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2 Alamanos, Y., Voulgari, P. V., et al., Survival and mortality rates of systemic lupus erythematosus patients in northwest Greece. Study of a 21-year incidence cohort. *Rheumatology.* 2003;42(9):1122-1123.

3 Arbuckle, M. R., McClain, M. T. et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *New England Journal of Medicine*. 2003;349(16):1526-1533.

4. Pollock W, Toh BH. Routine immunofluorescence detection of Ro/SS-A autoantibody using HEp-2 cells transfected with human 60 kDa Ro/SS-A. *J.Clin.Pathol*. 1999;52:684-687.

5. Bossuyt X, Meurs L., et al., Screening for autoantibodies to SS-A/Ro by indirect immunofluorescence using HEp-2000® cells. *Ann Clin Biochem*. 2000;37:216-219.

6. Fritzler MJ, Hanson C., et al., Specificity of autoantibodies to SS-A/Ro on a transfected and overexpressed human 60 kDa Ro autoantigen substrate. *J.Clin.Lab.Anal*. 2002;16:103-108.

7. Bossuyt X, Frans J., et al. Detection of Anti-SSA Antibodies by Indirect Immunofluorescence. *Clin Chem*. 2004;50(12):2361-2369.

Suggested Reading

Testing Guidelines

1. Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. *Arch.Pathol.Lab.Med.* 2000;124:71-81.

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4. Tozzoli R, Bizzaro N, Tonutti E, et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *Am.J.Clin.Pathol.* 2002;117:316-324.

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HEp-2000®

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12. Bossuyt X, Frans J, Hendrickx A, et al. Detection of Anti-SSA Antibodies by Indirect Immunofluorescence. *Clin Chem.* 2004;50(12):2361-2369.

immuno concepts

Part No. IC000779

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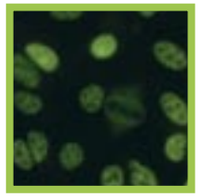
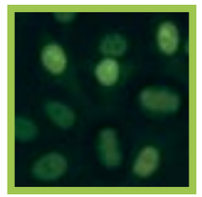
Fluorescent Patterns

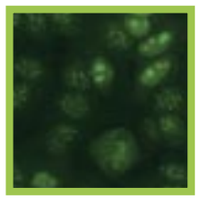
Associated with Antinuclear Antibody Detection



Speckled and SSA/Ro



Homogeneous and SSA/Ro



Centromere and SSA/Ro

SSA/Ro

immuno concepts

Systemic Rheumatic Disease

Identification of the antinuclear antibody (ANA) pattern remains a crucial step in the process of diagnosing the systemic rheumatic diseases. ANA patterns often give the clinician insight into which autoantibodies are present and indications of disease likelihood.

It has now been over 55 years since the LE cell was first described by Hargraves (1). During this time, substantial improvements in physician awareness, diagnostics and treatments have increased the survival rate for lupus patients from a 5 year rate of only 50% to a 10 year rate of >90% (2).

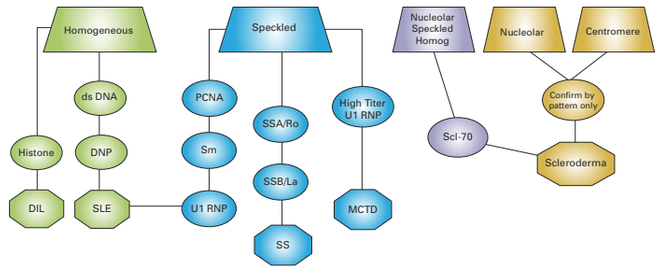
There is growing evidence that the appearance of autoantibodies may precede the onset of the disease, often by many years. Early detection of these autoantibodies may offer the opportunity for earlier diagnosis and treatment, improving the length and quality of life for the patient.

A recent study in the New England Journal of Medicine reported that, on average, at least one antinuclear antibody was present 3 years prior to the clinical diagnosis of SLE being made and at the time of diagnosis there were often 3 autoantibodies present (3). The earliest antibodies detected were ANA's, anti-SSA/Ro and anti-SSB/La antibodies. These antibodies are readily detected and reported using the HEp-2000® slide based assay.

The HEp-2000® substrate is Immuno Concepts' patented ANA substrate that has been consistently proven in independent studies to be superior to standard HEp-2 for the detection and identification of ANA's (4-7). When the unique SSA/Ro pattern is present on the HEp-2000® substrate the laboratory can immediately report the presence of anti-SSA/Ro antibodies to the clinician, potentially accelerating the correct diagnosis for the patient.

Flow Chart

ANA Pattern and Confirmatory Testing

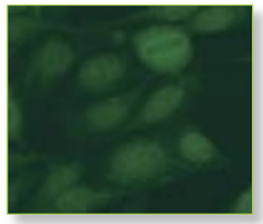


Antigen Chart

Associated with ANA Patterns

Pattern observed by indirect immuno fluorescence	Type of antibody	Disease in which antibodies seen	Characteristics of antigenic determinants	Tests used to confirm specific antibody
Homogeneous or peripheral	Double-stranded DNA (nDNA, dsDNA)	Characteristic of SLE, lower levels in other rheumatic diseases	Double-stranded DNA	IF using Crithidia luciliae, RIA, ELISA, HA, CF
	Deoxynucleoprotein, soluble form	SLE, Drug-induced LE	DNA-histone complex	LE cell prep, RIA, ID, HA
	Histone	Drug-induce LE, SLE, others	Different classes of histone	ELISA, RIA
Unusual Homogeneous	Nuclear Envelope or Nuclear Membrane	Lupoid hepatitis	Nuclear Lamins A, B, C	none
Speckled	Sm	Marker antibody for SLE	Core proteins of the U1, U2, U4, U5, U6, U7, U11, and U12 snRNPs	ID, ELISA, HA, CF
	U1-RNP	High levels in MCTD and SLE, low levels in other diseases	A, C, and 70kDa proteins complexed with U1 RNA	ID, ELISA, HA, CF
	SSA/Ro	High prevalence in Sjogren's syndrome sicca complex, lower prevalence in other rheumatic diseases	52 and 60 kDa proteins complexed with Y1-Y5 RNA's	Confirmatory with HEp-2000® staining pattern; rule out other ENAs with ID, ELISA, CIEP, IP, western blot
	SSB/La	High prevalence in Sjogren's syndrome sicca complex, lower prevalence in other rheumatic diseases	48 kDa protein complexed with RNA polymerase III	ID ELISA, CIEP, IP, western blot
	Scl-70	Marker antibody for Scleroderma	70 kDa protein of topoisomerase	ID, ELISA
	PCNA	Marker antibody for SLE	Auxiliary protein to DNA polymerase δ	ID, CIEP
	Matrix	Seen in some patients with evolving rheumatic disease	Heterogeneous nuclear RNP (hn RNP), others	Confirmed by staining pattern
Unusual Speckled	NSp I, sp-100, MND, PBC 95	Some association with Primary Biliary Cirrhosis	95-100 kDa protein	none
	NSp II, CENP F	unknown	367 kDa protein	none
	Midbody	Low percentage of patients with scleroderma	Unidentified proteins of midbody region	none
	p 80 (coilin)	unknown	80 kDa protein in the coiled body	none
Centromere	Centromere	Seen in 57 - 82% of patients with CREST variant of scleroderma	CENP A, CENP B, CENP C,	Confirmed by staining pattern
Nucleolar	Clumpy Nucleolar Speckled Nucleolar Smooth Nucleolar	Scleroderma Scleroderma Polymyositis/Scleroderma	Fibrillarin, others? RNA polymerase I, others? PM-1 (PM/Scl), others?	none none none

Negative 1



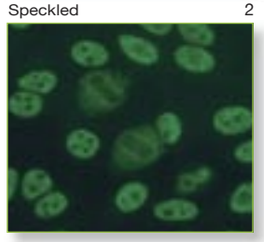
Report as: ANA Negative

Suspected antigen specificity: None

Clinical significance: None

Follow-up testing: None

Speckled 2



Report as: Speckled

Suspected antigen specificity: Sm, U1-RNP, others?

Clinical significance: Sm positive: marker antibody seen in 4 - 40% of SLE patients. RNP positive: seen in high titers in patients with MCTD and SLE, low titers in other diseases.

Follow-up testing: Confirm by ENA testing

Homogeneous 3

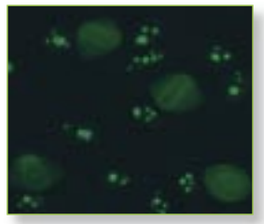
Report as: Homogeneous

Suspected antigen specificity: nDNA, DNP, Histone, DNA binding proteins, others?

Clinical significance: nDNA positive: marker antibody seen in 60% of SLE patients

Follow-up testing: Confirm nDNA antibodies

Nucleolar (clumpy) 4



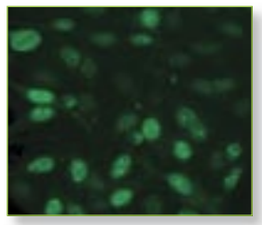
Report as: Nucleolar

Suspected antigen specificity: Fibrillarin, others?

Clinical significance: May be seen in patients with systemic sclerosis.

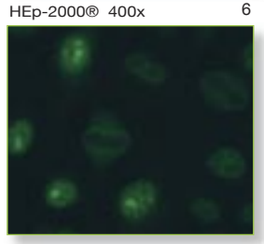
Follow-up testing: None required

HEp-2000® 200x 5



A distinct speckled and nucleolar pattern seen in 10 - 20% of the interphase nuclei. These are the hyperexpressing cells. The remaining 80-90% of the interphase cells may or may not demonstrate staining. The chromosome region of the metaphase mitotic cells is negative. Original total magnification was 200x.

HEp-2000® 400x 6



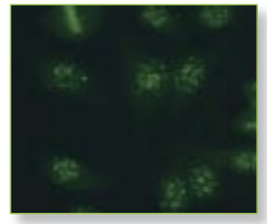
Report as: SSA/Ro pattern

Suspected antigen specificity: SSA/Ro

Clinical significance: SSA/Ro positive: seen in 60-70% of patients with primary Sjögren's syndrome, 30 - 40% of patients with SLE.

Follow-up testing: This pattern is confirmatory for SSA/Ro antibodies. Suggest ENA testing to rule out the presence of antibodies to other ENAs

Centromere 7



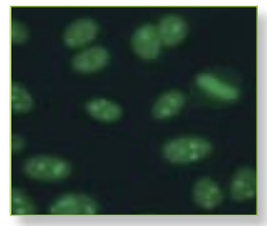
Report as: Centromere

Suspected Antigen Specificity: Centromere protein A, B, or C

Clinical significance: Seen in 57 - 96% of patients with the limited form of scleroderma (CREST).

Follow-up testing: None required, pattern confirmed by staining pattern

Scl-70 8



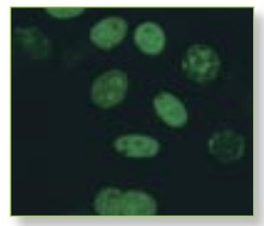
Report as: Homogeneous, speckled, and nucleolar

Suspected antigen specificity: Topoisomerase I

Clinical significance: Topoisomerase I positive: marker antibody seen in 15 - 20% of patients with scleroderma.

Follow-up testing: Confirm by ENA testing

PCNA 9



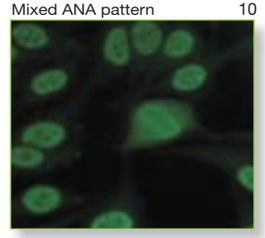
Report as: Speckled, possible PCNA

Suspected antigen specificity: DNA polymerase δ (cyclin)

Clinical significance: PCNA positive: marker antibody seen in 2 - 10% of SLE patients

Follow-up testing: Confirm by ENA testing

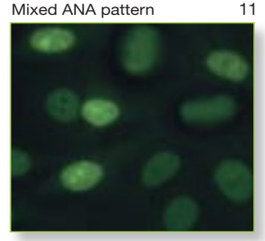
Mixed ANA pattern 10



Report as: Homogeneous and Speckled

Mixed ANA patterns can be caused by autoantibodies to several different antigens or by antibodies to an antigen located in several different areas of the cell nucleus. Titering and appropriate confirmatory testing for each pattern is recommended.

Mixed ANA pattern 11

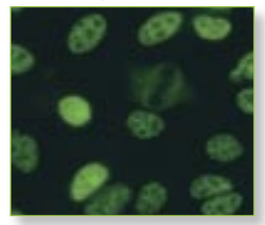


Mixed ANA pattern on HEp-2000®

Report as: Homogeneous and SSA/Ro

Suspected antigen specificity: SSA/Ro—confirm by pattern; Homogeneous—dsDNA, histone or others?

Mixed ANA pattern 12



Mixed ANA pattern on HEp-2000®

Report as: Speckled and SSA/Ro

Suspected antigen specificity: SSA/Ro—confirm by pattern; Speckled—Sm, U1-RNP, SSA/Ro, SSB/La, others