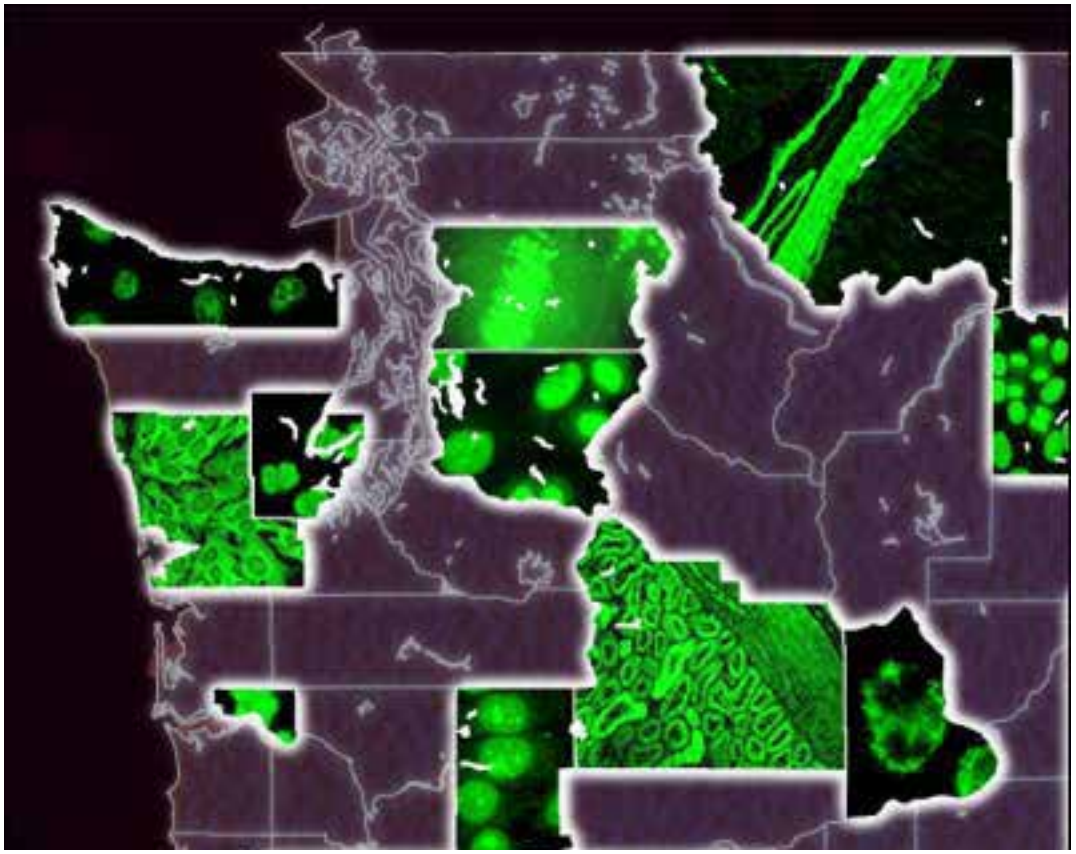
BIO-RAD

Autoantibody Atlas



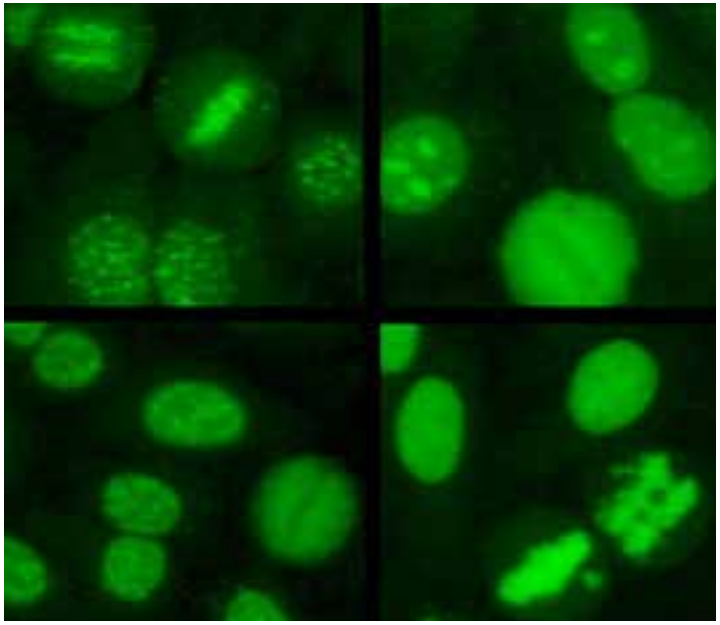
DEVELOPED BY

The University Of Washington
Department of Laboratory Medicine
Seattle, Washington

Kathleen W. Hutchinson, MS, MT(ASCP)
Mark H. Wener, MD
Bruce G. Gilliland, MD
Michael L. Astion, MD, PhD

List of Antibodies

ANA



Sample ANA staining patterns on HEp-2 Substrate

Antibody
ANA
Anti nuclear antibodies

Antigen
Many different antigens that are found in the cell nucleus.

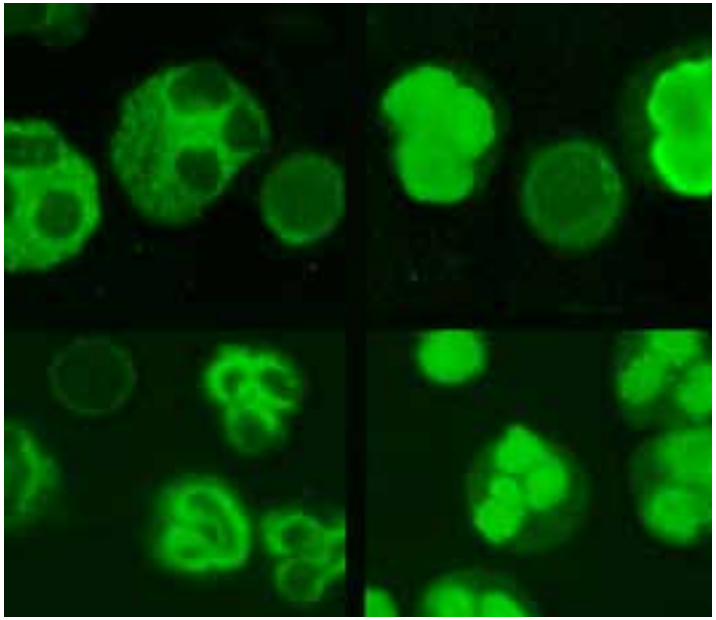
Disease Associations
Dependent upon the antibody specificity and antibody level: systemic autoimmune diseases and autoimmune liver disease. May also be seen in patients with some acute or chronic infections, in association with some medications, and in healthy individuals.

Testing Methods
IFA and ELISA (screening, not specific identification), Multiplexing.

Typical IFA Pattern
Many. See ANA Tutor

Notes
The sensitivity and specificity is very dependent upon the method used for ANA screening. The titer and frequency of anti nuclear antibodies in healthy individuals increases with age.

ANCA



Sample ANCA staining patterns

Antibody

ANCA
Anti neutrophil cytoplasmic antibodies

Antigen

A group of several different antigens that are found in neutrophils. Includes proteinase-3 (PR3) and myeloperoxidase (MPO). Some people also include other neutrophil specific autoantibodies (NSA) such as elastase (EL), and bacterial permeability increase (BPI) protein, Cathepsin G, lysozyme, lactoferrin, and catalase.

Disease Associations

Dependent upon the antibody specificity: ANCA-associated vasculitis, inflammatory bowel disease (Crohn's disease, ulcerative colitis), systemic autoimmune diseases.

Testing Methods

IFA (screening, not specific identification)

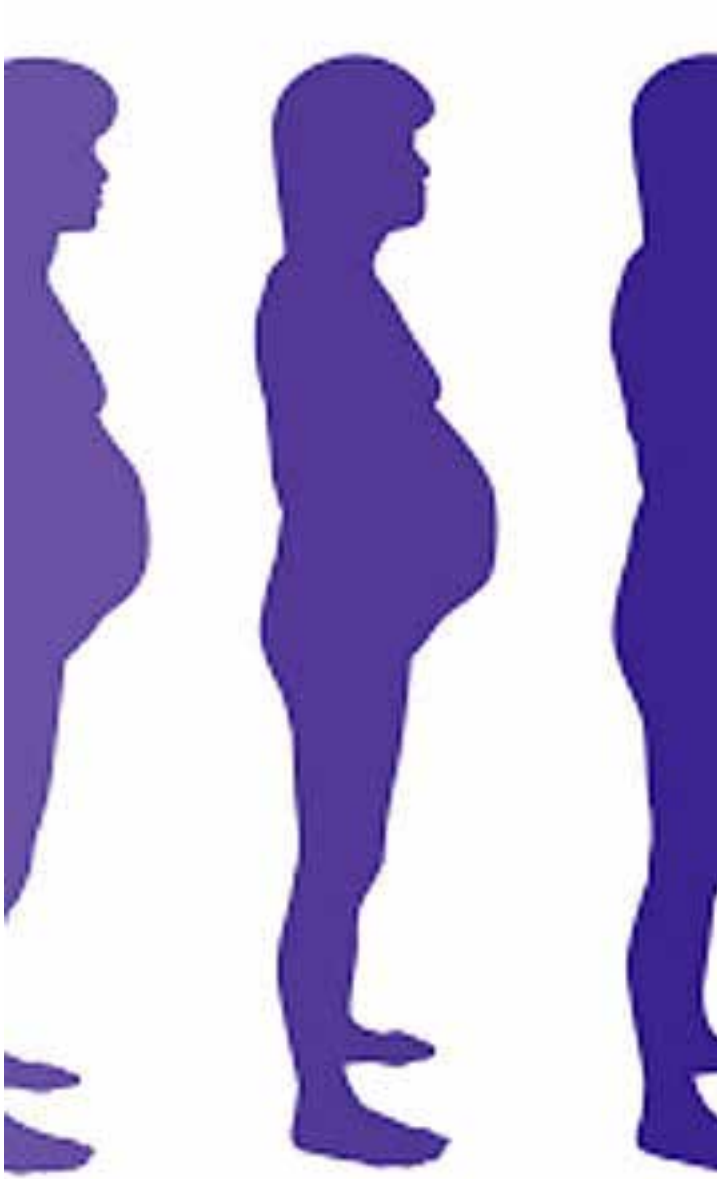
Typical IFA Pattern

Dependent on antibody specificity: C-ANCA, P-ANCA, atypical ANCA (see ANCA Tutor)

Notes

Clinical assays are not available for specific identification of many of these antibodies. ANA staining may resemble ANCA staining.

Anti Beta-2 Glycoprotein I



Antibody
IgG anti beta-2 glycoprotein I,
IgA anti beta-2 glycoprotein I,
IgM anti beta-2 glycoprotein I

Alternate Names
Anti ß2GPI, aß2GPI

Antigen
Glycoprotein that binds to cardiolipin and other anionic phospholipids (cardiolipin cofactor). Also called apolipoprotein H.

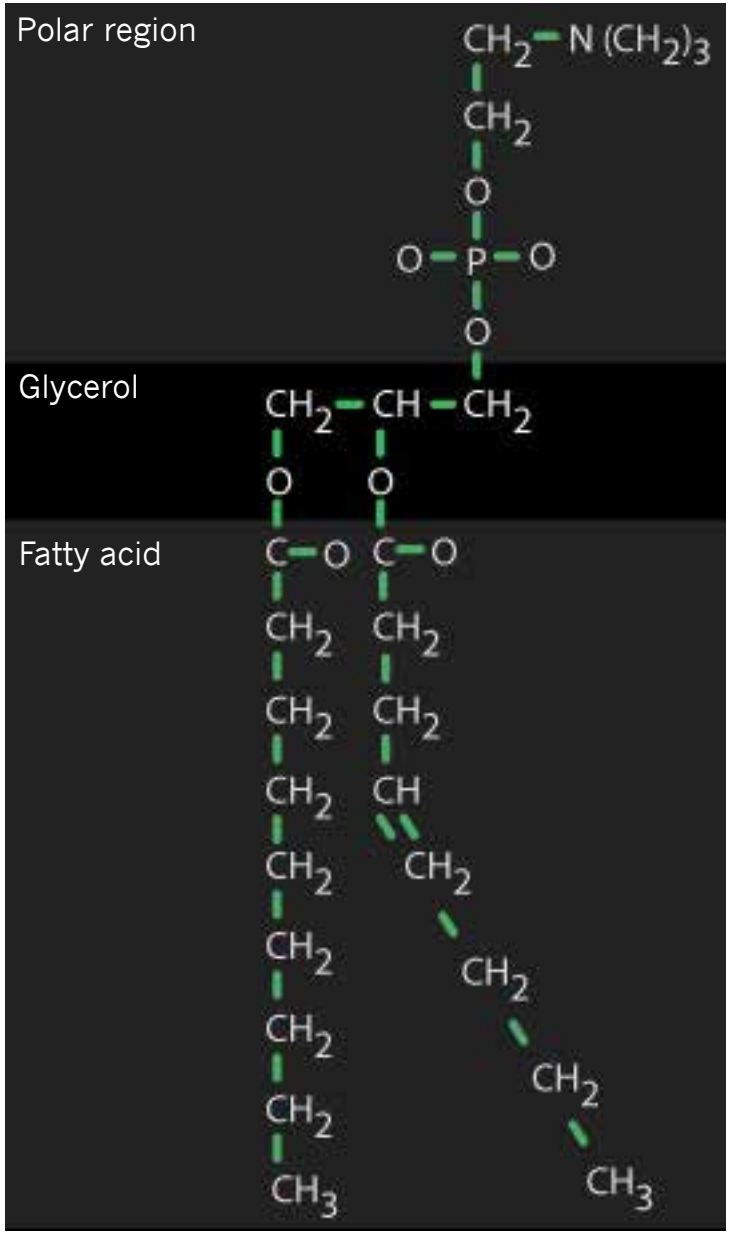
Disease Associations
Anti phospholipid syndrome (APS), systemic lupus erythematosus (SLE).

Testing Methods
ELISA

Typical IFA Pattern
Not applicable

Notes
Assays for different isotypes are available (i.e. IgG, IgA, IgM). IgG and IgM antibodies results are included in the 2006 APS Classification Criteria. Thought to be more specific for APS than anti-cardiolipin. APS is characterized by recurrent thrombosis. In pregnant women, antibodies to phospholipids have been associated with repeated, unexplained fetal loss.

Anti Cardiolipin



Phospolipid

Antibody
Anti Cardiolipin
IgG anti cardiolipin, IgA anti cardiolipin, igM anticardiolipin

Alternate Names
aCL

Antigen
Diphosphatidylglycerol lipid. Originally derived from cow heart tissue and used as the antigen in the Wassermann test for syphilis.

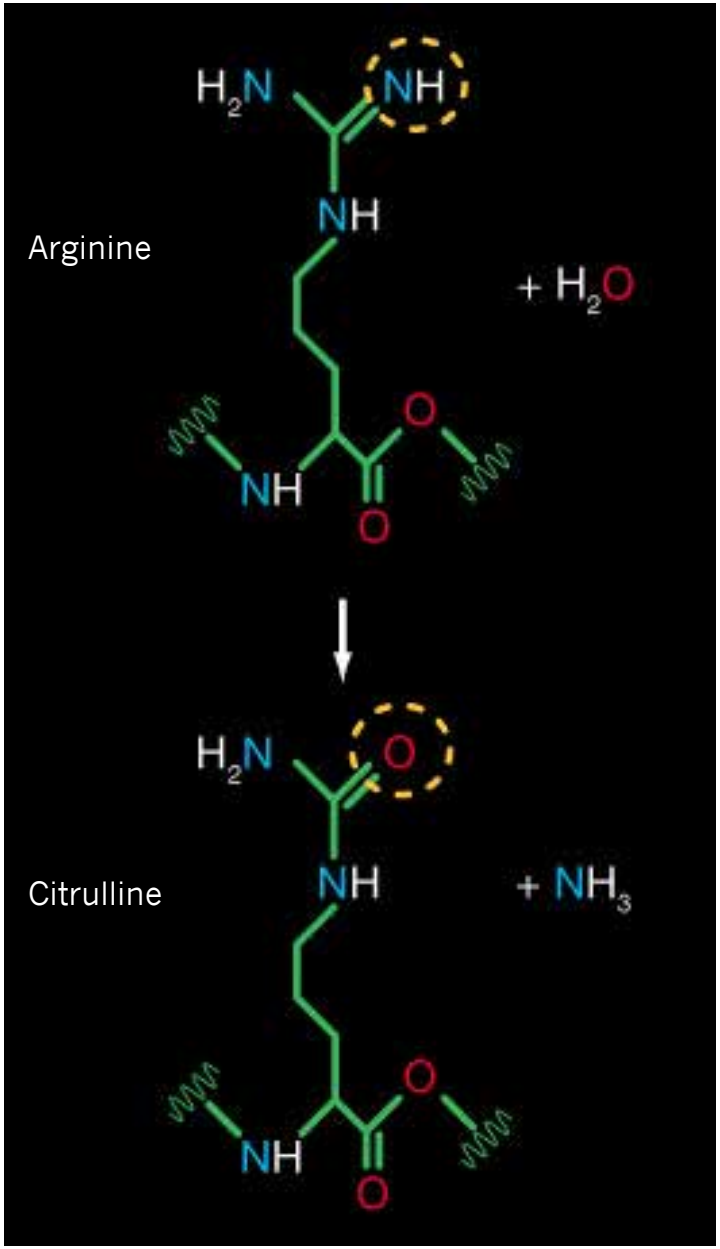
Disease Associations
Anti phosopholipid syndrome (APS), systemic lupus erythematosus (SLE), syphilis. IgM assay may be elevated in patients with infections, rheumatoid factor, or cryoglobulins.

Testing Methods
ELISA

Typical IFA Pattern
Not applicable

Notes
Assays are available for differentimmunoglobulin classes (i.e. IgG, IgA, IgM). IgG and IgM antibodies results are included in the 2006 APS Classification Criteria. One of many aPL (anti phospholipids) that include antibodies to cardiolipin, B2GP1, phosphatidylserine/prothombin, and lupus anticoagulant. May cause a biological false positive syphilis test.

Anti CCP



Antibody
Anti CCP
Anti cyclic citrullinated peptide

Alternate Names
Anti-citrullinated peptide antibody (ACPA), Anti-Sa

Antigen
Antibodies directed against citrullinated proteins and peptides(ACPA) are present in sera from a majority of patients with rheumatoid arthritis. The most common method of testing for ACPA is by detecting antibodies to synthetic cyclic (ring structured) citrullinated peptides (CCP). There are a variety of synthetic peptides that are used for clinical testing. Successive generations of tests are assigned different numbers (e.g. CCP2, CCP3, etc.). Some citrullinated peptides used in testing are derived from the sequences in filaggrin or vimentin.

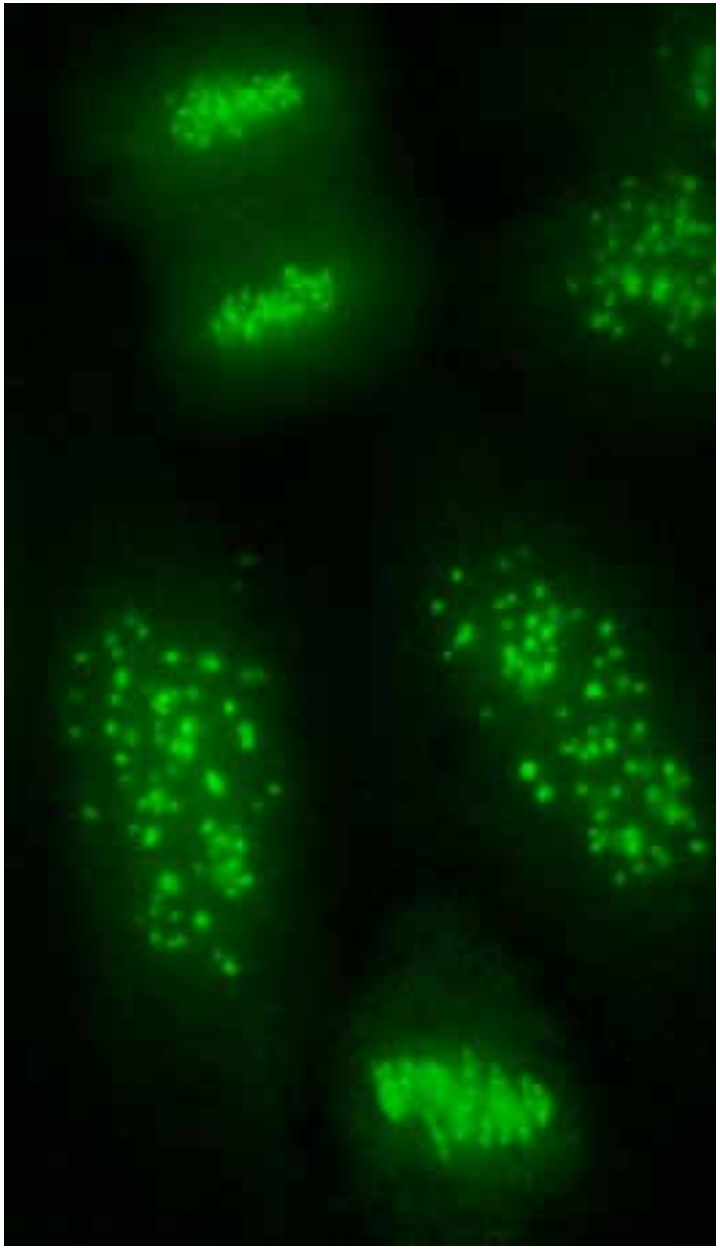
Disease Associations
Rheumatoid arthritis (RA).

Testing Methods
ELISA, Western blot (anti-Sa)

Typical IFA Pattern
Oral mucosal cells: Anti perinuclear factor (APF), Esophageal epithelial cells: anti keratin (AKA) (Not routinely used for clinical testing)

Notes
Anti CCP has better specificity for RA than rheumatoid factor does. Anti-CCP levels may correlate with disease prognosis. Citrulline is formed by a post translational modification of arginine.

Anti Centromere



HEp-2 Centromere Pattern, 100x Objective

Antibody
Anti Centromere

Alternate Names
Anti centromere associated protein (CENP)

Antigen
Proteins present in the centromere (kinetochore) region of the chromosome that includes CENP-A, CENP-B, CENP-C.

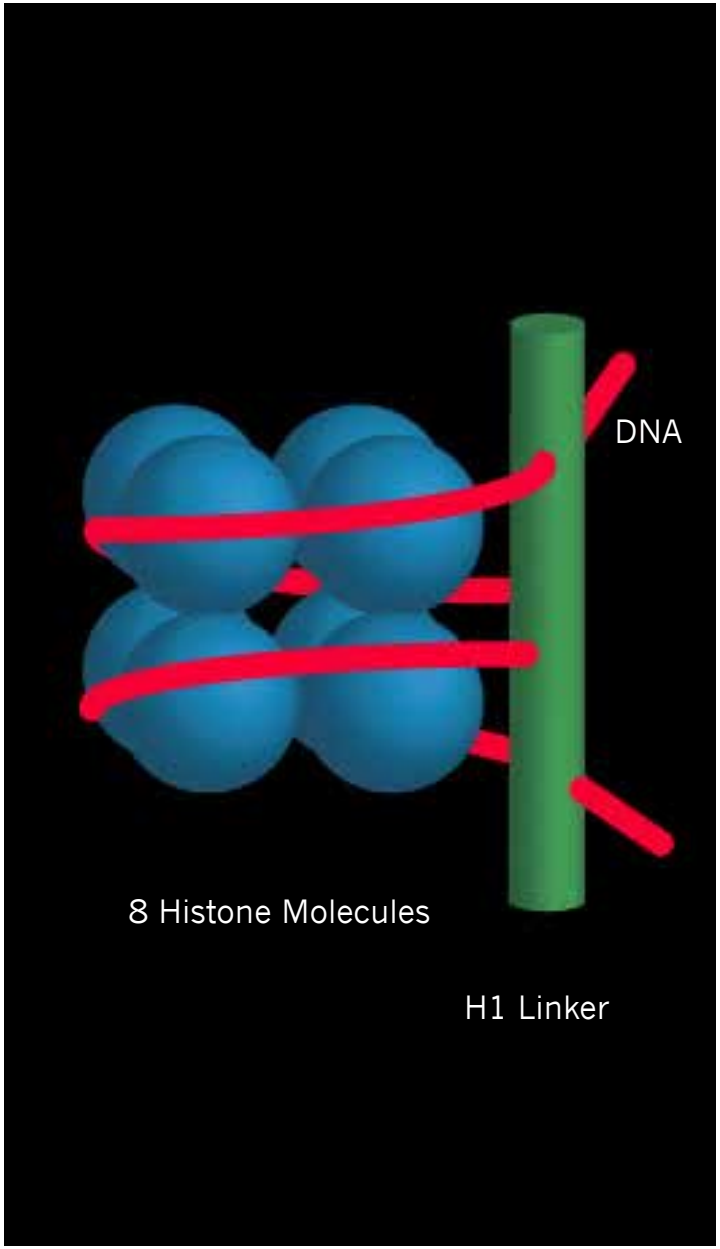
Disease Associations
Most common association is with the limited cutaneous form of scleroderma (CREST syndrome). May be present in other systemic autoimmune diseases and primary biliary cirrhosis (PBC). Patients with primary Raynaud's phenomenon and anti-centromere have an increased risk for later development of a systemic autoimmune disease.

Testing Methods
ELISA, Multiplexing, IFA

Typical IFA Pattern
HEp-2: Discrete speckles of the interphase cell nuclei and dividing cell chromatin. May be difficult to identify iin the presence of other nuclear or cytoplasmic antibodies.

Notes
Most patient sera with centromere antibodies react with CENP-A, CENP-B and CENP-C.

Anti Chromatin



Nucleosome structure

Antibody
Anti Chromatin

Alternate Names
Anti Nucleosome

Antigen
Epitopes present on histones that are bound to DNA in the nucleosome structure,i.e. the DNA-histone complex.

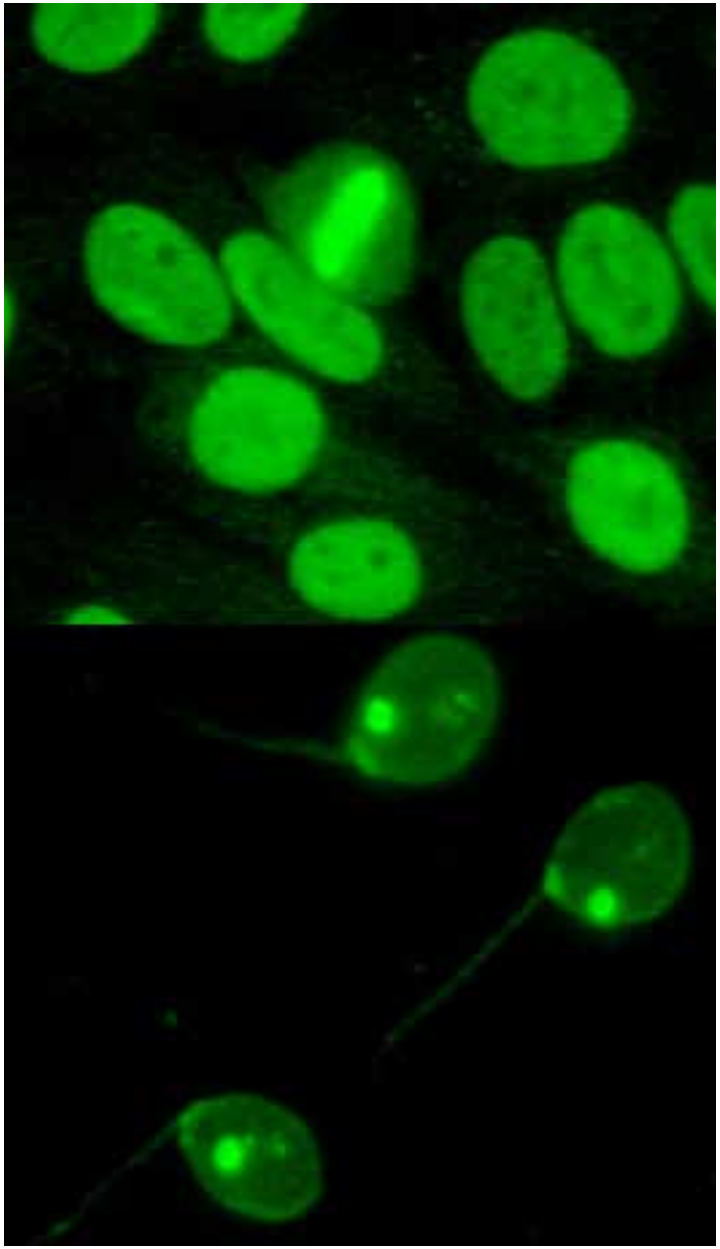
Disease Associations
Present in the sera of patients with drug induced lupus, SLE, and several other systemic autoimmune diseases. Some reports indicate that the antibody may play a pathogenic role and be helpful in the diagnosis and establishing prognosis of SLE patients.

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification)

Typical IFA Pattern
HEp-2: Homogeneous ANA, pattern is not unique therefore does not permit specific identification.

Notes
Anti chromatin refers to a family of autoantibodies that react to histones bound to DNA.

Anti dsDNA



HEp-2 (top), Crithidia (bottom)

Antibody
Anti dsDNA
Anti double stranded deoxyribonucleic acid

Alternate Names
Anti native DNA

Antigen
Double stranded DNA (deoxyribonucleic acid)

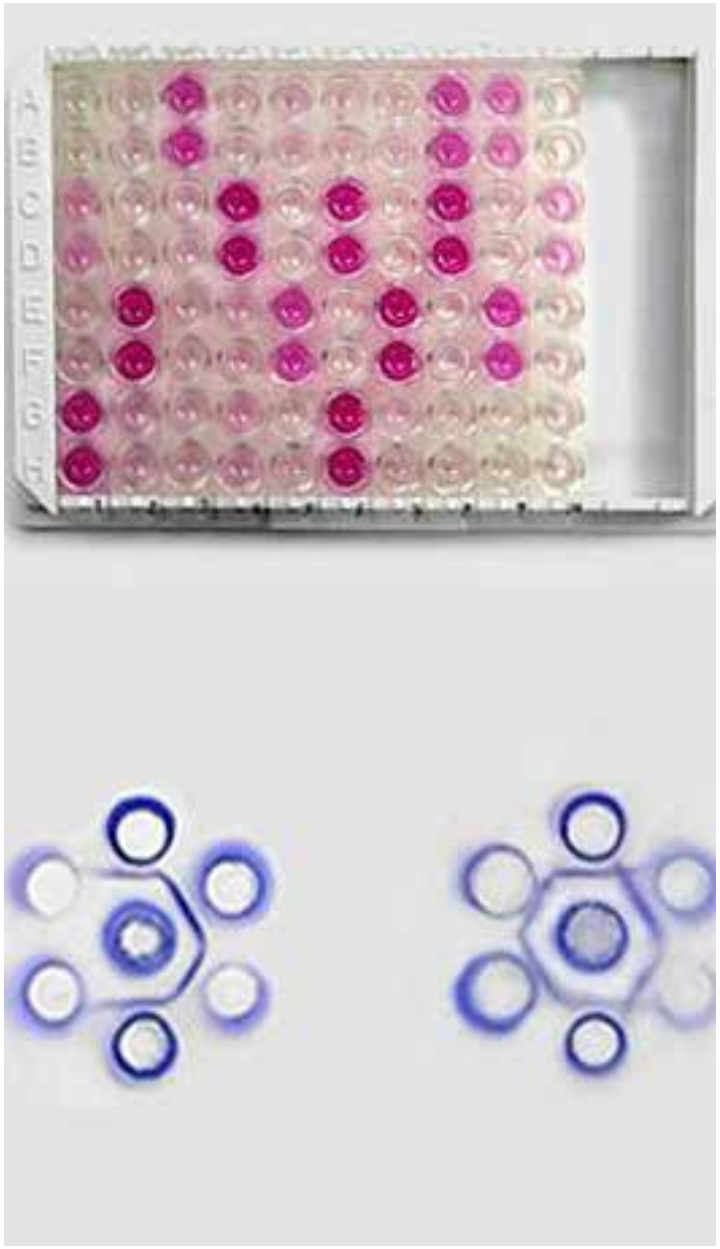
Disease Associations
SLE

Testing Methods
ELISA, Multiplexing, IFA on Crithidia, ANA IFA (screening, not specific identification)

Typical IFA Pattern
HEp-2: Homogeneous ANA, pattern is not unique therefore does not permit specific identification. Crithidia: Staining of the kinetoplast.

Notes
Antibody levels often correlate with disease activity, especially high avidity antibodies. Many assays detect both low and high avidity antibodies.

Anti ENA



ELISA plate (top), Double diffusion gel (bottom)

Antibody
Anti ENA
Anti Extractable Nuclear Antigens

Antigen
Group of several different antigens that are easily extracted from the cell nucleus including Sm/RNP, SS-A, SS-B, Scl-70, and Jo-1.

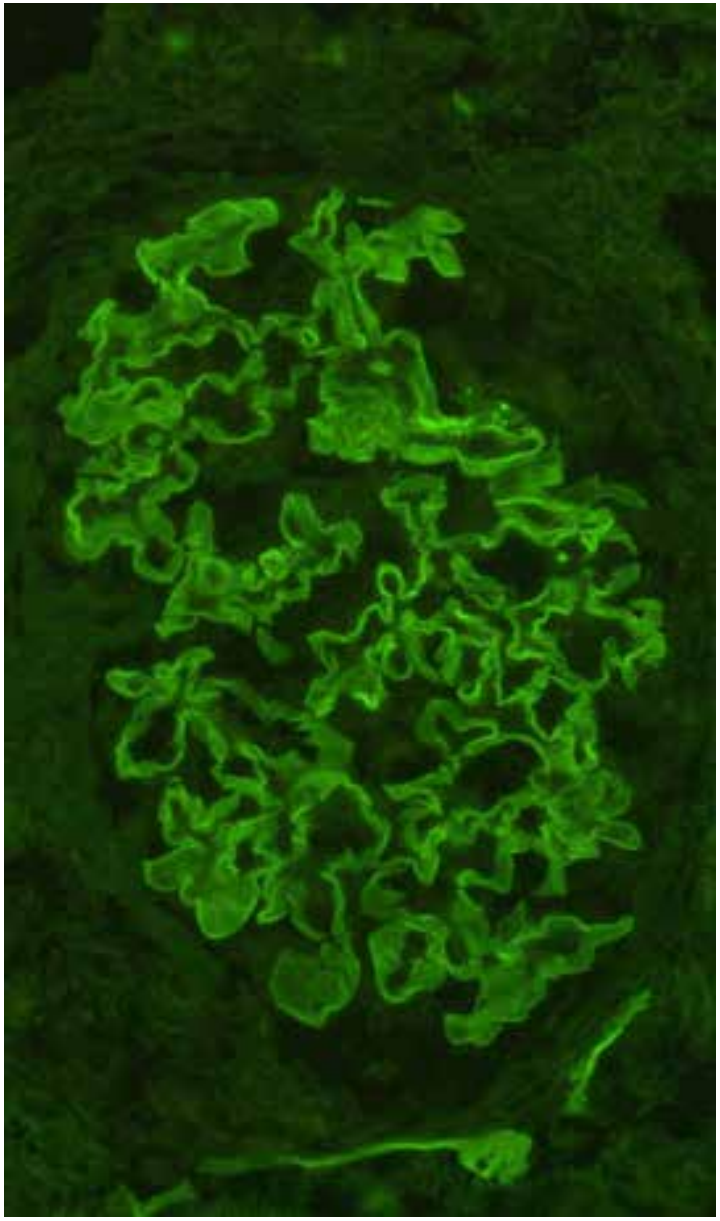
Disease Associations
Systemic autoimmune diseases

Testing Methods
ELISA, Multiplexing, Double Diffusion, CIE (counterimmunoelectrophoresis)

Typical IFA Pattern
HEp-2: most, but not all produce speckled nuclear staining.

Notes
It is common to use a screening method and then proceed to follow-up testing for specific antibodies if the screen is positive. The screening method used will determine which antibodies can be detected.

Anti GBM



Antibody
Anti GBM
Anti glomerular basement membrane

Alternate Names
Goodpasture-antibodies

Antigen
Type IV collagen. Most antibodies react with a cryptic epitope located on the C-terminal alpha 3 NC1 domain.

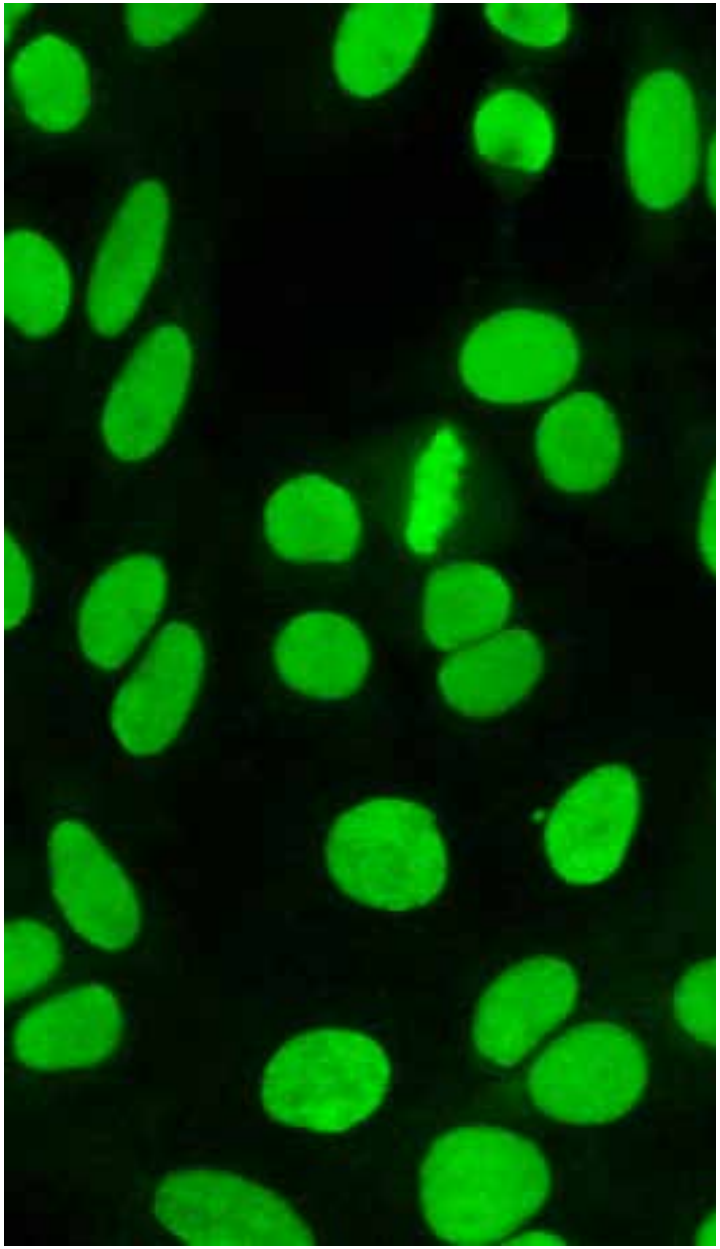
Disease Associations
Goodpasture's syndrome.

Testing Methods
ELISA, Mulitplexing, IFA on primate kidney

Typical IFA Pattern
Primate Kidney: Linear staining of the glomerular basement membrane

Notes
Anti-GBM levels may correlate with disease activity. Patients with acute renal failure and/or severe lung hemorrhage are often screened for ANCA and GBM antibodies.

Anti Histone



HEp-2 Homogeneous Pattern, 40x Objective

Antibody
Anti Histone

Antigen
Histone proteins (H1, H2A, H2B, H3, H4, H5)

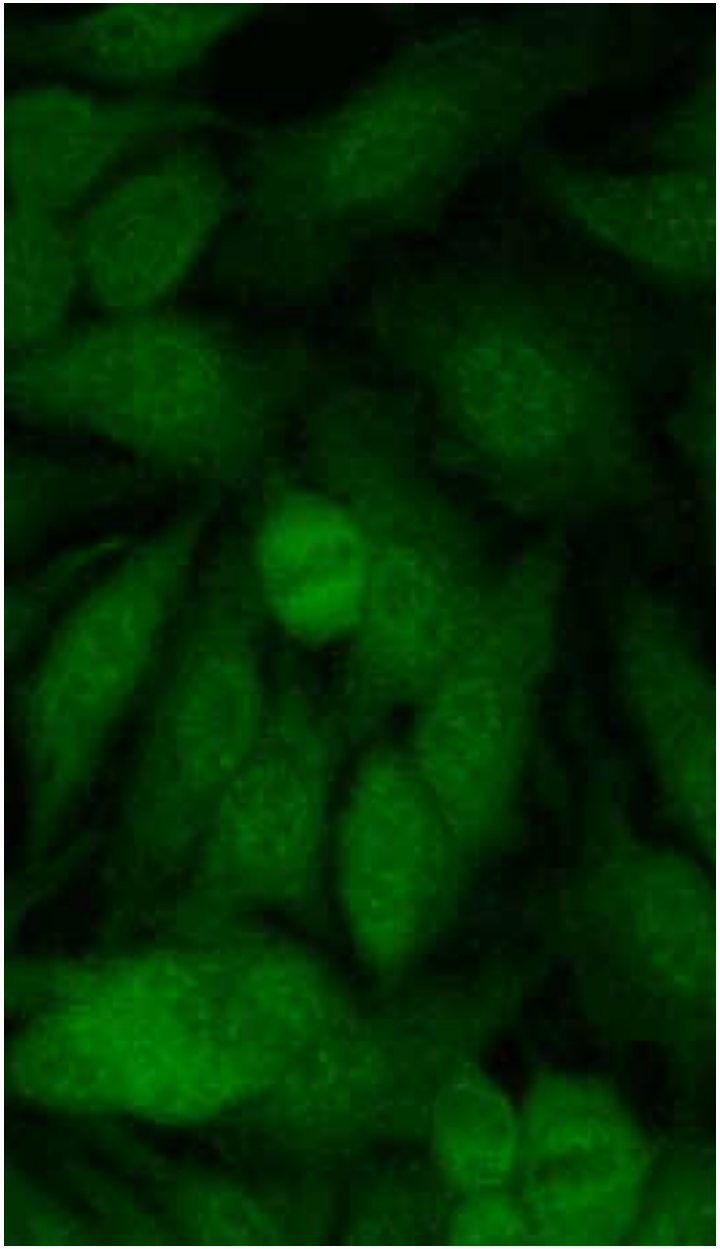
Disease Associations
Present in the sera of patients with drug induced lupus, spontaneous SLE, and several other systemic autoimmune diseases.

Testing Methods
ELISA, IFA (screening, not specific identification)

Typical IFA Pattern
HEp-2: Homogeneous ANA, pattern is not unique enough for specific identification.
Crithidia: Negative

Notes
Autoantibodies that only react to histones bound to DNA are usually called anti-nucleosome or anti-chromatin (see anti-chromatin section).

Anti Jo-1



HEp-2 Negative with weak cytoplasmic staining

Antibody
Anti Jo-1

Alternate Names
Anti histidyl-tRNA synthetase

Antigen
Anti histidyl-tRNA synthetase

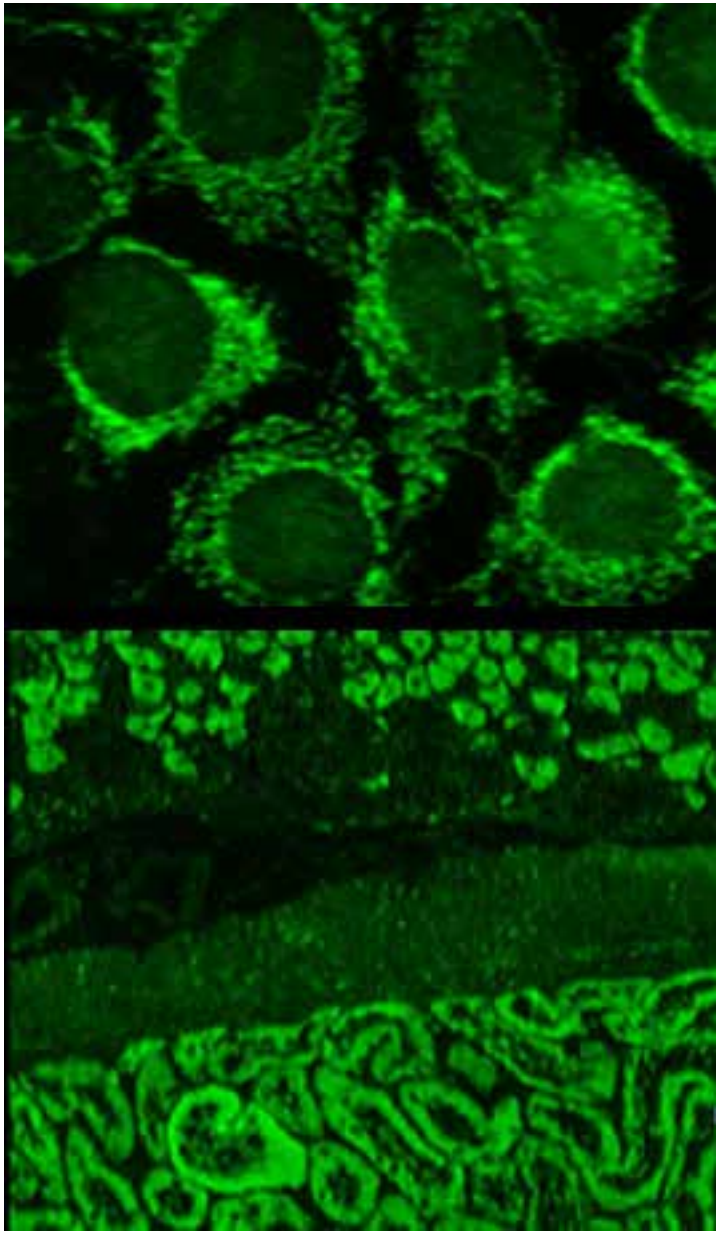
Disease Associations
Polymyositis and dermatomyositis

Testing Methods
ELISA, Multiplexing

Typical IFA Pattern
HEp-2: negative ANA, may have weak cytoplasmic staining that is not unique therefore does not permit specific identification.

Notes
Usually considered to be one of the ENA (extractable nuclear antigens) antibodies. Although the antibody is only present in the sera of approximately 20% of patients with myositis, it is the most common of the myositis specific antibodies (MSA).

Anti Mitochrondria



HEp-2 (top), Mouse Stomach Kidney (bottom)

Antibody
Anti - mitochondria

Alternate Names
AMA

Antigen
Nine different subtypes have been identified (M1-M9). Antibodies to M2 are considered to be the most clinically significant. Antibodies to M2 react with the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2).

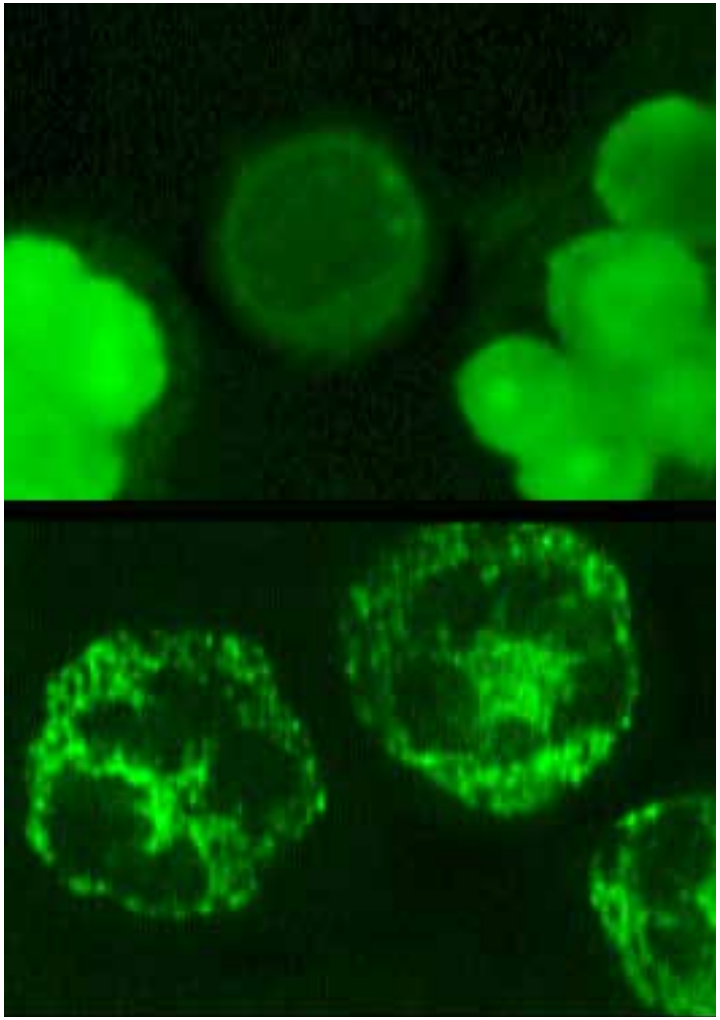
Disease Associations
Over 90% of patients with primary biliary cirrhosis(PBC) have mitochondrial antibodies.

Testing Methods
ELISA (anti-M2) IFA using mouse stomach kidney substrate (detects multiple subtypes)
Immunoblot (not generally available in clinical labs)

Typical IFA Pattern
MSK: cytoplasmic staining of the renal tubules and stomach parietal cells.
HEp-2: numerous granular speckles in the cytoplasm.

Notes
There are other antibodies that may resemble the mitochondrial staining seen on HEp-2. Different techniques should be used to identify the antibody.

Anti MPO



Ethanol (top) and Formalin fixed neutrophils

Antibody
Anti MPO
Anti myeloperoxidase

Antigen
Myeloperoxidase. A cationic enzyme found in the azurophilic granules of neutrophils.

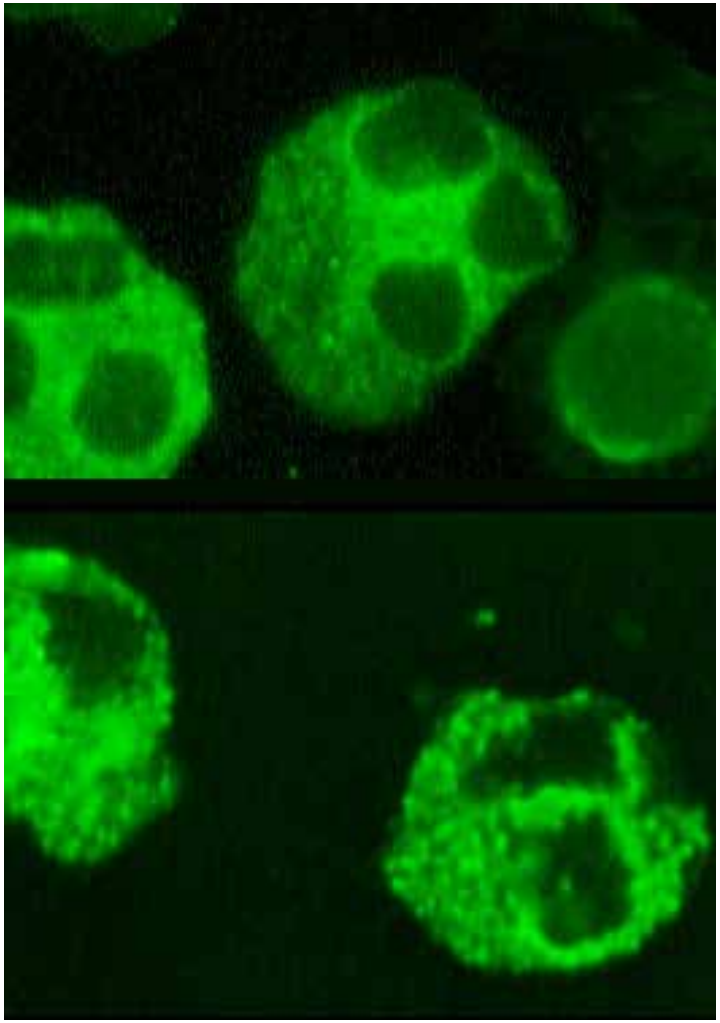
Disease Associations
ANCA-associated vasculitis. Strongest association is with idiopathic crescentic glomerulonephritis (NCGN) and microscopic polyangiitis (MPA), less common in and Churg-Strauss Syndrome (CSS) and Wegener's granulomatosis (WG).

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification)

Typical IFA Pattern
Ethanol Fixed Neutrophils: P-ANCA (perinuclear) Formalin Fixed Neutrophils: cytoplasmic granules

Notes
One of the anti-neutrophil cytoplasmic antibodies (ANCA). Anti-MPO is the most common antigen associated with the P-ANCA pattern. ANAs may resemble P-ANCA staining (see ANCA Tutor). Anti-MPO levels may correlate with disease activity. Patients with acute renal failure and/or severe lung hemorrhage are often screened for ANCA and GBM antibodies.

Anti PR3



Ethanol (top) and Formalin fixed neutrophils

Antibody
Anti PR3
Anti Proteinase 3

Antigen
Proteinase 3. A cationic enzyme found in the azurophilic granules of neutrophils.

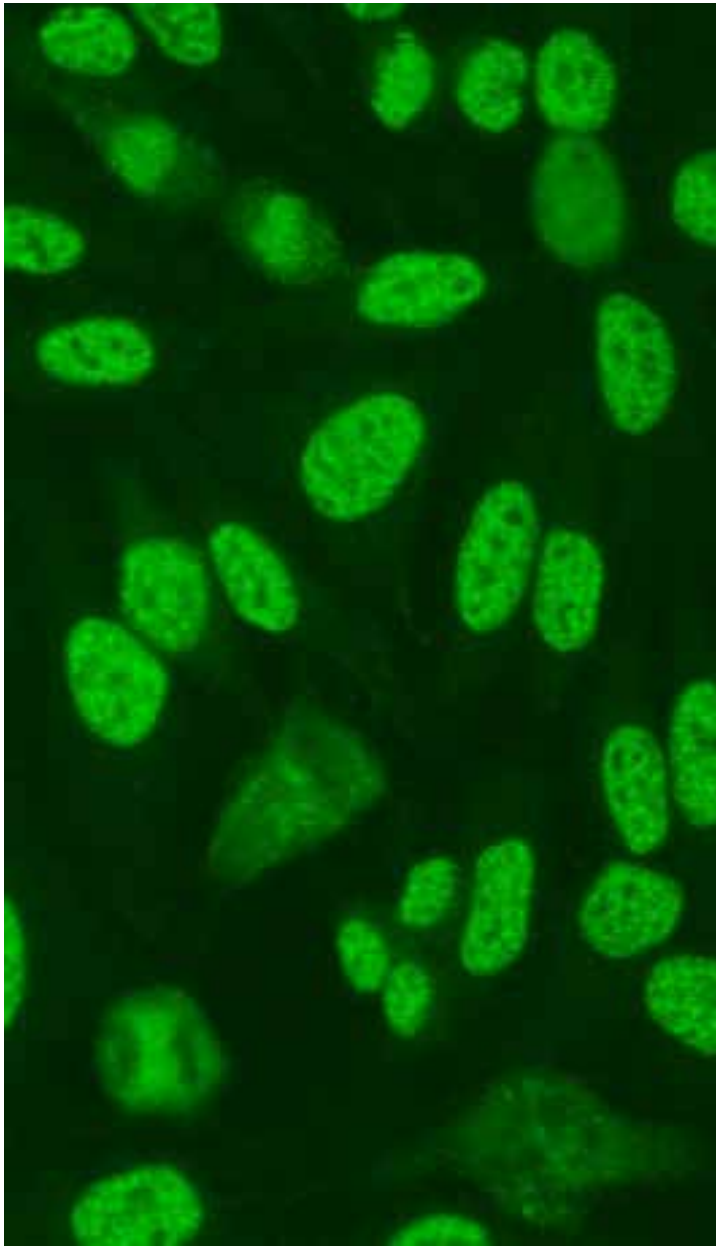
Disease Associations
ANCA-associated vasculitis. Strongest association is with Wegener's granulomatosis (WG), less often seen in microscopic polyangiitis (MPA) and Churg-Strauss Syndrome (CSS).

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification)

Typical IFA Pattern
Ethanol Fixed Neutrophils: C-ANCA (cytoplasmic) Formalin Fixed Neutrophils: cytoplasmic granules

Notes
One of the anti-neutrophil cytoplasmic antibodies (ANCA). Antibody levels often correlate with disease activity. Patients with acute renal failure and/or severe lung hemorrhage are often screened for ANCA and glomerular basement membrane (GBM) antibodies.

Anti Parietal Cell



Mouse stomach kidney

Antibody
Anti parietal cell

Alternate Names
PCA (parietal cell antibodies), GPC antibodies (gastric parietal cell antibodies)

Antigen
α and ß subunits of the H+/K+ ATPase

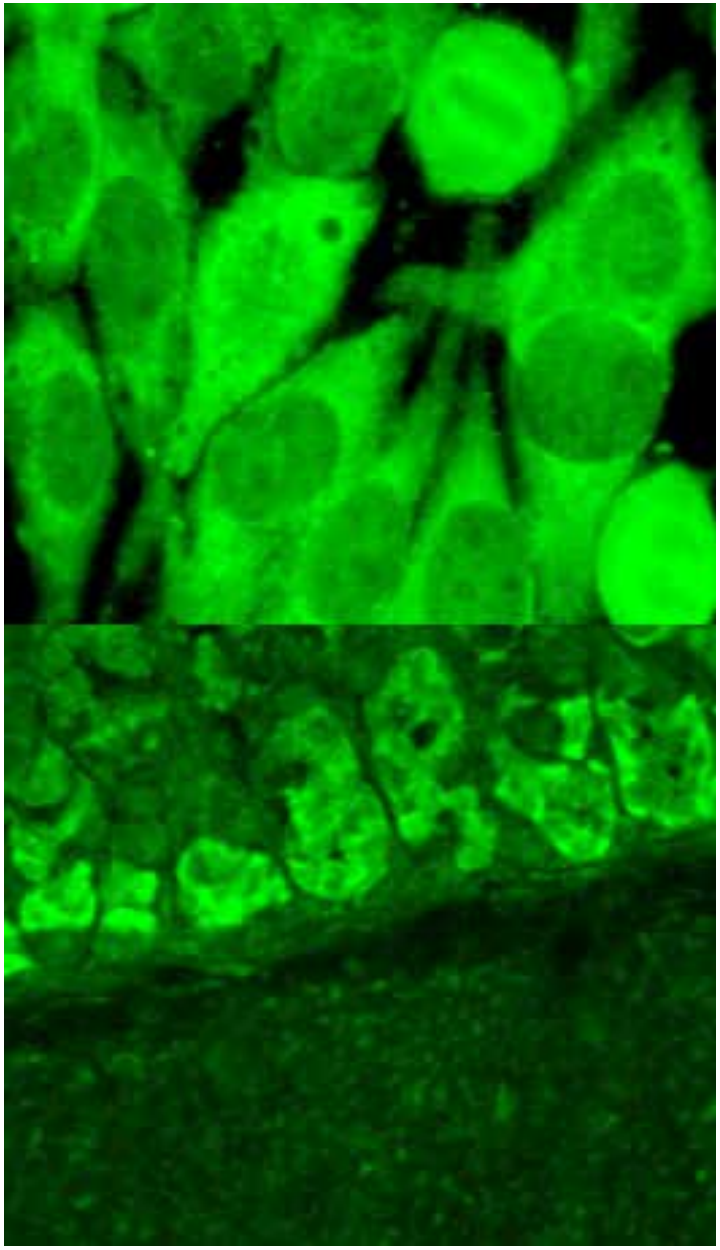
Disease Associations
Pernicious anemia

Testing Methods
IFA using mouse stomach kidney substrate
ELISA (anti-GPC)

Typical IFA Pattern
MSK: cytoplasmic staining of the stomach parietal cells. Renal tubules are negative.

Notes
This antibody cannot be detected by IFA if antibodies to mitochondria are present. The antibody frequency in the normal population increases with age, and the antibody is more common in women than in men.

Anti Ribosomal-P



HEp-2 cytoplasmic (top) MSK chief cells (bottom)

Antibody
Anti Ribosomal P

Antigen
Ribosomal proteins P0, P1, and P2

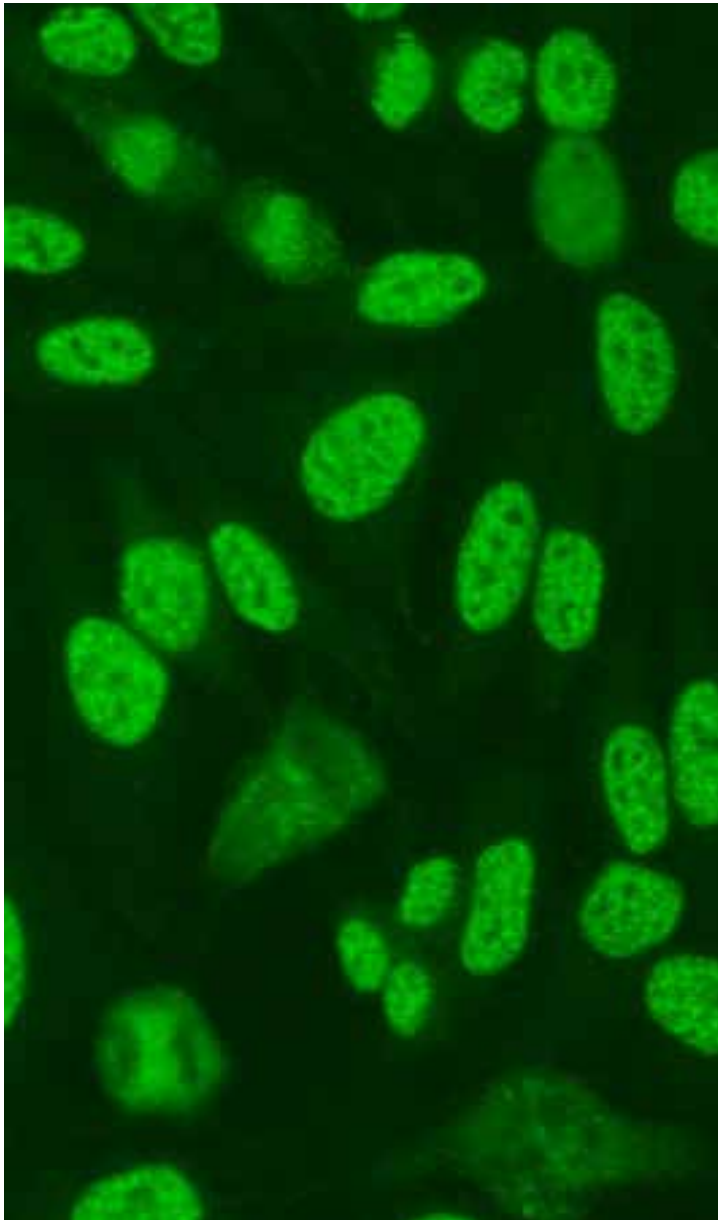
Disease Associations
SLE

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification)

Typical IFA Pattern
HEp-2: cytoplasmic staining that is not unique therefore does not permit specific identification, may have coexisting nucleolar staining. MSK: cytoplasmic staining of the chief cells

Notes
Antigen is present in thymus extract used for ENA (extractable nuclear antigens) antibody testing by double diffusion methods, but is not typically included in ELISA ENA screening kits.

Anti RNP



HEp-2 Speckled Pattern, 40x Objective

Antibody
Anti RNP
Anti ribonucleoprotein

Alternate Names
Anti U1-RNP, anti U1 snRNP

Antigen
Small ribonucleoproteins in spliceosomes, several proteins including 68-70kd, A, and C

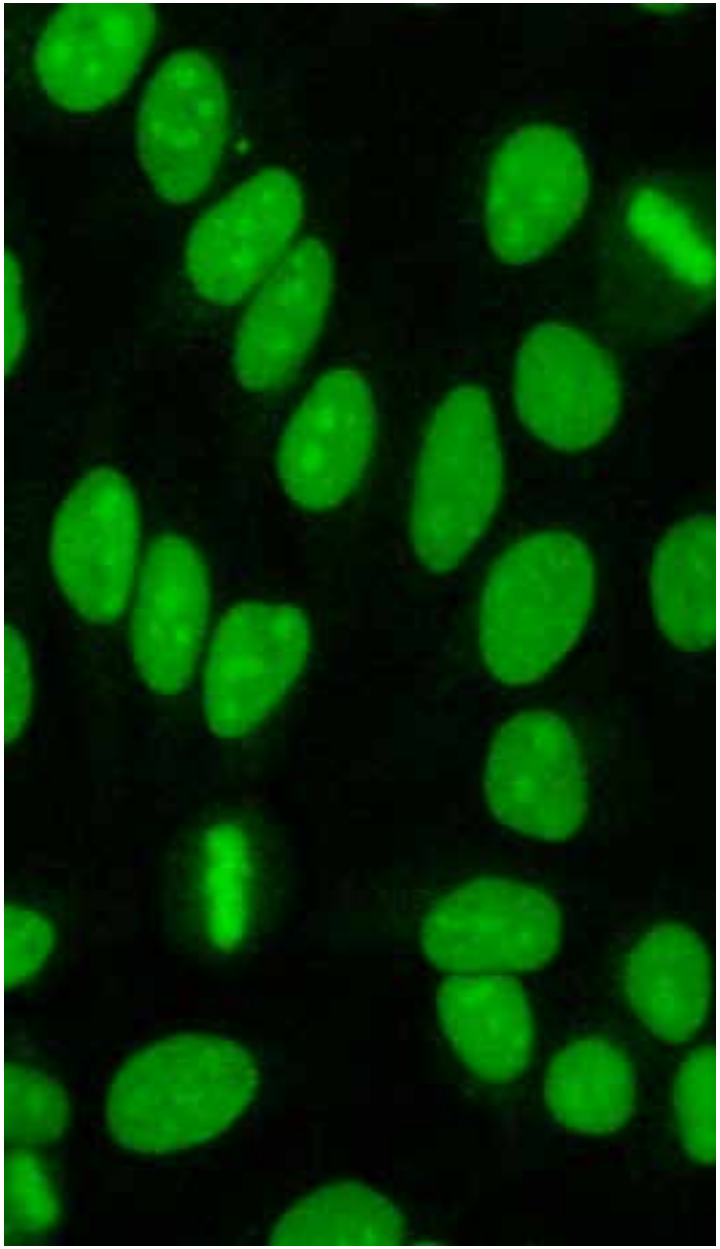
Disease Associations
Several systemic autoimmune diseases including SLE, Sjögren's Syndrome, scleroderma, myositis, occasionally rheumatoid arthritis. Patients with mixed connective tissue disease tend to have very high levels of the antibody, without other autoantibodies.

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification), immunodiffusion.

Typical IFA Pattern
HEp-2: Speckled ANA, pattern is not unique, therefore does not permit specific identification

Notes
Difficult to prepare the RNP antigens without Sm epitopes also present. Some assays use antigens that have both the Sm and RNP epitopes, these assays are called anti Sm/RNP. One of the ENA (extractable nuclear antigens) antibodies.

Anti Scl-70



HEp-2, Homogenous with nucleolar staining

Antibody
Anti Scl-70
Anti Scleroderma 70

Alternate Names
Anti Topo I, Anti topoisomerase I

Antigen
DNA topoisomerase I

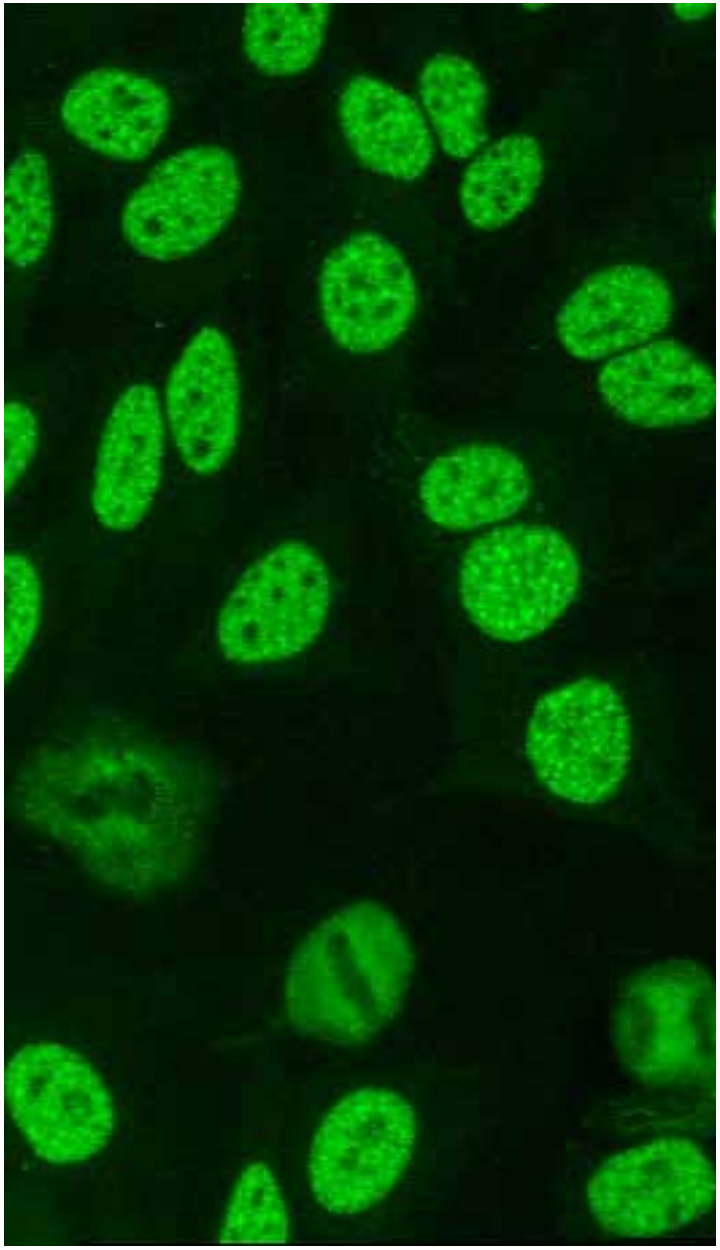
Disease Associations
Most common association is with the diffuse cutaneous form of scleroderma. Occasionally present in the serum of patients with limited cutaneous scleroderma.

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification)

Typical IFA Pattern
HEp-2: Homogeneous ANA, often with coexisting nucleolar staining. Pattern is not unique therefore does not permit specific identification.

Notes
One of the ENA (extractable nuclear antigens) antibodies.

Anti Sm



HEp-2 Speckled Pattern, 40x Objective

Antibody
Anti Sm

Alternate Names
Anti Smith

Antigen
Small ribonucleoproteins in spliceosomes, several proteins including B'/B and D

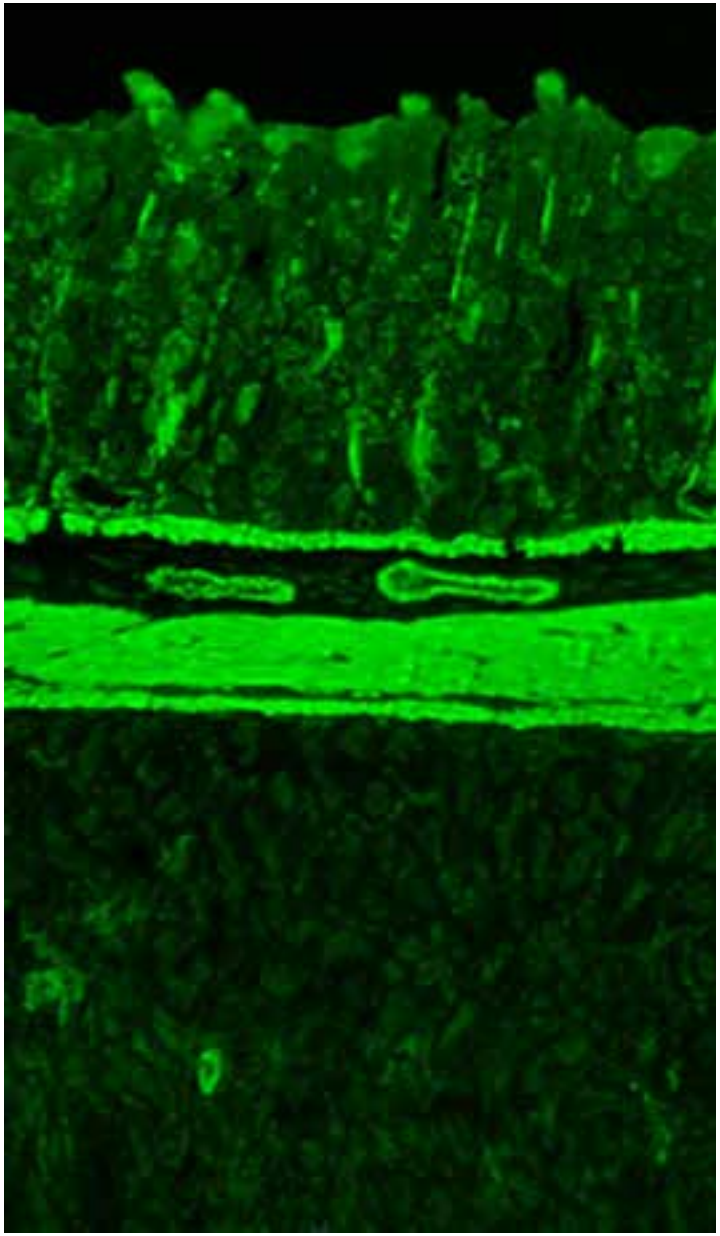
Disease Associations
SLE

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification), immunodiffusion

Typical IFA Pattern
HEp-2: Speckled ANA, pattern is not unique therefore does not permit specific identification.

Notes
Almost all samples with anti Sm have coexisting antibodies to RNP. One of the ENA (extractable nuclear antigens) antibodies. Some assays use antigens that have both the Sm and RNP epitopes, these assays are called anti Sm/RNP.

Anti Smooth Muscle



Mouse stomach kidney

Antibody
Anti-smooth muscle

Alternate Names
SMA

Antigen
Several potential antigens, including F-actin

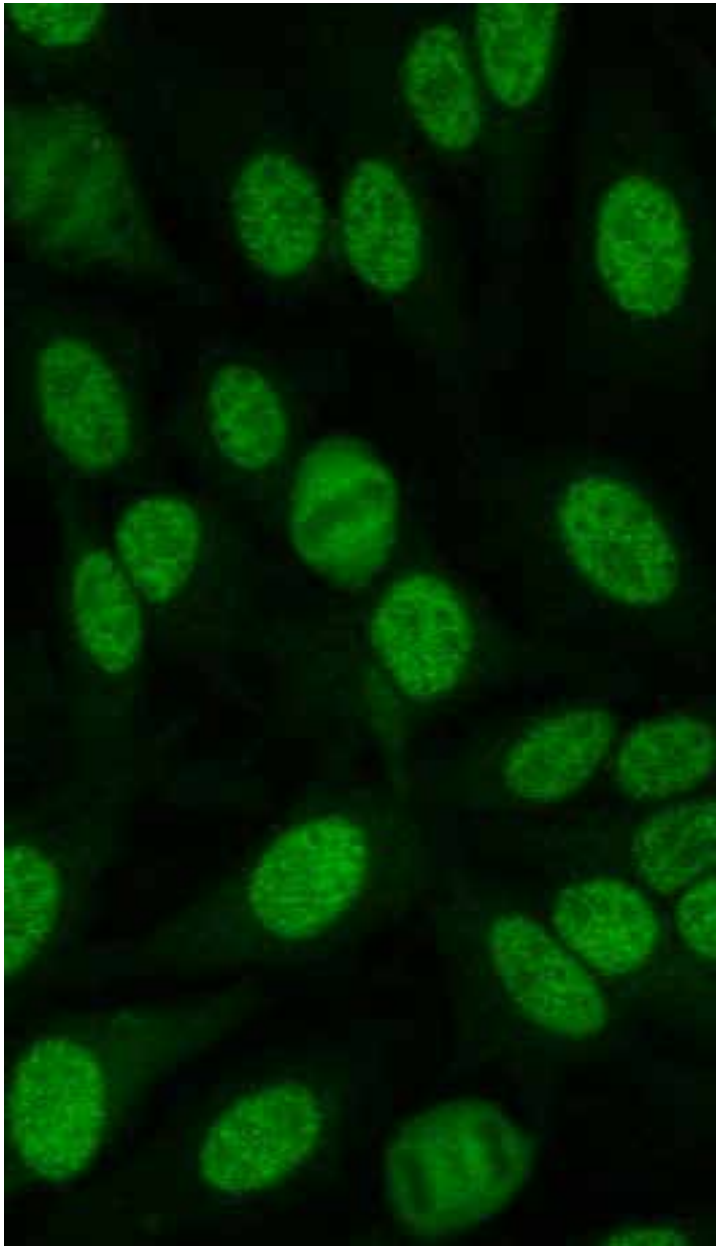
Disease Associations
Autoimmune hepatitis (type 1)

Testing Methods
IFA using mouse stomach kidney substrate, ELISA (anti-F-actin)

Typical IFA Pattern
MSK: cytoplasmic staining of the stomach smooth muscle cells and staining of the arterial blood vessel walls.

Notes
Low level staining of the smooth muscle layer is fairly common. Reference range studies should be performed to insure the proper cut-off is used.

Anti SS-A



HEp-2 Speckled Pattern, 40x Objective

Antibody
Anti SS-A
Anti Sjögren's Syndrome A

Alternate Names
Anti SSA, Anti Ro, Anti SS-A/Ro, Anti- Ro/SS-A

Antigen
Small ribonucleoproteins, two different epitopes: 52kd and 60 kd

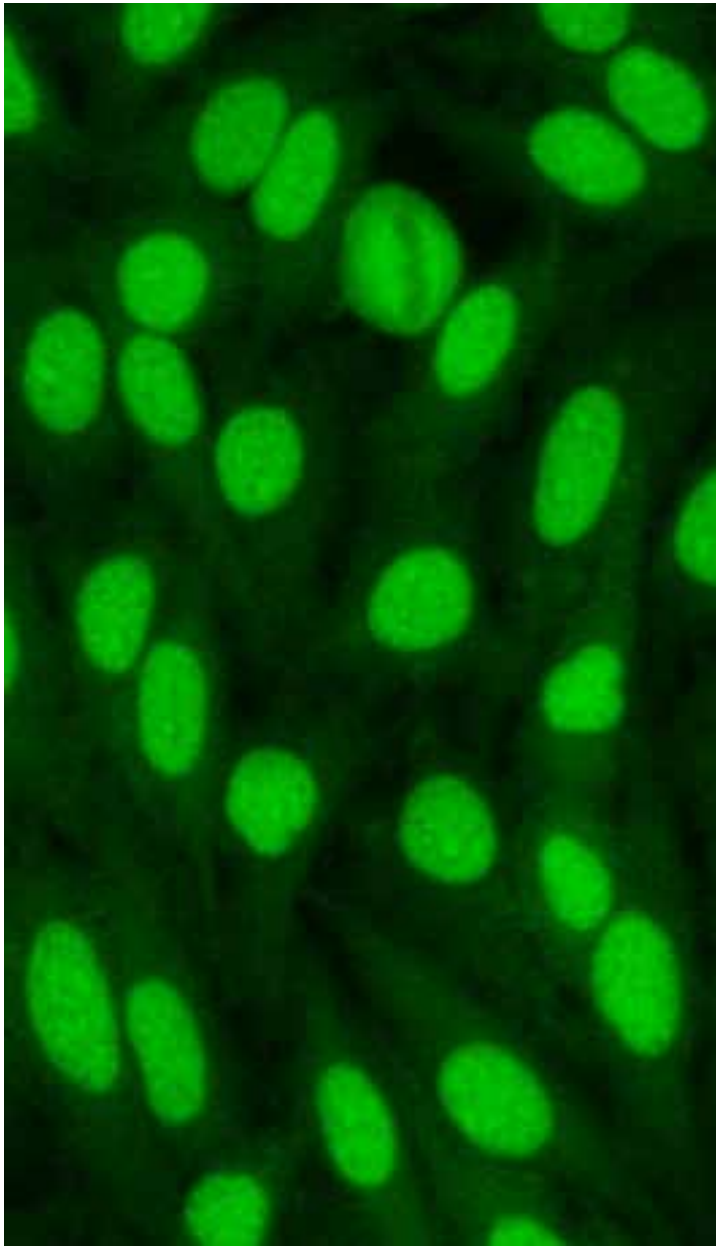
Disease Associations
Sjögren's Syndrome, SLE (particularly subacute cutaneous lupus variant), Neonatal Lupus, Congenital Heart Block

Testing Methods
ELISA, Multiplexing

Typical IFA Pattern
HEp-2: Speckled ANA, pattern is not unique therefore does not permit specific identification.

Notes
ANA by IFA may be weak or absent for some anti-SSA antibodies. One of the ENA (extractable nuclear antigens) antibodies.

Anti SS-B



HEp-2 Speckled Pattern, 40x Objective

Antibody
Anti SS-A
Anti Sjögren's Syndrome A

Alternate Names
Anti SSB, Anti La

Antigen
Small ribonucleoproteins

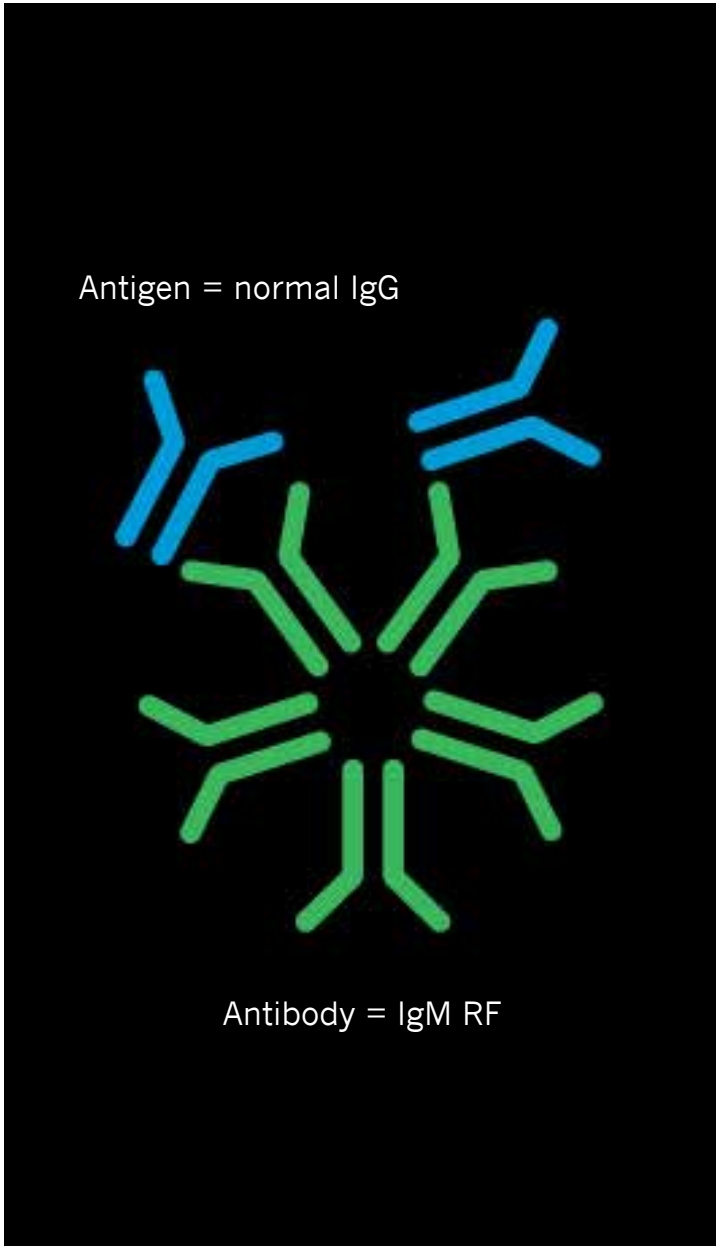
Disease Associations
Sjögren's Syndrome, SLE, Neonatal Lupus, Congenital Heart Block

Testing Methods
ELISA, Multiplexing, IFA (screening, not specific identification)

Typical IFA Pattern
HEp-2: Speckled ANA, may also have some nucleolar staining, pattern is not unique therefore does not permit specific identification.

Notes
Most samples with anti SS-B have coexisting antibodies to SS-A. One of the ENA (extractable nuclear antigens) antibodies.

Rh



IgM RF binding to normal IgG

Antibody
RF
Rheumatoid factor

Antigen
Epitopes are in the constant region of the IgG heavy chain.

Disease Associations
Rheumatoid arthritis (RA) and Sjögren's syndrome. Also seen in the sera of patients with other systemic autoimmune disease, mixed cryoglobulinemia, some chronic bacterial and viral infections, and in the sera of healthy individuals (particularly the elderly) in low titer.

Testing Methods
Agglutination, Nephelometry/Turbidometry, ELISA

Typical IFA Pattern
Not applicable

Notes
The name rheumatoid factor was given before the discovery that it was an antibody to IgG. There are several different isotypes of rheumatoid factor, IgM, IgA, and IgG (including the subclasses IgG1, IgG2, IgG3, and IgG4). Most clinical assays in the US detect only IgM RF.