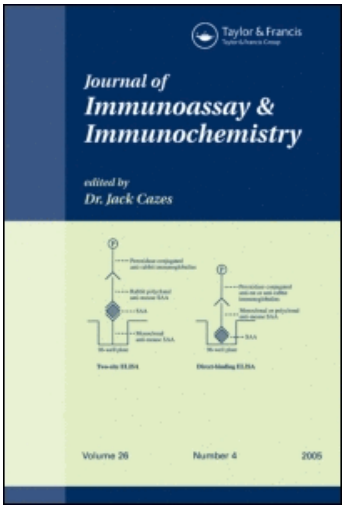

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

A Comparison of Two Fixatives on IFA HEp-2 Slides for the Detection of Antinuclear Antibodies

Napoleon M. Moncé[a]; Virginia L. Cappel[a]; Cora B. Saqueton[a]
[a] Helix Diagnostics, Inc., West Sacramento, CA

To cite this Article Moncé, Napoleon M. , Cappel, Virginia L. and Saqueton, Cora B.(1994) 'A Comparison of Two Fixatives on IFA HEp-2 Slides for the Detection of Antinuclear Antibodies', Journal of Immunoassay and Immunochemistry, 15: 1, 55 — 68

To link to this Article: DOI: 10.1080/15321819408009571

URL: http://dx.doi.org/10.1080/15321819408009571

JOURNAL OF IMMUNOASSAY, 15(1), 55-68 (1994)

A COMPARISON OF TWO FIXATIVES ON IFA HEp-2 SLIDES FOR THE DETECTION OF ANTINUCLEAR ANTIBODIES

Napoleon M. Moncé, Jr., Virginia L. Cappel, and Cora B. Saqueton
Helix Diagnostics, Inc., 3148 Industrial Blvd.
West Sacramento CA. 95691

ABSTRACT

A comparative evaluation of two fixatives on HEp-2 slides that detect antinuclear antibodies via indirect immunofluorescence was undertaken. The sensitivities of these two methods were compared to determine which of the two is more efficient in screening for anti-SS-A (Ro) antibodies. Fixing HEp-2 cells with a pure acetone solution resulted in a 97.5% sensitivity when anti-SS-A (Ro) positive samples were tested while only an 81.3% sensitivity was seen on HEp-2 cells fixed in an alcohol/acetone solution when detecting anti-SS-A (Ro) antibodies. In sera with only anti-SS-A (Ro) antibodies present, the fluorescence was more pronounced on the acetone fixed slides which made it easier to read than the alcohol/acetone fixed slides.

(KEY WORDS: Antinuclear Antibody, Immunofluorescence, SS-A (Ro), Fixation, HEp-2, SLE, Neonatal Lupus)

INTRODUCTION

Antinuclear antibodies (ANAs) directed against a variety of macromolecules occur in extraordinarily high frequency in systemic rheumatic diseases. ANAs are a spectrum of autoantibodies that react with various nuclear molecules including

deoxyribonucleic acid (DNA), ribonucleic acid (RNA), histones, acidic nuclear proteins or complexes of these molecular elements (1). Many of the rheumatic diseases are charaterized by the presence of one or more of these antinuclear antibodies (2). Because many ANAs are relatively specific serologic markers for particular systemic rheumatic diseases, identification of the specific antibody is useful in the diagnosis, management, and treatment of the disease.

Several techniques have been developed for the detection of ANAs. These include hemagglutination, immunodiffusion, enzyme immunoassays (EIA) and indirect immunofluorescence (IFA) tests (3-6). Many laboratories first use the IFA technique to screen sera before other techniques are used to define antibody specificity because IFA detects a variety of antibodies to different nuclear antigens (7).

Recently, negative results of IFA tests have been observed in patients with Systemic Lupus Erythematosus (SLE), Sjogren's Syndrome, Rheumatoid Arthritis (RA), Polymyositis and Scleroderma (2,8,9). The reason for these IFA ANA-negative/disease state positive results, is the inability of the IFA test to detect antibodies to a particular nuclear antigen known as SS-A (Ro) (10).

The Ro antigen was first described in 1969 by Clark et al. in patients with connective tissue diseases, the majority of whom had SLE and Sjogren's Syndrome (11). In 1975, sera from patients with Sjogren's Syndrome were shown to contain an antibody which reacted with soluble extracts from human lymphocyte cell line and the antigen was designated SS-A. Later, it was found that the Ro antigen was identical with the SS-A antigen. The SS-A (Ro) antigen is described as a soluble nuclear non-histone protein antigen consisting of two proteins of 60 and 52 kilodaltons complexed with four to five small cytoplasmic RNA's called YRNA's (12), and is present in several diseases (Table 1).

The inabililty of the IFA test to detect antibodies to the SS-A (Ro) antigen may be due to the diverse array of fixatives used. The SS-A (Ro) particle's antigenicity is partially destroyed during substrate fixation (2). Reagent alcohol, acetone, ethanol, methanol, periodate/lysine/paraformaldehyde (PLP) and mixtures of these solutions are the more commonly used fixatives (13,25,30). The effects of these fixatives on HEp-2 cells have been studied (10,30,31). It has been found that alcohol, when used as the fixative alone or when mixed with other fixatives like acetone, diminishes the cells staining intensity with anti-SS-A (Ro) sera (10). Similar results were seen when either methanol or ethanol was used as the fixative. Fixation in PLP resulted in a non-specific immunofluorescent pattern of the entire cell when anti-SS-A (Ro) sera was tested (25). The best result was seen when

TABLE 1

Percentage of SS-A antibodies detected in certain clinical disease.

CLINICAL ASSOCIATION	PERCENTAGE	REFERENCE(S)
ANA Negative SLE	60-70%	10, 13-15
Cerebral spinal fluid of patients with diffuse cerbral vasculitis who displayed Moya Moya	----	2
Chronic Hepatitis	----	1, 12
Extraglandular disease	40-62%	10, 16
Homozygous Complement C2 and C4 deficiency	75-80%	10, 12, 14, 15, 17
Infants with mothers who have SLE	90-95%	15, 17
Leukopenia	----	10
Lupus Nephritis	40-50%	10, 14, 17
Lympadenopathy	----	10
Mothers giving birth to infants with congenital heart block	77-83%	9, 18
Neonatal Lupus Erythematosus	----	2, 10, 12, 14, 19-21
Polymorphous Light Eruption (PMLE)	22%	22
Polymyositis	----	16
Primary Biliary Cirrhosis	19%	12, 13, 16, 17
Rheumatoid Arthritis (RA)	5-10%	1, 16
Rheumatoid Arthritis and SS	39%	13
Scleroderma	20%	1, 16
Sjogrens Syndrome (SS)	40-70%	1, 8, 10, 13-15, 23-26
Subcutaneous Lupus Erythematosus (SCLE)	63-70%	10, 12, 13, 15, 21
Systemic Lupus Erythematosus (SLE)	25-40%	1, 8, 9, 14, 21, 24, 25
SLE with interstitial pneumonitis	----	10
SS and SLE	58%	13
Vasculitis	----	10, 14, 16, 21

acetone alone was used as the fixative. Anti-SS-A (Ro) sera exhibited discrete nuclear speckles with no cytoplasmic staining when acetone alone was used as a fixative.

The goal of this investigation is to perform a detailed analysis of anti-SS-A (Ro) sera that yield discordant immunofluoresent results between HEp-2 slides fixed in acetone and slides fixed in an alcohol/acetone mixture in order to further define the sensitivity of the immunofluorescence test.

MATERIALS and METHODS

Serum Samples

Human serum samples were obtained from several medical centers and reference laboratories. Samples were from patients on whom previous ANA tests had been performed as requested by physicians. Twenty one were purchased characterized serum samples along with 30 normal samples obtained from a local blood bank. Samples were aliquoted and stored at -20 degrees Celcius until tested.

Immunofluorescence HEp-2 Slides

Heavy teflon coated autoclavable slides were used to culture HEp-2 cells (Cell Line Associates, Inc. New Field, NJ). The cells were grown in Eagle's minimal essential medium (MEM) supplemented with fetal bovine serum (JRH Biosciences, Lenexa, Kansas). The slides were then transfered into racks and fixed accordingly.

Acetone Fixation

This fixative consist of a 99% HPLC grade acetone solution (Baxter Diagnostics, McGaw Park, IL.). Slides were fixed in this solution for five minutes and fan dried.

Alcohol/Acetone Fixation

This fixative consists of 10% reagent alcohol (an Ethyl-Methyl-Isopropyl mixture)

and 90% acetone mixture (Baxter Diagnostics, McGaw Park, IL.). Slides were fixed in this mixture for five minutes and fan dried.

Immunofluorescence Test Procedure

Detection of ANA pattern and titer was accomplished via HEp-2 indirect immunofluorescence (Helix Diagnostics, Inc. West Sacramento, CA). The antibody measurements were run according to the package insert instructions. In general, serial dilutions of patient samples starting at 1:40 were done using phosphate buffered saline (PBS). Reaction wells were layered with 0.02ml of each dilution and allowed to incubate at room temperature in a moist chamber for 30 minutes. Each slide contained a positive and negative control. After this initial incubation period, slides were washed with a strong stream of PBS and layered with fluorescent conjugated goat antihuman IgG (heavy and light chains) and incubated again for 30 minutes at room temperature in a moist chamber. After this incubation period, slides were again washed with a strong stream of PBS and mounted with glycerol, coverslipped, and read. Slides were read on the same day they were prepared using an Olympus BH2 fluorescent microscope with a 100 watt high pressure mercury light source, a dichroic beam splitter, and a BP-490 exciter filter. A serum is considered positive when the fluorescent intensity of the cell's nuclei is greater than the negative control and there is a clearly discernible pattern in the nucleus.

Hemagglutination Procedure

Antibodies against SS-A (Ro), SS-B(La), Sm, and Sm/RNP were determined using hemagglutination tests (Hemagen Diagnostics, Waltham, MA). The test was run according to the package insert instructions provided with each kit.

In general, samples were diluted 1:50, 1:100, and 1:200 in SeraTest Serum Diluent. 0.05ml of each dilution was added into a V-well microtiter plate. Each plate contained a positive and negative control (included in the kit). One free-falling drop of Sera Cell (red cell suspension) was then added into each well. After a 90 minute undisturbed incubation period at room temperature, each sample was read according to the criteria given.

Western Blot Assay

This assay was performed to confirm the presence of antibodies against SS-A (Ro). SS-A antigen coated nitrocellulose strips and reagents were provided by ImmunoVision, Inc. Springdale, AR. The assay run according to the package insert instructions provided. In general, a 1:100 dilution of the samples were added to the nitrocellulose strips and allowed to incubate for one hour at room temperature. After washing the strips with wash buffer, the conjugate solution was added to the strips and again allowed to incubate for one hour at room temperature. After another washing of the strips, the color development solution was added to the strips. A serum is considered positive if a visible band is present on the nitrocellulose strip.

EIA ANA Procedure

Samples were screened for the presence of ANAs using a commercial enzyme immunoassay antinuclear antibody (EIA ANA) screening test (Helix Diagnostics, Inc. West Sacramento, CA). In general, after 1:40 dilutions of patient samples and controls with serum diluent were done, 0.1ml of each was added into antigen coated polystyrene microtiter wells and allowed to incubate for 30 minutes at room temperature. Wells were then washed five times with PBS-Tween. 0.1ml of horse radish peroxidase (HRP) conjugated antihuman IgG was then added to each well. After another 30 minute incubation period, wells were again washed as before. 0.1ml of color development solution was then added to each well and the reaction was stopped after 10 minutes by adding 0.1ml of stopping solution. Wells were read using a Bio Tek microplate reader set at 450nm with a maximum optical density (O.D.) reading capability of 2.000. A serum is considered positive when the absorbance is greater or equal to the absorbance of the endpoint cutoff control.

EIA Anti-dsDNA Test Procedure

Detection of antibodies to double stranded DNA (dsDNA was accomplished using an anti-dsDNA test (Helix Diagnostics, West Sacramento, CA). The procedure is similar to that of the EIA ANA assay except that patient samples and

controls are diluted 1:100. The antigen used is a highly purified plasmid dsDNA bound to microtiter wells.

RESULTS

Serum samples (n=345) were randomly selected then screened for the presence of antinuclear antibodies using the EIA ANA screening test (5). Of the 345 samples tested, the EIA ANA screening test showed 254 samples to be positive for ANAs and 91 samples to be negative for ANAs. The EIA ANA positive samples were then screened for the presence of SS-A (Ro) antibodies using a commercial anti-SS-A (Ro) hemagglutination test. Of the 254 EIA ANA positive samples, 46 were positive on the anti-SS-A hemagglutination assay. An anti-SS-A western blot assay was performed on the positive hemagglutination samples to confirm the presence of anti-SS-A antibodies. Of the 46 hemagglutination anti-SS-A positives, 39 displayed a visible anti-SS-A band on the western blot assay (Table 2). These 39 anti-SS-A (Ro) positive samples, along with 30 normal blood bank donor samples, were then tested on immunofluorescence test utilizing the alcohol/acetone fixed and acetone fixed slides. Both slide fixations were done at the same time to reduce any variability. Two different technologists were used to blindly read the IFA slides in order to determine any inter-observer variation. With the exception of three pattern conflicts, the results of both technologist were similar.

Of the 39 sera with anti-SS-A (Ro) antibody, nine (22.5%) failed to demonstrate an IFA staining pattern on the alcohol/acetone fixed slides, while only one (2.6%) failed on the acetone fixed slides (Table 3). Thirty samples showed positive fluorescence and one showed no fluorescence on both the acetone fixed and alcohol/acetone fixed slides. However, the acetone fixed slides did detect eight additional anti-SS-A (Ro) positive samples which the alcohol/acetone fixed slides called negative (Table 3). Defining sensitivity as the ability of a test to give a positive result for positive samples, [true positive/true positive + false negative (TP/TP + FN)] we found the acetone fixed slides to be 97.5% sensitive for the detection of anti-SS-A (Ro) antibodies and only 81.3% sensitive with the alcohol/acetone fixed slides.

To further identify the 39 anti-SS-A (Ro) positive samples, an anti-dsDNA EIA test was performed along with hemagglutination tests for the presence of SS-B (La), Sm, and Sm/RNP antibodies. Of the 39 anti-SS-A (Ro) positive samples, 10 (25.6%) were also positive for dsDNA, 14 (35.9%) for SS-B (La), 1 (2.6%) for

TABLE 2

Results of the 345 serum samples.

	ANA POSITIVE	ANA NEGATIVE
EIA ANA Screen	254	91
	SS-A Positive	SS-A Negative
Hemagglutination anti-SS-A †	46	208
Western Blot anti-SS-A *	40	6

†Testing the EIA ANA positive samples.
*Testing the anti-SS-A hemagglutination positive samples for confirmation.

TABLE 3

2x2 table comparing acetone fixed IFA slides and alcohol/acetone fixed IFA slides on SS-A positive sera.

		ACETONE FIXED +	ACETONE FIXED -
ALCOHOL/ACETONE FIXED	+	30	0
	-	8	1

Sm, 6 (15.2%) for Sm/RNP, with 17 (43.6%) samples being mono-specific for the anti-SS-A (Ro) antibody (table 4). We found that the majority of the samples with more than one antibody present were easily detected on both the alcohol/acetone fixed slides and the acetone fixed slides. Seven of the nine samples that the alcohol/acetone fixed slides called negative were mono-specfic for the anti-SS-A (Ro) antibody, with the remaining two also having anti-SS-B (La) antibodies present (Table 4). Both fixatives resulted in no fluorescence when the 30 normal blood bank donor samples were tested.

The majority of the 39 anti-SS-A (Ro) samples showed a speckled IFA pattern, however, homogeneous and nucleolar patterns were also observed. The samples which were previously characterized for antibody specificity (anti-SS-B, anti-Sm, anti-RNP, and anti-dsDNA), and IFA patterns (homogeneous, speckled, nucleolar, centromere, and peripheral) were tested on both fixatives. No distinct differences were seen when both the specific antibody samples and different IFA patterns were tested. However, the acetone fixed slides showed a brighter and easier to read fluorescence resulting in higher IFA titers for a few samples (Table 4).

DISCUSSION

Detection of antinuclear antibodies (ANAs) in human serum has proven useful in the confirmation of autoimmune disease diagnoses. Consequently, sensitive methods for the detection and characterization of these antibodies are required. The indirect immunofluorescent assay (IFA) is widely used in the clinical laboratory to detect the presence of ANAs. In fact, in most laboratories, the detection of ANAs by IFA is the initial screening test. Recently however, negative results of standard IFA tests have been observed in patients with Sjogren's Syndrome, SLE, RA, Polymyositis and Scleroderma. The inability of the IFA test to detect anti-SS-A (Ro) antibodies may account for a significant percentage of these "ANA-negative" cases.

The SS-A (Ro) antigen is described as a soluble nuclear non-histone protein present on subcellular ribonucleoprotein particles which is associated with numerous diseases (Table 1). Therefore, it is important to detect SS-A (Ro) antibodies to aide in the diagnosis, management and treatment of the disease.

The inablilty of the IFA test to detect antibodies to the SS-A (Ro) antigen may be due to the substrates used. Cultured human epithelial (HEp-2) cells and cryostat frozen tissue sections from rat kidney and mouse liver are among the more commonly used substrates. However, cryostat tissue sections may be of variable thickness, making interpretation of patterns more difficult and the SS-A (Ro) antigen is absent or in very low concentration in cells of mouse, rat, hamster and chicken. Cultured HEp-2 cells are more sensitive and reliable than cryostat tissue sections. Cultured HEp-2 cells are capable of detecting most nuclear and cytoskeletal related ANAs and can also differentiate among cytoplasmic antigens such as ribosomes, mitochondria and golgi apparatus.

TABLE 4

Characteristics of the sera studied for anti-SS-A (Ro) and other antibodies.

Sample Number	Alcohol/Acetone Fixed IFA Titer & Pattern	Acetone Fixed IFA Titer & Pattern	Other Antibodies Detected*
1	Negative	Negative	SS-B
2	Negative	**Speckled 1:40**	None
3	Negative	**Speckled 1:40**	None
4	Negative	**Speckled 1:40**	None
5	Negative	**Speckled 1:80**	None
6	Negative	**Speckled 1:80**	None
7	Negative	**Speckled 1:80**	None
8	Negative	**Speckled 1:160**	SS-B
9	Negative	**Speckled 1:80**	None
10	Speckled 1:80	Speckled 1:80	SS-B
11	Nucleolar 1:≥640	Nucleolar 1:≥640	None
12	Speckled 1:≥640	Speckled 1:≥640	Sm/RNP
13	Homogeneous 1:≥640	Homogeneous 1:≥640	SS-B, Sm/RNP, dsDNA
14	Speckled 1:160	Speckled 1:160	SS-B
15	Nucleolar 1:160	Nucleolar 1:160	None
16	Speckled 1:320	Speckled 1:320	SS-B
17	Speckled 1:160	**Speckled 1:320**	None
18	Speckled 1:80	**Speckled 1:160**	SS-B
19	Speckled 1:160	**Speckled 1:320**	None
20	Atypical cytoplasmic	Atypical cytoplasmic	SS-B
21	Nucleolar 1:160	Nucleolar 1:160	None
22	Homogeneous 1:160	Homo/Speck 1:160	None
23	Speckled 1:160	Speckled 1:160	SS-B
24	Speckled 1:40	**Speckled 1:80**	None
25	Speckled 1:80	Speckled 1:80	None
26	Speckled 1:80	**Speckled 1:160**	None
27	Speckled 1:80	Speckled 1:80	SS-B, dsDNA
28	Speckled 1:160	Speckled 1:160	SS-B
29	Speck/Nuc 1:320	Speckled 1:320	SS-B
30	Homogeneous 1:160	Homogeneous 1:160	Sm/RNP, dsDNA
31	Homogeneous 1:320	Homogeneous 1:320	SS-B
32	Homogeneous 1:320	Homogeneous 1:320	Sm/RNP, dsDNA
33	Speckled 1:≥640	Speckled 1:≥640	Sm/RNP, dsDNA
34	Homogeneous 1:320	Homogeneous 1:320	None
35	Homogeneous 1:320	Homogeneous 1:320	SS-B, dsDNA
36	Homogeneous 1:320	Homogeneous 1:320	Sm, dsDNA
37	Speckled 1:320	Speckled 1:320	dsDNA
38	Homo/Speck 1:320	Speckled 1:320	Sm/RNP, dsDNA
39	Speckled 1:320	Speckled 1:320	SS-B, dsDNA

*Sera tested for antibodies to SS-B, Sm, and Sm/RNP by hemagglutination and for anti-double stranded DNA (anti-dsDNA) by ELISA anti-dsDNA.

In this study, we compared two solutions commonly used as fixatives for IFA HEp-2 substrates to determine which would detected anti-SS-A (Ro) sera more effectively. We initially screened 345 serum samples using a commercial EIA ANA screening test and found 245 samples to be positive. The 254 EIA ANA positives were then tested on a commercial hemagglutination anti-SS-A (Ro) assay, and confirmed by western blot, resulting in 39 anti-SS-A (Ro) positive samples (Table 2). These 39 anti-SS-A (Ro) positive samples were then tested on IFA HEp-2 slides fixed in an acetone solution and on slides fixed in an alcohol/acetone mixture (10% alcohol - 90% acetone). The acetone fixed slides detected 38 of the 39 anti-SS-A (Ro) positives while the alcohol/acetone fixed slides only detected 30 of the 39 anti-SS-A (Ro) positive samples (Table 3). The sensitivities for these two fixatives were calculated to be 97.5% and 81.3%, respectively.

Anti-dsDNA EIA and hemagglutination tests detected antibodies for dsDNA, SS-B (La), Sm, and Sm/RNP in 22 of the 39 samples with the remainder being mono-specific for anti-SS-A (Ro) antibodies (Table 4). The alcohol/acetone fixed slides failed to detect seven of these mono-specific anti-SS-A samples and none were missed by the acetone fixative slides

The acetone fixation method does not adversly affect the results of sera positive for other antibodies. In fact, when characterized sera for antibody specificity and IFA patterns were tested, the acetone fixation method resulted in a brighter and easier to read fluorescence.

In 1991, Bylund and Nakmura suggested that each clinical laboratory develop an IFA test quality assurance program to detect anti-SS-A (Ro) antibodies (10). Each laboratory using IFA tests should also determine from the manufacturer the type of substrate and fixative being used. As we have demonstrated from our results, IFA HEp-2 slides fixed in an acetone solution provides a significant improvement in screening for ANAs and provides a greater sensitivity in detecting sera positive for anti-SS-A (Ro) antibodies. In conclusion, fixation in a pure acetone solution will decrease the number of IFA false negatives seen in many laboratories and may therefore decrease a portion of the so called "ANA-negatives".

ACKNOWLEDGEMENTS

The authors wish to thank Kevin Clark and the staff at ImmunoVision for their donation of the Western Blot assay.

Address all correspondences and reprint request to:

Napoleon M. Moncé, Jr.

3148 Industrial Blvd.

West Sacramento, CA

95691

REFERENCES

1. White RH, Robbins DL: Clinical significance and interpretation of ANA. Western Journal of Medicine. 1987; 147: 210-213.

2. Mongey AB, Hess EV: Antinuclear antibodies and disease specificity. Adv Intern Med. 1991; 36: 151-169.

3. Sharp GC, Irvin WS, LaRoque R, Velez C, Daly V, Kaiser A, Holman A: Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. J Clin Invest. 1971; 50: 350-359.

4. Nakamura RM, Greenwald CA, Peebles CL, Tan EM (eds.): Autoantibodies to Nuclear Antigens (ANA): Advances in Laboratory Tests and Significance in Systemic Rheumatic Diseases. American Society of Clinical Pathologist Press, Chicago, Illinois, 1978.

5. Moncé NM, Bogusky RT, Cappel NN: An enzyme immunoassay screening test for the detection of total antinuclear antibodies. J Clin Lab Anal. 1991; 5: 439-442.

6. Friou GJ: Antinuclear Antibodies: Diagnostic Significance and Methods. Arthr Rheum. 1967; 10: 151-159.

7. McDougal JS, Kennedy S, McDuffie FC: Standards and quality assurance for immunologic tests. Standards and reference materials for autoantibodies to nuclear antigens. In: Diagnostic Immunology: Technology Assessment and Quality Assurance. College of American Pathologist. 1983.

8. Nakamura RM, Tan EM: Autoantibodies to nonhistone nuclear antigens and their clinical significance. Hum Path. 1983; 14(5): 392-400.

9. Nakamura RM, Tan EM: Recent advances in laboratory tests and the significance of autoantibodies to nuclear antigens in systemic rheumatic diseases. Clinics in Lab Med. 1986; 6(1): 41-53.

10. Bylund DJ, Nakamura RM: Importance of detection of SS-A/Ro autoantibody in screening immunofluorescence test for autoantibodies to nuclear antigens. J Clin Lab Anal. 1991; 5: 212-218.

11. Clark G, Reichlin G, Tomasi TB: Characterization of a soluble cytoplasmic antigen reactive with sera form patients with SLE. J Immunol. 1969; 102: 117-122.

12. Tan EM: ANA: Diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol. 1989; 44: 93-151.

13. Harmon C: ANA in autoimmune disease: significance and pathogenicity. Med Clin North America. 1985; 69: 547-563.

14. Reichlin M: Molecular definition of the Ro(SSA) particles(s): A Frequent target of autoimmunity in Systemic Lupus Erythematosus and Sjogren's Syndrome. British J Rheum. 1991; 30 (suppl 1): 58-62.

15. Provost TT, Levin LS, Watson RM, Mayo M, Ratrie H: Detection of anti-Ro (SSA) antibodies by gel double diffusion and a 'sandwich' ELISA in Systemic and Subacute Cutaneous Lupus Erythematosus and Sjogren's Syndrome. J Autoimmun. 1991; 4: 87-96.

16. Reichlin M, Wasicek CA: Clinical and biologic significance of antibodies to Ro/SSA. Human Path. 1983; 14: 401-405.

17. Reichlin M, Harley JB: Antibodies to Ro(SSA) and the heterogeneity of SLE. J Rheumatol. 1987; 14 (suppl 13): 112-117.

18. Lipsmeyer EA: Significance of a positive ANA: A clinical review. J Arkansas Med Society. 1988; 85(1): 51-53.

19. Litsey SE, Noonan JA, O'Connor WN, Cottrill CM, Mitchell B: Maternal connective tissue disease and congenital heart block. N Engl J Med. 1985; 312: 98-100.

20. Lieu TS, Reimer CB, Sontheimer RD: Immunoglobulin class and subclass profile of the Ro/SS-A autoantibody response. J Invest Dermat. 1988; 90(2): 158-164.

21. Boire G, Lopez-Long FJ, Lapointe S, Menard HA: Sera form patients with autoimmune disease recognize conformational determinants on the 60-kd Ro/SS-A protein. Arth Rheum. 1991; 34(6): 722-730.

22. Kiss M, Husz S, Dobozy A: The occurence of antinuclear, anti-SSA/Ro and anti-SSB/La antibodies in patients with Polymorphous Light Eruption. Acta Derm Venereol (Stockh). 1991; 71: 341-343.

23. Alspaugh M, Tan EM: Antibodies to cellular antigens in Sjogren's Syndrome. J Clin Invest. 1975; 55: 1067-1073.

24. Tan EM: Autoantibodies to nuclear antigens (ANA): Their immunobiology and medicine. Adv Immunol. 1982; 33: 167-240.

25. Harmon CE, Deng J, Peebles CL, Tan EM: The importance of tissue substrate in the SS-A/Ro antigen-antibody system. Arth Rheum. 1984; 27(2): 166-173.

26. Cleymaet JE, Nakamura RM: Indirect immunofluorescent antinuclear antibody test: Comparison of sensitivity and specificity of different substrates. Am J Clin Pathol. 1972; 58: 388-393.

27. Lubiniecki AS, Dorsch CA: Comparison of mouse liver sections and cultured fibroblasts as substrates for the detection of antinuclear antibodies. Am J Clin Pathol. 1976; 66: 892-896.

28. Hahon N, Eckert HL, Stewart J: Evaluation of cellular substrates for antinuclear antibody determination. J Clin Microbiol. 1975; 2: 42-45.

29. McCarty GA: Autoantibodies and their relation to rheumatic diseases. Med Clin N Amer. 1986; 70: 237-261.

30. Prost AC, Abuaf N, Rouquette-Gally AM, Homberg JC, Combrisson A: Comparing HEp-2 cell line with rat liver in routine screening test for antinuclear and antinucleolar autoantibodies in autoimmune diseases. Ann Biol Clin. 1987; 45: 610-617.

31. Chan PK, Aldrich MB, Yung BJM: Nucleolar protein B23 translocation after doxorubicin treatment in murine tumor cells. Cancer Res. 1987; 47: 3798-3801.