS-A) antibodies are characteristic of two CTDs, namely, lupus erythematosus and Sjögren's syndrome.[27,95-97,108,109,113-115] The reported incidence of this antibody varies with the technique used in the study. The incidence of positive anti-Ro(SS-A) antibody in a specific disorder is lower by immunodiffusion compared with ELISA. Most of the old reports utilized radial immunodiffusion.[108-110,113,116-118] The more recent reports provide incidences based primarily on ELISA testing[95,108,119,120] and are therefore higher than those reported by immunodiffusion. By radial immunodiffusion, anti-Ro(SS-A) antibodies are detected in approximately 50% of patients with Sjögren's syndrome[11,95,96,109,113] and a varying percentage of patients with the various subsets of lupus erythe-

matosus[11,96,109,110,115,116,121-123] (Table IV). Anti-Ro(SS-A) antibodies are strongly associated with photosensitivity,[95,96,118] especially in patients with subacute cutaneous lupus erythematosus (SCLE) of the idiopathic as well as the drug-induced types.[95,96,108,124-126] Anti-Ro(SS-A) antibodies may be associated with a higher incidence of vasculitis.[95,96,108,113] There appears to be a genetic predisposition for the presence of anti-Ro(SS-A) antibodies. Patients have a higher incidence of HLA-DR3,[115] -DQ2,[96] and -DRw52.[95,101]

Anti-La(SS-B) antibodies are closely related to anti-Ro(SS-A) antibodies. More than 90% of sera with anti-La(SS-B) antibodies are also positive for anti-Ro(SS-A) antibodies.[116] The diseases associated with antiLa(SS-B) antibodies are similar to those associated with anti-Ro(SS-A) antibodies, namely, lupus erythematosus and Sjögren's syndrome. The incidence of anti-La(SS-B) antibodies in these disorders, however, is approximately half that of anti-Ro(SS-A) antibodies.[101,113,116]

Indications for ordering anti-Ro(SS-A) and anti-La(SS-B) antibody testing. There are several indications in dermatological practice to order anti-Ro(SS-A) and anti-La(SS-B) antibody testing (Table V). Anti-Ro(SS-A) and anti-La(SS-B) antibodies are occasionally helpful in the diagnostic work-up of a patient with photosensitivity,[95,96,118] especially when the clinical and histologic findings are not characteristic. Anti-Ro(SS-A) and anti-La(SS-B) antibody testing may also be helpful in the initial baseline evaluation of patients with cutaneous lupus erythematosus with features of photosensitivity. Anti-Ro(SS-A) and anti-La(SS-B) antibodies are helpful in confirming the clinical diagnosis of a disease that is known to be highly associated with these antibodies, such as SCLE, neonatal lupus erythematosus, and Sjögren's syndrome.[11,95,96,109,124-126] An occasional patient with chronic idiopathic vasculitis may be revealed to have underlying undiagnosed Sjögren's syndrome, making it appropriate to obtain testing for anti-Ro(SS-A) and

Table VI. Indications for fluorescent ANA testing in dermatological practice

Work-up for photosensitivity
Baseline in patients with discoid LE
Clinical suspicion of CTD
Baseline for phototherapy
Work-up of chronic vasculitis

anti-La(SS-B) antibodies in patients with chronic idiopathic vasculitis.[95,96,108,113] Finally, anti-Ro(SS-A) and anti-La(SS-B) antibodies are useful in the evaluation of a patient with the clinical manifestations of SLE or SCLE if the screening fluorescent antinuclear antibody (ANA) test is negative,[11,117,118] since the ANA test may be negative despite the presence of anti-Ro(SS-A) and/or anti-La(SS-B) antibodies.

Antibodies to U_1RNP and Sm

Antibodies to U_1RNP are present in the sera of patients with MCTD and SLE. By definition, antibodies to U_1RNP are detected in 100% of patients with MCTD[15,23-25,127] and approximately 30% of patients with SLE.[92,93] They have also been reported rarely in neonatal lupus erythematosus.[128,129] As will be discussed in more detail later, the presence of U_1RNP antibodies in MCTD is to the exclusion of other types of antinuclear antibodies.[23,92] In contrast, patients with SLE who have U_1RNP antibodies usually have ANAs with other specificities as well.[92] This observation is important when attempting to differentiate between MCTD and SLE. U_1RNP antibodies are very rarely detected in patients with SSc.[130-132] Because the incidence of SLE is much higher than that of MCTD, the majority of patients with U_1RNP antibodies have SLE rather than MCTD. The presence of U_1RNP antibodies is usually associated with sclerodactyly, Raynaud's phenomenon, esophageal dysmotility, low incidence of renal disease, pulmonary dysfunction, arthritis, and myositis.[132,133]

Antibodies to Sm by immunodiffusion are diagnostic of SLE.[16,107] They have not been reported in patients with other CTDs. The incidence of Sm antibodies in SLE is only 15% to 40%.[15,16,104,134-136] Most patients with antibodies to Sm will also have antibodies to U_1RNP.[93,137] The converse of this observation, however, is not true. Most patients with U_1RNP antibodies do not have Sm antibodies.[127,131]

Antibodies to U_1RNP and Sm are indicated when attempting to confirm the diagnosis of MCTD[23-25,92,127] and SLE,[16,93,107] respectively.

OTHER AUTOANTIBODIES

Several other autoantibodies have been reported in patients with CTD. The diagnostic value of most of these antibodies is limited; only two are discussed in this review. Scl-70 antibodies are directed against the enzyme topoisomerase-I.[138-140] This is a 100-kd basic protein that affects the tertiary structure of DNA molecules. Scl-70 antibodies are characteristic of SSc and help differentiate patients with extensive cutaneous and systemic involvement from those with limited disease.[131,141-143] The incidence of Scl-70 antibodies, however, is low (approximately 10%-20% by radial immunodiffusion).[141,142] Scl-70 antibodies may be viewed as a marker for SSc when compared with patients with CREST syndrome who have another marker antibody, namely, anti-centromere antibody (see section on fluorescent antinuclear antibody testing).[5,28,142-144]

Jo-1 antibodies are directed against the enzyme histidyl tRNA synthetase (150 kd) and are detected in a small number of patients with dermatomyositis (and polymyositis).[145-148] The presence of Jo-1 antibodies is often associated with pulmonary involvement and possibly the mechanic's hand skin lesions.[147,149,150]

FLUORESCENT ANA TEST

The fluorescent ANA test is a very good screening test for most of the previously discussed antibodies.

Testing technique

The ANA test is an indirect immunofluorescence test that utilizes a substrate rich in nuclear material. A positive ANA test indicates the presence of ANAs. It does not indicate the specific type of antibody, although close examination of the pattern of positivity may be helpful in suggesting the specific type of ANA that is present in the tested serum.

The indications for ordering an ANA test in dermatology include the work-up of patients with photosensitivity, work-up of patients with chronic vasculitis, a baseline for patients with discoid lupus erythematosus, clinical suspicion of CTD, and baseline for patients undergoing phototherapy (Table VI).

Interpretation of results

When an ANA test result is obtained, 3 parameters are evaluated; these include the substrate used, the titer of a positive test, and the pattern of fluorescence.

ANA substrate. There are two major types of substrate for ANA testing. Until two decades ago, most ANA tests were performed on animal substrates, such as mouse kidney or rat liver.[10,11,42,151] Sera of some patients with SLE were reportedly negative on such substrates. It became clear that human substrates (cultured human cells) are more sensitive

Table VII. Conditions other than autoimmune CTDs with positive ANA

Elderly persons[12,153]
Pregnant women[154,155]
Relatives of patients with CTD[12,156]
Other autoimmune diseases (eg, primary biliary cirrhosis, autoimmune thyroiditis)[14,196]
Drugs (eg, procainamide, hydralazine)[68-83,157]
Chronic infections[10,14]
Neoplasms[10,14]
Healthy persons[9,11,12,153]

Table VIII. Positive fluorescent ANA test in healthy persons (on HEp-2 cells)[9]

Titer	Prevalence
1:40	32%
1:80	13%
1:160	5%
1:320	3%

Table IX. ANA patterns and their antigen and disease associations

ANA	Predominant antigen	Disease	Reference Nos.
Peripheral	nDNA	SLE	10,14,161
Homogeneous	nDNA, histones	SLE	14,161
Nucleolar	Nucleolar RNA	SSc, SLE	14,158,161
Centromere	Kinetochore	CREST	14,159
Speckled	Various ribo-nucleo-proteins	MCTD, SLE, SSc, Sjögren's Syndrome	14,161

than animal substrates.[11,30,32,38] Most SLE sera that were negative on animal substrates were positive on human substrate. Because of this observation, most laboratories use cultured human cell substrates. Presently, the vast majority of laboratories use a specific type of cultured human cells referred to as HEp-2 cells.[11,30,32,38] These are obtained from cultured esophageal squamous cell carcinoma cells. The cells are available commercially, prefixed on glass slides. Because an occasional laboratory may still be using animal substrates for ANA testing, it is essential to pay attention to the substrate being used by each of the various laboratories from which a physician may receive results. A serum that is negative on animal substrate may be positive when tested on cultured human cells.

ANA titer. As mentioned earlier, the presence of ANAs is not diagnostic of CTD. The amount of antibody (and the specificity) have significant value in the interpretation of an ANA test.[9,11,13] The ANA titer is an indirect measure of the total amount of serum antibodies. The higher the titer, the higher the amount of antibodies. Generally, the ANA test is negative or very low in young and healthy persons.[9,12,152,153] It is generally high in patients with systemic CTD.[11-13] The ANA titer is intermediate in some patients with CTD as well as in persons with a wide variety of conditions (Table VII). These include old age,[12,153] pregnancy,[154,155] close relatives of patients with systemic CTD,[12,156] patients taking drugs that are known to induce SLE (who do not have manifestations of CTD),[68-83,157] and healthy persons.[9,11,12,153] The incidence of positive ANA in healthy persons at various titers is shown in Table VIII.[9] Accordingly, a titer of 1:80 or less is of no diagnostic value because of the high prevalence of positive ANA tests at such titers in the general population. A reasonable cut-off point is around 1:160 to 1:320. An ANA test at such titers or higher may help confirm the clinical diagnosis of a CTD. There are, however, healthy persons who have ANA titers above 1:320. The diagnosis of a CTD should not be made solely on the titer of an ANA test.

ANA patterns. The patterns of fluorescence of the nuclei in an ANA test are usually associated with specific antinuclear antibodies (Table IX) (Fig 2).[10,11,14,20,158-161] For example, the peripheral or rim pattern is associated with antibodies to nDNA and thus correlates with the diagnosis of SLE. The homogeneous pattern is associated with antibodies to nDNA or antibodies to histones, which are seen frequently in patients with SLE.

ANA-negative SLE

ANA-negative SLE was reported in patients who had cutaneous and/or systemic manifestations of SLE, but who were negative by ANA testing on animal substrates.[162,163] Most of these patients were later found to have positive ANA on human substrate. Many of these patients had photosensitivity and some of them were later reported as having SCLE with anti-Ro(SS-A) antibodies.[163] Another reason for the ANA test to be negative in a patient with SLE is if the patient's ANAs are solely against ssDNA. Because the fluorescent ANA substrate has intact nuclei without single strands of DNA, the test is expectedly negative.

DIAGNOSTIC VALUE OF THE FLUORESCENT ANA TEST

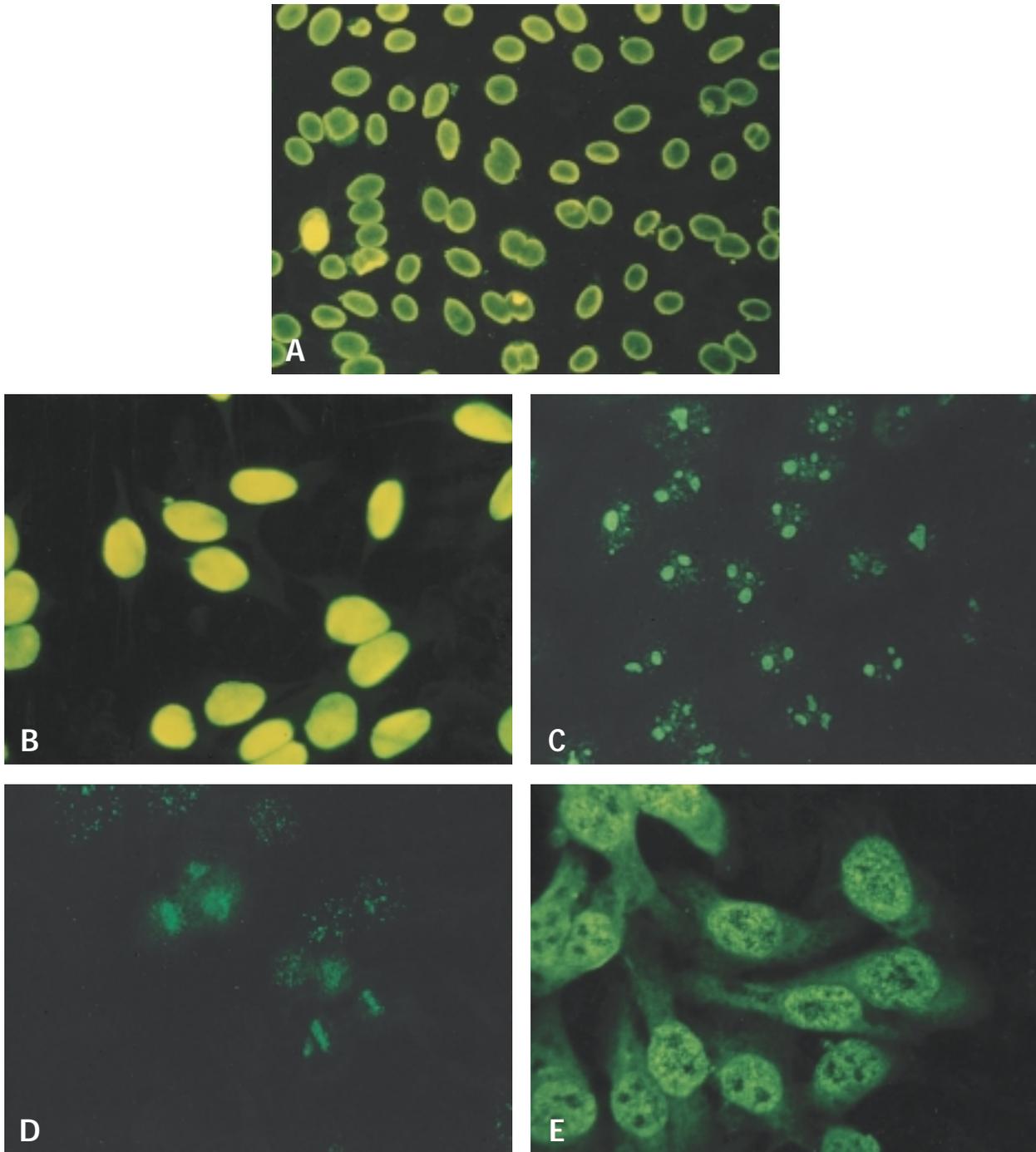There are several parameters that indicate the

Fig 2. The different patterns of fluorescence on HEp-2 cells include **(A)** peripheral, **(B)** homogeneous, **(C)** nucleolar, **(D)** centromere, and **(E)** speckled.

value of a certain test. These include sensitivity, specificity, positive predictive value, negative predictive value, and marginal benefit. Sensitivity refers to the probability of a test to have a positive result in a patient with the disease (true positives ÷ [true positives + false negatives]).[12] Specificity refers to the probability of a test to have a negative result in a person without disease (true negatives ÷ [true negatives + false positives]).[12] Positive predictive value refers to the probability of a person with positive test to have disease (true positives ÷ [true positives + false positives]).[12] The positive predictive value is directly cor-

related with test sensitivity and prevalence of disease in the test population.[12,164] Negative predictive value refers to the probability of a person with negative test to be free of disease (true negatives ÷ [true negatives + false negatives]).[12] Tests with high specificity will have high predictive value when positive, since false positivity is very low. Tests with high sensitivity will have high predictive value when negative, since false negativity is very low. Marginal benefit of a test refers to the posttest disease probability compared with pretest probability.[165]

The value of the fluorescent ANA test in the diagnosis of SLE and other CTDs has been evaluated repeatedly. The primary focus of the published studies is on SLE. The sensitivity of ANA tests for SLE is very high. Almost all patients with SLE have positive ANA tests.[12,19,164,166] The negative predictive value for SLE is also very high. A patient with a negative ANA test is highly unlikely to have SLE.[12,164,166] The positive predictive value for SLE, however, is generally low, especially at low titers[12,164,166,167] because the specificity of the ANA test for SLE, especially at low titers, is low. As discussed earlier, a positive ANA test especially at a low titer may be seen in several conditions and persons without SLE or other CTDs.[11,12,68-73,75-83,153-157] In the case of a patient with clinical findings suggestive of SLE or other systemic CTDs in which the ANA test is negative or with a low titer, more selective testing for individual antinuclear antibodies (eg, DNA, ribonucleoprotein) may be helpful in confirming the diagnosis.

Of all the parameters to evaluate a test, the marginal benefit is of high practical value for the physician who is attempting to confirm or exclude a diagnosis by ordering a certain test. The marginal benefit of the fluorescent ANA test is minimal when the pretest probability of disease is very low or very high. For example, persons with no clinical findings to suggest SLE are highly unlikely to benefit from an ANA test. In such a setting, the test is almost invariably negative or with very low titer and thus will not confirm a diagnosis of SLE. Similarly, the marginal benefit from the fluorescent ANA test in a patient with the characteristic multiple organ involvement of SLE is low because the diagnosis is already known and the test will invariably be strongly positive. The marginal benefit of the fluorescent ANA test is maximal when the pretest probability of disease is intermediate.[168] For example, the diagnosis of a patient with some cutaneous and/or systemic manifestations suggestive of SLE may be confirmed or excluded by the result of an ANA test. A strongly positive ANA test will help confirm the diagnosis, whereas a negative test may exclude SLE. These observations were supported in a recent study in which the usefulness of the ANA test was investigated in a group of more than 1000 inpatients and outpatients in whom the ANA test was ordered.[164] One hundred fifty-three patients with a positive ANA test were compared with an equal number of patients with a negative ANA test. Patients with positive ANA were generally older than those with negative ANA. The ANA test was ordered primarily in patients suspected of having a CTD or vasculitis. The negative predictive value was 100% for SLE and 97% for other CTDs. The positive predictive value was 11% for SLE and 22% for other CTDs. The predictive value was lower for patients who were older than 65 years compared with those younger than 65 years. The conclusion of the study was that the diagnostic value of the ANA test depends on the clinical setting in which it is ordered,[164] and clinicians should be aware that in the setting of a low prevalence of CTD an ANA test's positive predictive value is low.

RECENT SCREENING ANA TESTS

In the past few years, attempts have been made to replace the fluorescent ANA test with ELISA screening tests. There have been many ELISAs that have been reported to be of value for screening ANA tests. Some of these ELISAs utilize extracts of tissue containing various nuclear components. Other ELISAs utilize molecules synthesized by recombinant technology. Some ELISAs utilize individual recombinant molecules such as Ro(SS-A), whereas others utilize combinations of various molecules to increase the sensitivity of the test. In a recent study, the performance of the various ELISA ANA tests was compared with the "gold standard" fluorescent ANA test.[169] Sera that were positive by fluorescent ANA test were tested by the various ELISA techniques. The agreement that a serum is ANA positive was 87% to 95% when comparing the various ELISA tests with the fluorescent ANA test results.[169] The sensitivity of the various ELISAs was 69% to 98% and the specificity ranged between 81% and 98%. These figures were arrived at using sera that were positive at 1:160 by the fluorescent ANA test. The above comparison figures were much lower for sera with fluorescent ANA titer of 1:40. Many ELISA techniques missed a low titer positive ANA as well as sera with specific ANAs (eg, anti-nDNA antibodies). Presently, ELISA screening ANA tests may be adequate to screen sera with intermediate to high titer.[169] It remains to be seen whether the performance of screening ANA tests by ELISA would match that by the fluorescent technique.

SEROLOGIC PROFILES IN CTDS

Each CTD has a rather specific autoantibody profile (Fig 1). Some of these profiles are "simple" in

Table X. Serologic profiles in CTDs[27]

Profile	nDNA	Sm	U$_1$RNP	Ro(SS-A)	La(SS-B)	Centromere	Scl-70	Histone	Disease
A	+	+							SLE
B	-	-	+						MCTD
C	-	-	-	+	+				SS, SCLE
D	-	-	-	-	-	+	+		SSc, CREST
E	-	-	-	-	-	-	-	+	Drug-SLE

SS, Sjögren's syndrome; *SCLE,* subacute cutaneous lupus erythematosus.

Table XI. Indications for APA testing*

Livedo reticularis
Purpura and necrosis
Ulcers
Internal organ thrombosis
Recurrent miscarriages
Screening in patients with SLE

*References 173, 174, 176, 182, 190, 191, 193, 194, 197, 198.

that they include one characteristic antibody (eg, anticentromere antibodies in patients with CREST[5,28,29,170,171] and anti-U$_1$RNP antibodies in patients with MCTD[15,23-25,127]). On the other hand, patients with SLE have a larger array of autoantibodies. Some of these antibodies are highly characteristic for SLE (nDNA antibodies[1,4,49,51,172] and Sm antibodies[15,16,104,107,134]), whereas others are less characteristic (screening fluorescent ANA test,[12,70,71,153-155] anti-Ro(SS-A) antibodies,[95-97,108-110] and U$_1$RNP antibodies[23,25,93]).

A recent study addressed the question whether the diagnosis of a CTD could be predicted among a group of patients suspected of having CTD and in whom extensive autoantibody testing was performed. The investigators created 5 profiles.[27] The 5 profiles are shown in Table X. Profiles were divided on the basis of positivity and negativity of individual ANA tests. Empty boxes in the table do not indicate negativity of those tests, but instead the irrelevance of the results of those tests. For example, profile A included patients who had antibodies to nDNA and/or antibodies to Sm.[27] These patients had SLE regardless of the results of their other ANA tests. Patients in profile B had antibodies to U$_1$RNP but were negative for nDNA antibodies and Sm antibodies. These patients had the diagnosis of MCTD or SLE.[27] The authors comment that these SLE patients with U$_1$RNP antibodies only may be classified by others as having MCTD. Patients in profile C were negative for antibodies to nDNA, Sm, and U$_1$RNP, but positive for antibodies to Ro(SS-A) and/or La(SS-B). These patients had either Sjögren's syndrome or SCLE.[27] Patients in profile D were negative for antibodies to nDNA, Sm, U$_1$RNP, Ro(SS-A), La(SS-B), and positive for antibodies to centromere and/or antibodies to Scl-70. These patients had SSc or CREST syndrome.[27] Finally, patients in profile D were negative for all antibodies except antihistone antibodies. Patients in this group had drug-induced SLE.[27] These data should be helpful to the practicing physician in the interpretation of the various ANA test results.

ANTIPHOSPHOLIPID ANTIBODIES

Antiphospholipid antibodies (APAs) are directed against negatively charged phospholipids, present in cell membranes.[58,173-177]

Testing technique

APAs are detected by various techniques. These antibodies cause the biologically false positive VDRL test for syphilis.[58,178,179] Thus VDRL is positive in many patients with APA. These patients will have negative fluorescent treponemal antibody test. In the 1950s, these antibodies were detected in the sera of patients with SLE, by their in vitro anticoagulant properties; thus the term *lupus anticoagulant* has been used[177,180] and remains one of the methods to assay for APA. Sera containing APAs delay the coagulation pathway of normal blood in vitro. It is interesting that the presence of the antibodies is associated clinically with thrombosis rather than bleeding diathesis. The most frequently used assay for APA is ELISA using bovine cardiolipin.[58,180-183] The term *anticardiolipin antibodies* is frequently used interchangeably with APAs. The sensitivity of the lupus anticoagulant assay and ELISA for anticardiolipin antibodies is 75% to 90% each.[174,176] It is interesting that some sera may be positive by one assay and negative by the other. Because of the high degree of sensitivity of the ELISA, it has been recommended as the screening test for APA. If the ELISA is negative in a patient who is highly suspected of having APA, the lupus anticoagulant assay may be obtained.[176,182]

Disease association

APAs are most prevalent in patients with SLE (approximately 50%).[58,173,174,178,184,185] Patients with other CTDs have a lower prevalence of these antibodies. APAs may also be seen in patients taking certain drugs (cocaine, interferon alfa, procainamide, hydralazine, phenothiazines, quinine, quinidine, fansidar, and phenytoin[182]), patients with chronic infections (syphilis, infectious mononucleosis, tuberculosis, leprosy, leptospirosis, malaria, typhus, trypanosomiasis,[173] schistosomiasis and filariasis,[186] cytomegalovirus infection,[187] HIV infection,[188] hepatitis C[189]), and occasionally in persons who do not have an associated condition (primary APA syndrome[174,190]). APAs have been associated with arterial and venous thrombosis in various organs, including the central nervous system, the heart, and the skin.[173,174,176,182,191-193] Young women with APAs are predisposed to recurrent miscarriages.[173,174,176,182,192] Many patients with APAs have thrombocytopenia. Patients with APAs who present to dermatological practice usually have livedo reticularis, purpura, necrosis, and ulcers.[182,190,193,194] The indications for obtaining APA testing are shown in Table XI.

Interpretation of results

The results of APA testing should be interpreted with caution. Low levels may be of no clinical relevance and should not be interpreted as the cause of leg ulcers and purpura in every patient who has low levels of antibodies.[195]

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■

Answers to CME examination

Identification No. 800-102

February 2000 issue of the Journal of the American Academy of Dermatology

Questions 1-29, Mutasim DF, Adams BB. J Am Acad Dermatol 2000;42:159-74.

1. c	16. c
2. c	17. a
3. c	18. c
4. b	19. c
5. b	20. a
6. d	21. a
7. e	22. a
8. c	23. c
9. d	24. a
10. e	25. d
11. b	26. a
12. d	27. e
13. d	28. b
14. b	29. c
15. d	

CME examination

Identification No. 800-102

Instructions for Category I CME credit appear in the front advertising section. See last page of Contents for page number.

Questions 1-29, Mutasim DF, Adams BB. J Am Acad Dermatol 2000;42:159-74.

Directions for questions 1-13: Give single best response.

1. Each of the following is true about antibodies in connective tissue diseases *except*
   a. the total amount of antibodies in a patient's serum is usually indicated by the titer.
   b. the specificity of each of the antibodies varies.
   c. antibodies are not found in healthy persons.
   d. the total amount of antibodies is larger in patients with connective tissue diseases compared with others.
   e. each connective tissue disease has a unique profile of antibodies.

2. Which of the following is true regarding radial immunodiffusion?
   a. It is less sensitive and less specific than enzyme-linked immunosorbent assay (ELISA).
   b. It is less sensitive and less specific than immunofluorescence.
   c. The diagnostic value of a positive test by radial immunodiffusion is higher than that by ELISA.
   d. Radial immunodiffusion will be positive even when small amounts of antibodies are presented.
   e. It is less subjective than ELISA.

3. Each of the following connective tissue disorders demonstrates a high incidence of anti-Ro(SS-A) antibodies *except*
   a. neonatal lupus erythematosus (LE)
   b. antinuclear antibody (ANA)–negative systemic LE (SLE)
   c. discoid LE
   d. mothers of infants with neonatal LE
   e. Homozygous C2 or C4 deficiency

4. Compared with radial immunodiffusion, characteristics of ELISA testing include each of the following *except*
   a. sensitive test
   b. specific test
   c. less labor intensive
   d. easy to screen large number of sera together
   e. less subjective

5. Which of the following is not an antibody to small ribonucleoproteins?
   a. Anti-Ro(SS-A) antibody
   b. Antihistone antibody
   c. Anti-nuclear ribonucleoprotein

   d. Anti-Sm antibody
   e. Anti-La(SS-B) antibody

6. Each of the following is true regarding native DNA (nDNA) antibodies *except*
   a. the ELISA for nDNA is more sensitive than indirect immunofluorescence.
   b. nDNA antibodies are characteristic of SLE.
   c. nDNA antibodies are associated with renal disease.
   d. nDNA antibodies detected by ELISA are diagnostic of SLE.
   e. the indirect immunofluorescence test is performed on *Crithidia.*

7. Each of the following statements is true *except*
   a. anti-Ro(SS-A) antibodies are associated with photosensitivity.
   b. anti-Ro(SS-A) antibodies are associated with subacute cutaneous LE.
   c. there is a genetic disposition for the presence of anti-Ro(SS-A) antibodies.
   d. anti-Ro(SS-A) antibodies are common in neonatal LE.
   e. anti-Ro(SS-A) antibodies can be detected reliably by the fluorescent ANA test.

8. Anti-Jo-1 antibodies are directed against
   a. topoisomerase
   b. gyrase
   c. histidyl transfer RNA synthetase
   d. phospholipase
   e. lysyl oxidase

9. ANA is *least* useful in evaluating
   a. patients with photosensitivity
   b. patients with chronic vasculitis
   c. patients undergoing phototherapy
   d. patients with facial eruptions
   e. patients with discoid LE

10. HEp-2 cells, used by many laboratories as a substrate for ANA testing, are obtained from
    a. mouse kidney
    b. rat liver
    c. hybridomas
    d. rat bladder
    e. cultured human cells

11. Which of the following statements about the ANA test is correct?
    a. The diagnostic value of the ANA test does not depend on the clinical presentation.

b. The positive predictive value of the ANA test for SLE is low.

c. The negative predictive value of the ANA test for SLE is low.

d. The marginal benefit of the ANA test is maximal when the pretest probability is low.

e. Tests with high sensitivity will have high predictive value when positive.

12. Regarding antiphospholipid antibodies, which of the following statements is true?
   a. The sensitivity of the ELISA is low.
   b. These antibodies are not related to false-positive VDRL.
   c. They are associated with a bleeding diathesis.
   d. In vitro these antibodies delay the coagulation pathway.
   e. These antibodies are directed against positively charged phospholipids.

13. Cutaneous manifestations of antiphospholipid antibody syndrome include each of the following *except*
   a. livedo reticularis
   b. ulcers
   c. purpura
   d. calcinosis
   e. necrosis

Directions for questions 14-17: For each numbered item, select the one lettered item that reflects the incidence of anti-Ro(SS-A) antibodies by radial immunodiffusion (each letter may be used once, more than once, or not at all).
   a. <5%
   b. 50%
   c. 70%
   d. 95%

14. Sjögren's syndrome

15. Neonatal LE

16. Subacute cutaneous LE

17. Systemic sclerosis

Directions for questions 18-20: For each numbered ANA pattern, select the one lettered answer that reflects the antigen closely associated with the ANA pattern (each letter may be used once, more than once, or not at all).
   a. Kinetochore
   b. Single-stranded DNA
   c. Double-stranded DNA

18. Peripheral

19. Homogeneous

20. Centromere

Directions for questions 21-24: For each numbered ANA pattern, select the one lettered item most closely related (each letter may be used once, more than once, or not at all).
   a. SLE
   b. Mixed connective tissue disease
   c. CREST syndrome
   d. Dermatomyositis

21. Peripheral

22. Homogeneous

23. Centromere

24. Nucleolar

Directions for questions 25-29: For each numbered item, select the one lettered item most closely related (each letter may be used once, more than once, or not at all).
   a. Anti-Jo-1 antibodies
   b. Anti-Ro(SS-A) antibodies
   c. Anti-Scl-70 antibodies
   d. nRNP antibodies
   e. Antihistone antibodies

25. Mixed connective tissue disease

26. Dermatomyositis

27. Drug-induced SLE

28. Neonatal LE

29. Systemic sclerosis