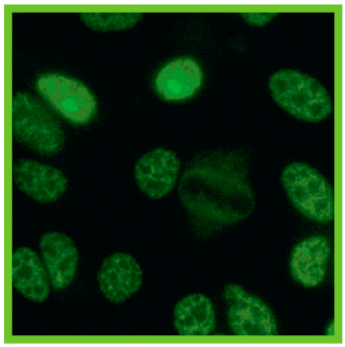
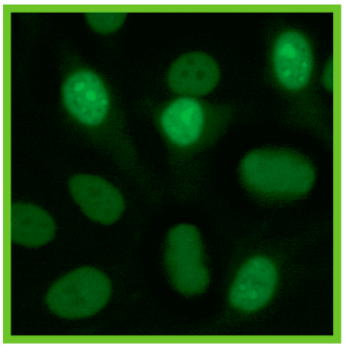
Fluorescent Patterns

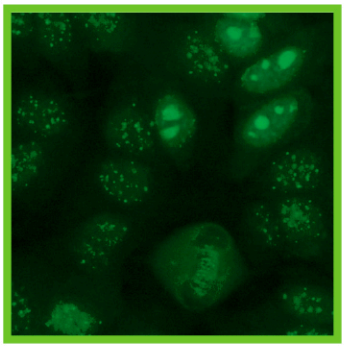
Associated with Antinuclear Antibody Detection



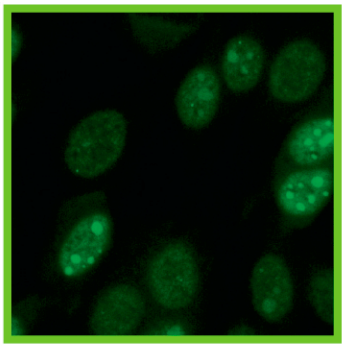
Speckled and SSA/Ro



Homogeneous and SSA/Ro



Centromere and SSA/Ro



SSA/Ro

immuno concepts

Antigen Chart

Associated with ANA Patterns

Pattern observed by indirect immuno fluorescence	Type of antibody	Disease in which antibodies are 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 70 kDa 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

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 indicate 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 were 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.

The photographs and charts presented here are designed to give the laboratorian a comprehensive guide to pattern recognition, antigen specificity, and disease association. Some of the more recently described patterns do not have disease association but are included for educational value.

References:

1. Hargraves, M., Richmond H., et al., Presentation of two bone marrow components, the tart cell and the LE cell. Mayo Clin Proc. 1948;27:25-28.

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.

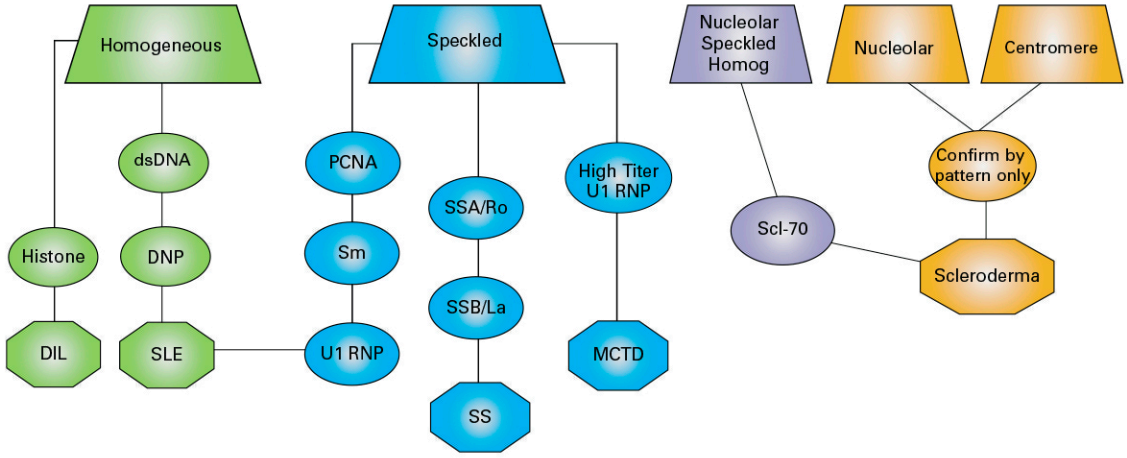
6. Fritzler MJ, Hanson C., et al., Specificity of autoantibodies to SS-A/Ro on a transfected and overexpressed human 60 kDa Ro autoantigen substrate. J.Clin.Lab.Anal. 2002;16:103-108.

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

Flow Chart

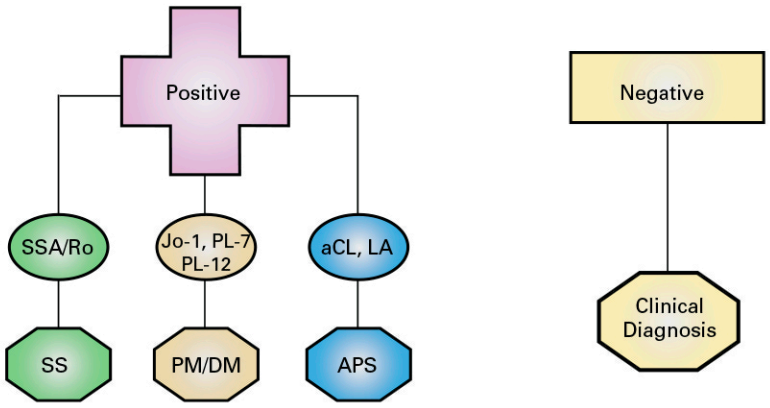
ANA Pattern and Confirmatory Testing

ANA Positive

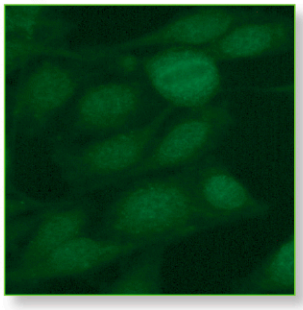


ANA Negative

If the ANA is negative, but disease is suspected, test for specific autoantibodies



Negative 1



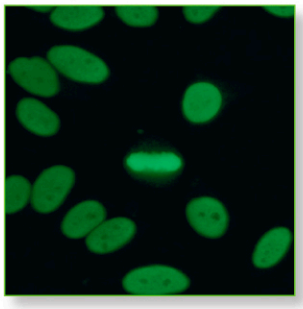
Report as: ANA Negative

Suspected antigen specificity: None

Clinical significance: None

Follow-up testing: None required

Homogeneous 2



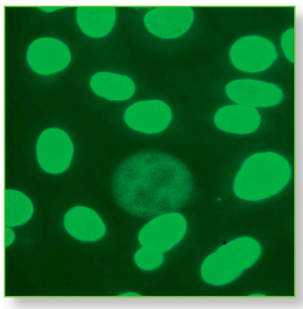
Report as: Homogeneous

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

Nuclear membrane 3



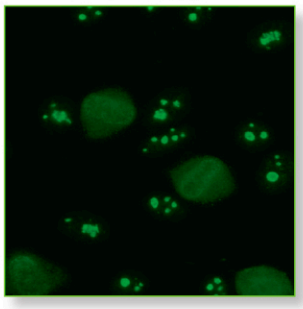
Report as: Nuclear Membrane

Suspected antigen specificity: Nuclear lamins, others?

Clinical significance: May be seen in patients with SLE, RA, autoimmune hepatitis

Follow-up testing: None required

Nucleolar (clumpy) 4



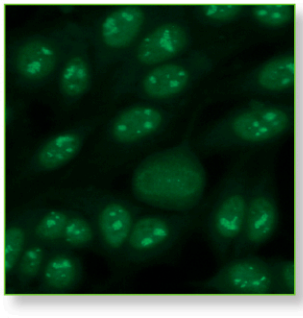
Report as: Nucleolar

Suspected antigen specificity: Fibrillarin, others?

Clinical significance: May be seen in patients with SLE, RA, autoimmune hepatitis

Follow-up testing: None required

Nucleolar (speckled) 5



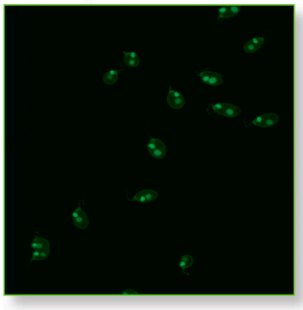
Report as: Nucleolar

Suspected antigen specificity: RNA Polymerase I, NOR 90, others?

Clinical significance: Diffuse scleroderma

Follow-up testing: None required

dsDNA positive 6



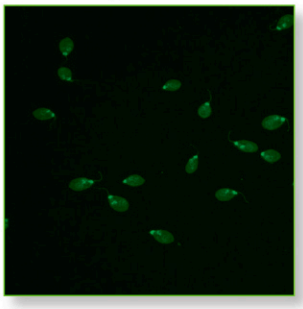
Pattern: Positive staining of the kinetoplast in the *Crithidia luciliae*

Report as: nDNA positive

Suspected antigen specificity: nDNA or dsDNA

Clinical significance: Marker antibody seen in 30 - 70% of SLE patients

dsDNA negative 7

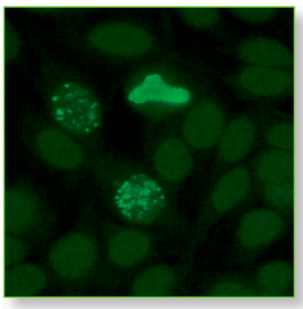


Pattern: No apparent staining of the kinetoplast in the *Crithidia luciliae*

Report as: nDNA negative

Suspected antigen specificity: None

Chromosomal Coating 8



Report as: ANA Negative, Suspect Chromosomal Coating Antibody

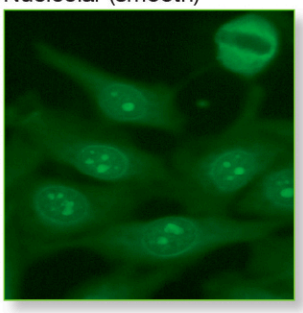
Pattern: Stains only the chromosomal area of metaphase mitotic cells

Suspected antigen specificity: Unknown

Clinical significance: Unknown

Follow-up testing: None required

Nucleolar (smooth) 9



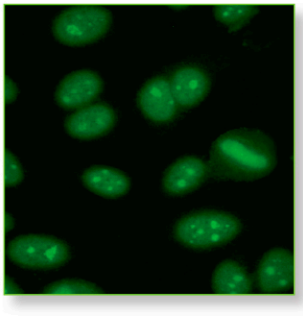
Report as: Nucleolar

Suspected antigen specificity: Pm/Scl

Clinical significance: Seen in patients with polymyositis / scleroderma overlap

Follow-up testing: None required

Scl-70 10



Report as: Homogeneous, speckled, and nucleolar

Suspected antigen specificity: Topoisomerase I

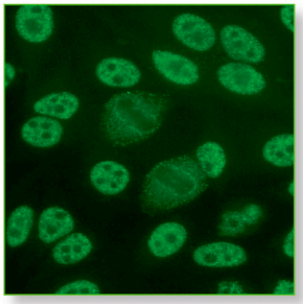
Clinical significance: Topoisomerase I positive—marker antibody seen in 15 - 20% of patients with scleroderma

Follow-up testing: Confirm by ENA testing

Fluorescent Patt

Associated with Antibo

Speckled 11



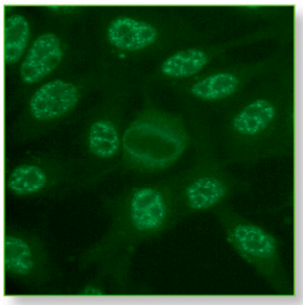
Report as: Speckled

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

Coarse Speckled 12



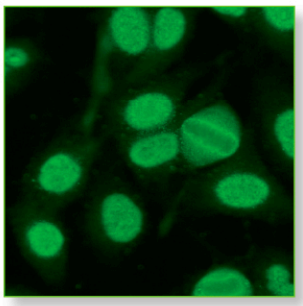
Report as: Speckled

Suspected antigen specificity: 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

Speckled (flat or fine) 13



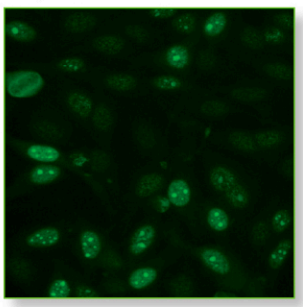
Report as: Speckled

Suspected antigen specificity: SSA/Ro, SSB/La, others?

Clinical significance: SSA/Ro and SSB/La seen in high percentage of patients with primary Sjögren's syndrome, 30 - 40% of patients with SLE

Follow-up testing: Confirm by ENA testing

HEp-2000® 200x 14

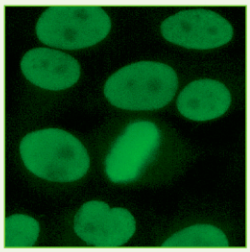


A distinct speckled and nucleolar pattern seen in 10 - 20% of the interphase nuclei. These are the hyperexpressing cells. The remaining 80-90% of the interphase cells may or may not demonstrate staining. The chromosome region of the metaphase mitotic cells is negative. Original total magnification was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

Mitosis

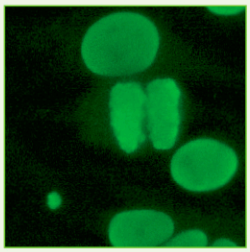
Stages of Cell Division

Metaphase



The double-stranded chromosomes align at the center of the cell and the spindle fibers (generated from the centrioles) attach to the centromeres. Because the nuclear membrane has dissolved, nonchromosome specific antigens redistribute to outer region of the cell.

Anaphase



Chromosomes move to opposite poles.

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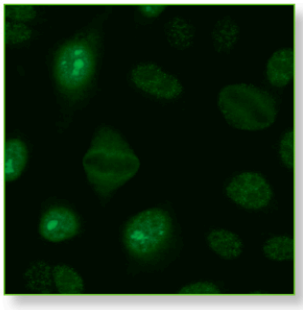
HEp-2000® 400x 15



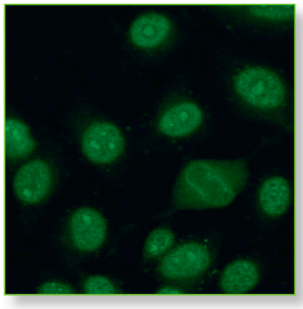
Report as: SSA/Ro pattern

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

Speckled & Nucleolar 16



(SSA/SSB on regular HEp-2)

Report as: Speckled and Nucleolar

Suspected antigen specificity: SSA/Ro, SSB/La, others?

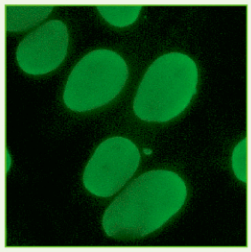
Clinical significance: SSA/Ro positive—see previous pattern. SSB/La positive—seen in 50-60% of patients with primary Sjögren's and 10-15% of SLE patients. Also seen in conjunction with SSA/Ro in patients with neonatal lupus.

Follow-up testing: Confirm by ENA testing

atterns

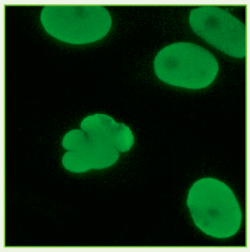
with Antinuclear ntibody Detection

Interphase



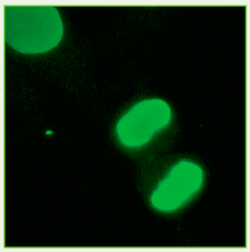
DNA synthesis and chromosome replication occurs. Centrioles replicate.

Prophase



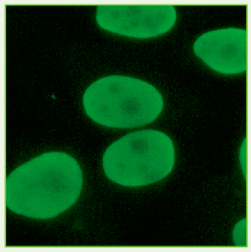
Chromosomes condense. Centrioles migrate to opposite ends of the cell. Nuclear membrane fragments.

Telophase



Spindle dissolves and nonchromosome specific antigens become contained within the chromosomal matrix as the nuclear membrane reforms.

Cytokinesis



Cell division is complete.

Note: chromosome staining demonstrated utilizing DNA specific antibody.

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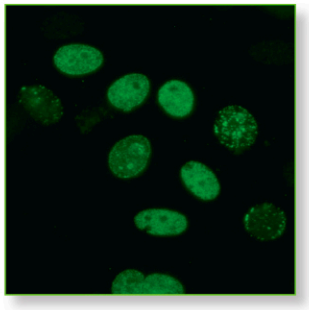
PCNA 17



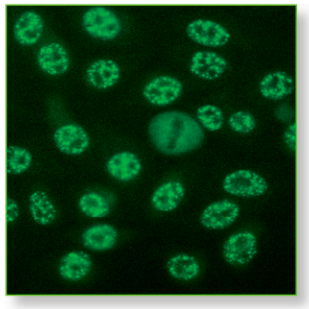
Report as: Speckled, possible PCNA

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

Nuclear Matrix 18



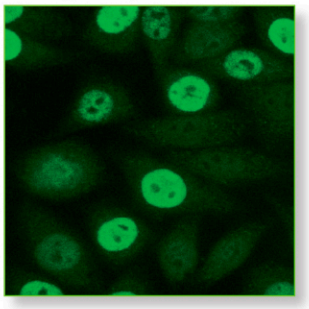
Report as: Speckled

Suspected antigen specificity: hnRNP, others?

Clinical significance: Seen in patients with evolving rheumatic diseases, also Chronic Fatigue Syndrome

Follow-up testing: None required

Cell Cycle Speckled 19



Unusual Cell Cycle Dependent Speckled

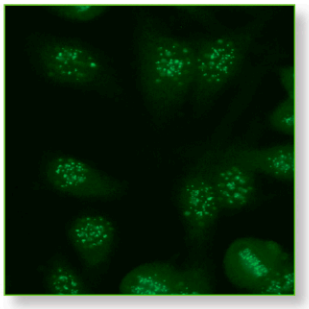
Report as: Speckled or Unusual Speckled or Cell Cycle Dependent Speckled

Suspected antigen specificity: unknown

Clinical significance: Unknown

Follow-up testing: None required

Centromere 20



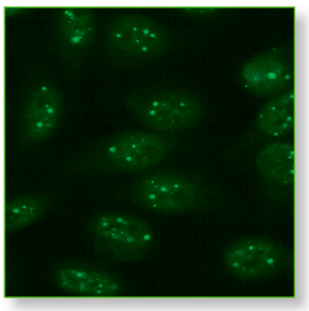
Report as: Centromere

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

Multiple Nuclear Dots 21



Multiple Nuclear Dots (NSp I, PBC 95)

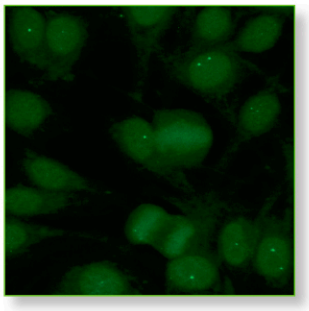
Report as: Atypical speckled

Suspected antigen specificity: 95 - 100 kD protein

Clinical significance: seen 27-44% of patients with PBC

Follow-up testing: None required

p80 coilin 22



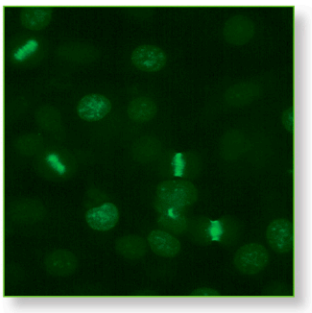
Report as: Atypical Speckled

Suspected antigen specificity: Coilin

Clinical significance: Unknown

Follow-up testing: None required

Midbody 23



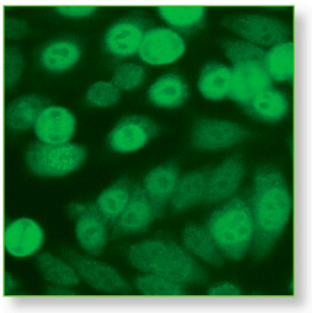
Report as: Atypical speckled, midbody

Suspected antigen specificity: Midbody

Clinical significance: May be seen in a low percentage of patients with scleroderma

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

NspII 24



NSp II (CENP F)

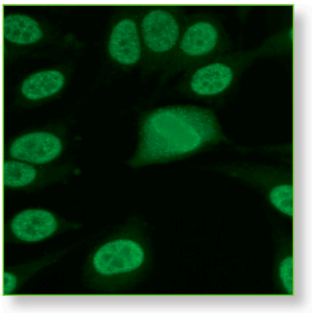
Report as: Atypical Speckled

Suspected antigen specificity: Centromere protein "F", others?

Clinical significance: Unknown, some association with malignancies

Follow-up testing: None required

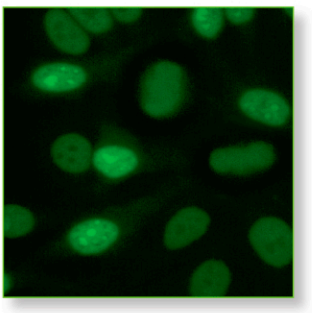
Mixed ANA pattern 25



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 26

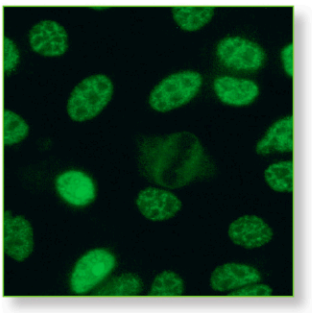


Mixed ANA pattern on HEp-2000®

Report as: Homogeneous and SSA/Ro

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

Mixed ANA pattern 27

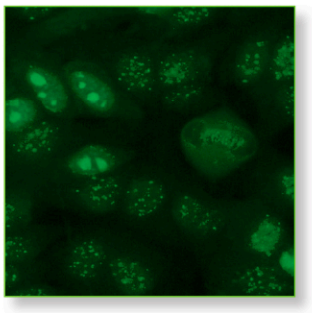


Mixed ANA pattern on HEp-2000®

Report as: Speckled and SSA/Ro

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

Mixed ANA pattern 28



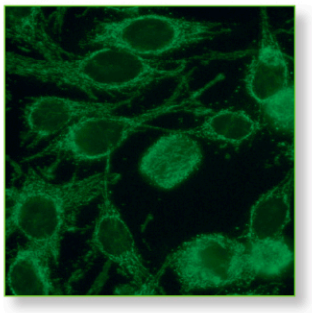
Mixed ANA pattern on HEp-2000®

Report as: Centromere and SSA/Ro

Suspected antigen specificity: SSA/Ro—confirm by pattern; Centromere—confirm by pattern

Clinical significance: See significance for each respective pattern

suspect Mitochondrial 29



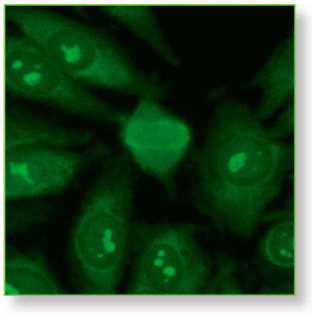
Report as: ANA Negative, suspect Mitochondrial

Suspected antigen specificity: Mitochondria

Clinical significance: Seen in up to 90% of patients with PBC

Follow-up testing: Confirm on AMA specific substrate such as rodent tissue

suspect Ribosomal P 30



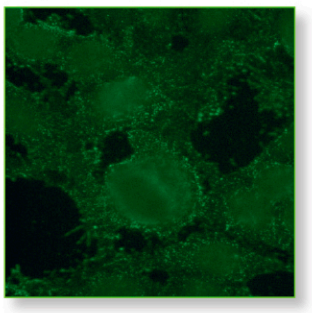
Report as: suspect Ribosomal P

Suspected antigen specificity: Ribosomal P proteins P0, P1, and P2

Clinical significance: Seen in up to 20% of patients with SLE

Follow-up testing: Confirm with anti-ribosomal specific assay

suspect Jo-1 31



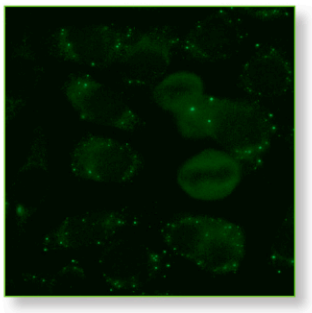
Report as: ANA Negative, cytoplasmic speckling

Suspected antigen specificity: Jo-1 (Histidyl tRNA synthetase)

Clinical significance: Seen in up to 35% of patients with myositis

Follow-up testing: Confirm with Jo-1 specific testing

suspect Endosome-GWB 32



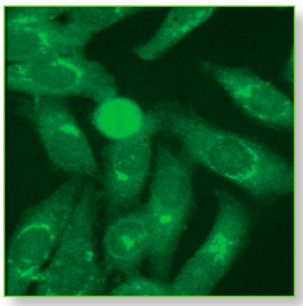
Report as: ANA Negative, cytoplasmic speckling

Suspected antigen specificity: Unknown

Clinical significance: Unknown

Follow-up testing: None required

suspect Golgi 33



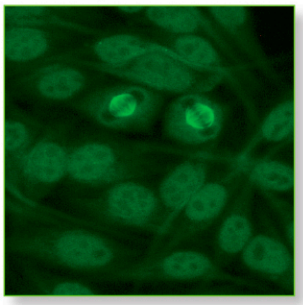
Report as: ANA Negative, suspect Golgi

Suspected antigen specificity: Golgi proteins

Clinical significance: Seen in patients with primary Sjögren's Syndrome and SLE.

Follow-up testing: None required

Mitotic Spindle 34



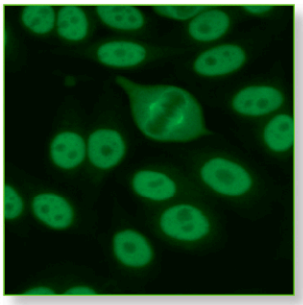
Report as: ANA Negative, Mitotic Spindle

Suspected antigen specificity: Mitotic spindles

Clinical significance: Seen in evolving rheumatic diseases

Follow-up testing: None required

NuMA 35



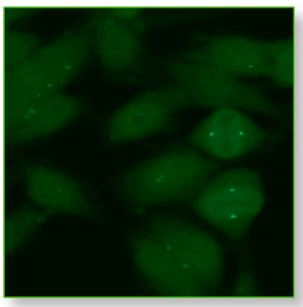
Report as: ANA positive, Speckled, Mitotic spindle also present

Suspected antigen specificity: Nuclear Mitotic Apparatus

Clinical significance: Sjögren's Syndrome, PBC, others?

Follow-up testing: None required

suspect Centriole 36



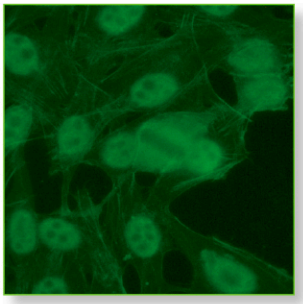
Report as: ANA Negative, suspect Centriole

Suspected antigen specificity: Centriole proteins

Clinical significance: Unknown

Follow-up testing: None required

suspect Cytoskeletal 37



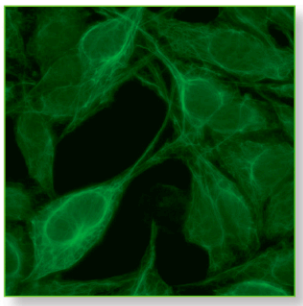
Report as: ANA Negative, suspect Cytoskeletal

Suspected antigen specificity: Actin.

Clinical significance: Seen in patients with autoimmune liver disease

Follow-up testing: Confirm on antigen specific substrate such as rodent tissue

suspect Cytoskeletal 38



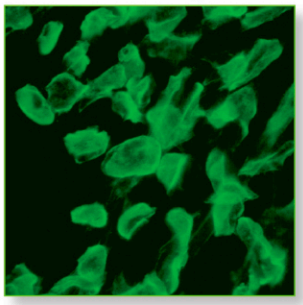
Report as: ANA Negative, suspect Cytoskeletal

Suspected antigen specificity: tubulin, vimentin, others?

Clinical significance: Seen in patients with autoimmune liver disease

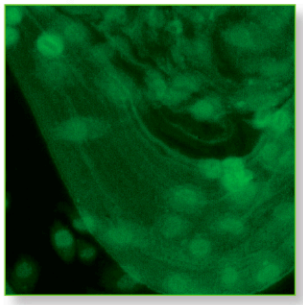
Follow-up testing: Confirm on antigen specific substrate such as rodent tissue

Mechanical Damage 39



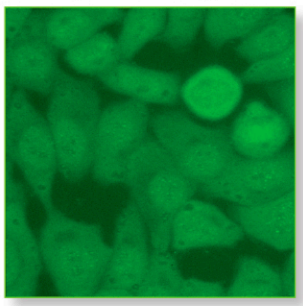
Damage to the substrate caused by moving the coverslip or by touching the substrate with a pipet tip.

Non-specific Film 40



Non-specific film caused by adsorption of serum proteins to the slide surface.

Heterophile Staining 41



Report as: ANA Negative, cytoplasmic speckling

A result of non-specific antibody binding to substrate

Evan's Blue counterstain 42

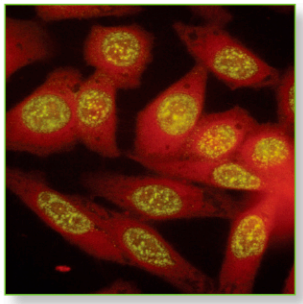


Image demonstrates the effect of Evan's Blue counterstain

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.

2. Kavanaugh AF, Solomon DH, Amer Coll Rheumatology Ad Hoc C. Guidelines for immunologic laboratory testing in the rheumatic diseases: Anti-DNA antibody tests. Arthritis & Rheumatism-Arthritis Care & Research. Oct 15 2002;47(5):546-555.

3. Solomon DH, Kavanaugh AJ, Schur PH, Amer Coll Rheumatology Ad Hoc C. Evidence-based guidelines for the use of immunologic tests: Antinuclear antibody testing. Arthritis & Rheumatism-Arthritis Care & Research. Aug 15 2002;47(4):434-444.

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.

5. Reveille JD, Solomon DH, Amer Coll Rheumatology Ad Hoc C. Evidence-based guidelines for the use of immunologic tests: Anticentromere, Scl-70, and nucleolar antibodies. Arthritis & Rheumatism-Arthritis Care & Research. Jun 15 2003;49(3):399-412.

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6. Keech CL, McCluskey J, Gordon TP. Transfection and overexpression of the human 60-kDa Ro/SS-A autoantigen in HEp-2 cells. Clin.Immunol.Immunopathol. 1994;73:146-151.

7. Fritzler MJ, Miller BJ. Detection of autoantibodies to SS-A/Ro by indirect immunofluorescence using a transfected and overexpressed human 60 kD Ro autoantigen in HEp-2 cells. J.Clin.Lab.Anal. 1995;9:218-224.

8. 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.

9. Bossuyt X, Meurs L, Mewis A, Mariën G, Blanckaert N. Screening for autoantibodies to SS-A/Ro by indirect immunofluorescence using HEp-2000® cells. Ann Clin Biochem. 2000;37:216-219.

10. Fritzler MJ, Hanson C, Miller J, Eystathioy T. 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.

11. Reiff A, Haubruck H, Amos MD. Evaluation of a recombinant antigen enzyme-linked immunosorbent assay (ELISA) in the diagnostics of antinuclear antibodies (ANA) in children with rheumatic disorders. Clinical Rheumatology. May 2002;21(2):103-107.

12. Bossuyt X, Frans J, Hendrickx A, et al. Detection of Anti-SSA Antibodies by Indirect Immunofluorescence. Clin Chem. 10 7 2004;50(12):2361-2369.

immuno concepts

Part No. IC000778