

# Autoimmune Diseases and Testing

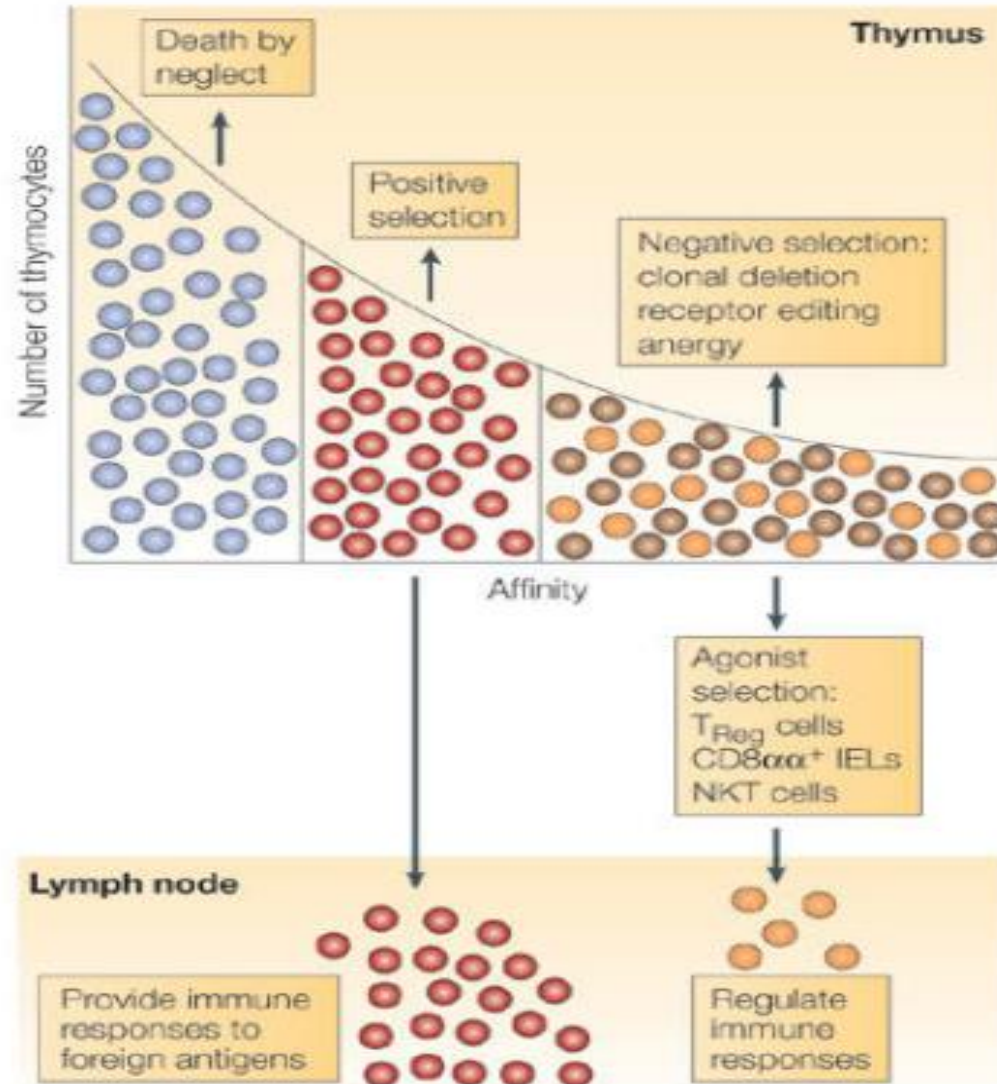
Sonja T. Bruketa MLS(ASCP)

# Objectives:

- Discuss mechanism of autoimmune disease and trends
- Cover testing done in Immunology Lab for connective tissue disease
- Cover ANCA testing
- Discuss Autoimmune Liver disease testing, Celiac disease and Rheumatoid Arthritis

- The role of the immune system is to keep the body healthy by destroying that which it perceives to be non-self.
- In Autoimmune Disease the body mistakes normal tissue for non-self and begins to attack the tissue in an attempt to destroy it.
- Activation of an immune response against self-tissue
- Specific breakdown of mechanisms responsible for tolerance to self-antigens

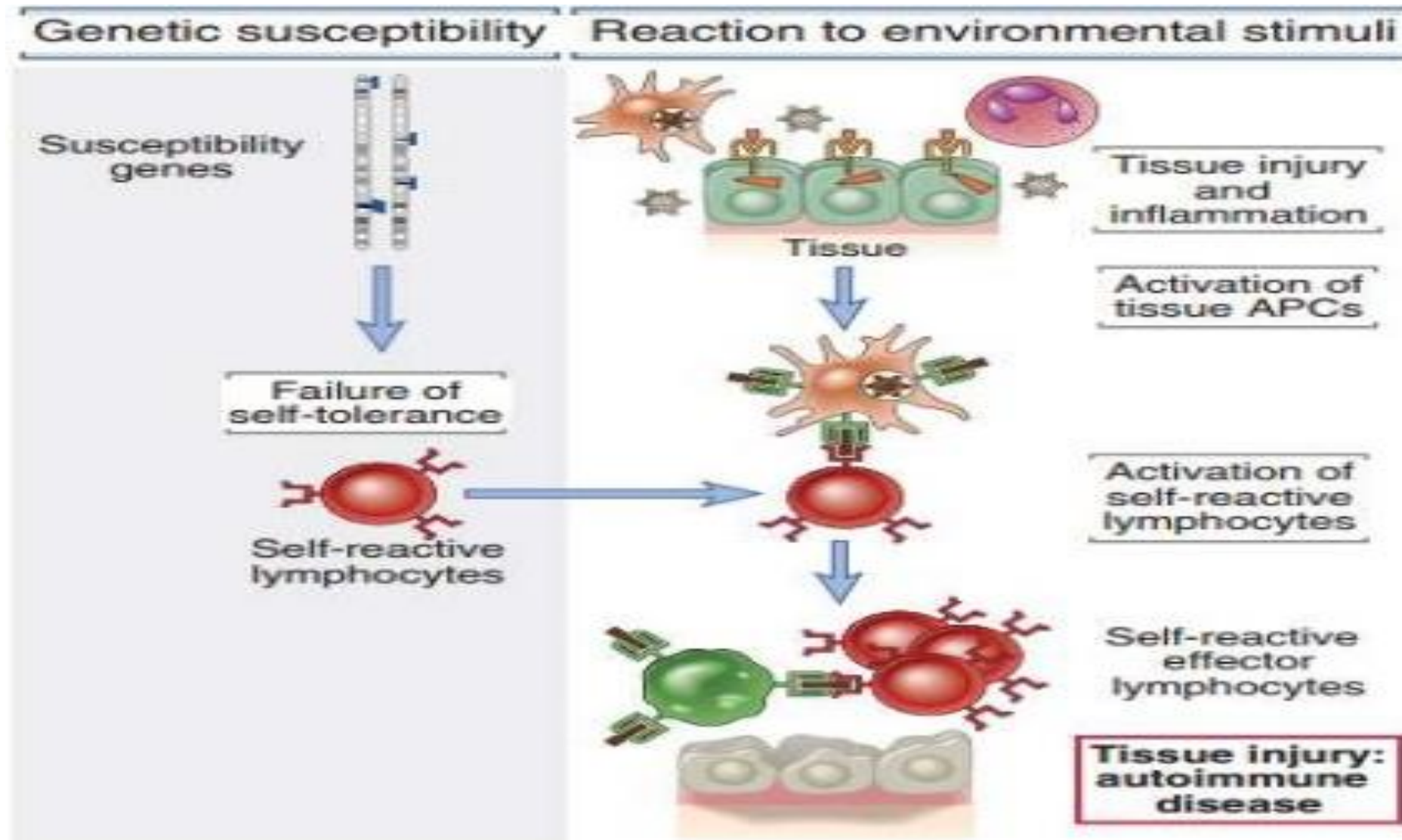
## Central Tolerance



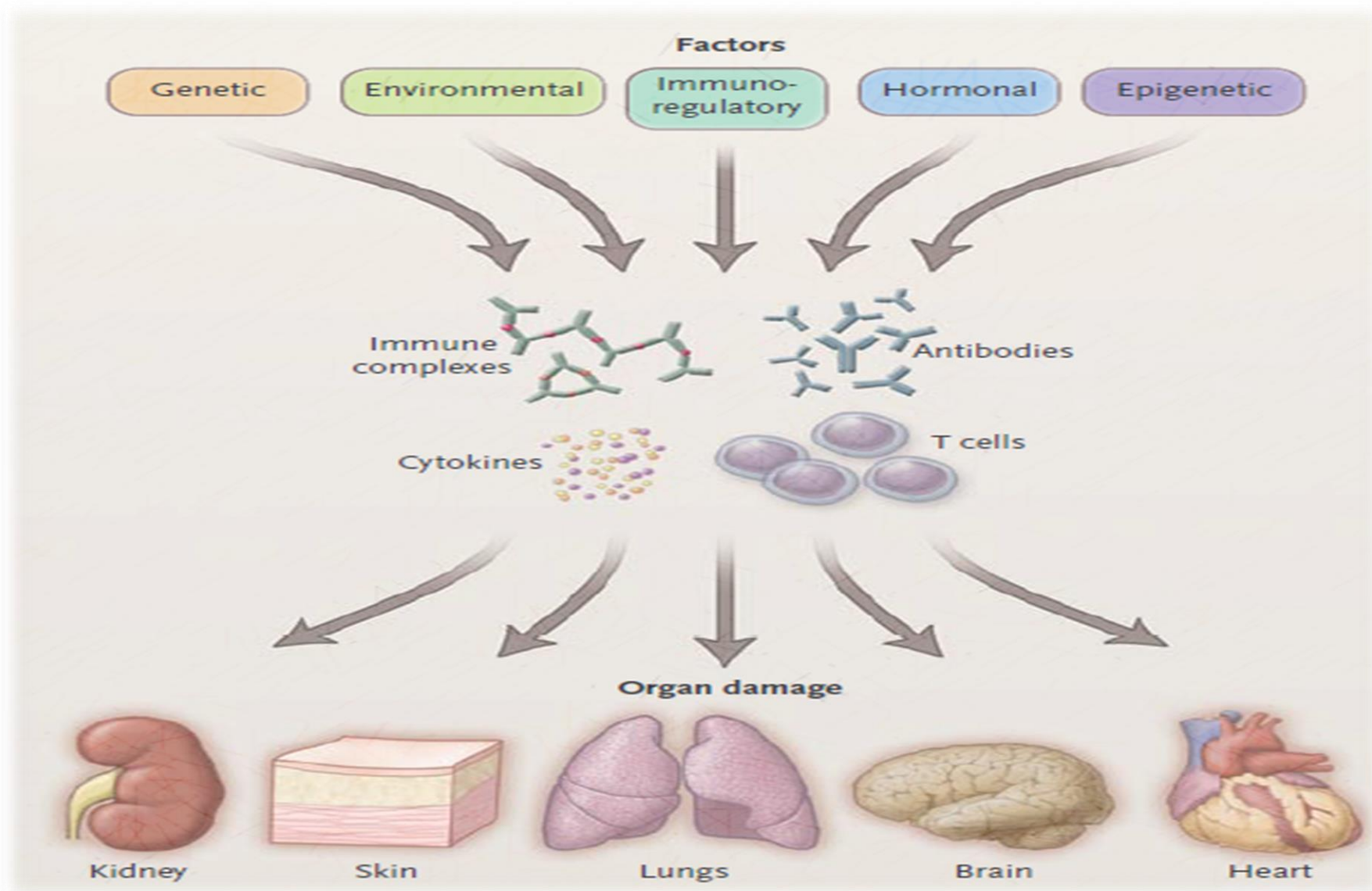
All prothymocytes migrate from bone marrow and enter thymus

1. Positive selection – must recognize self-MHC
2. Negative selection – must not recognize self-Ags

# Mechanism of Autoimmunity



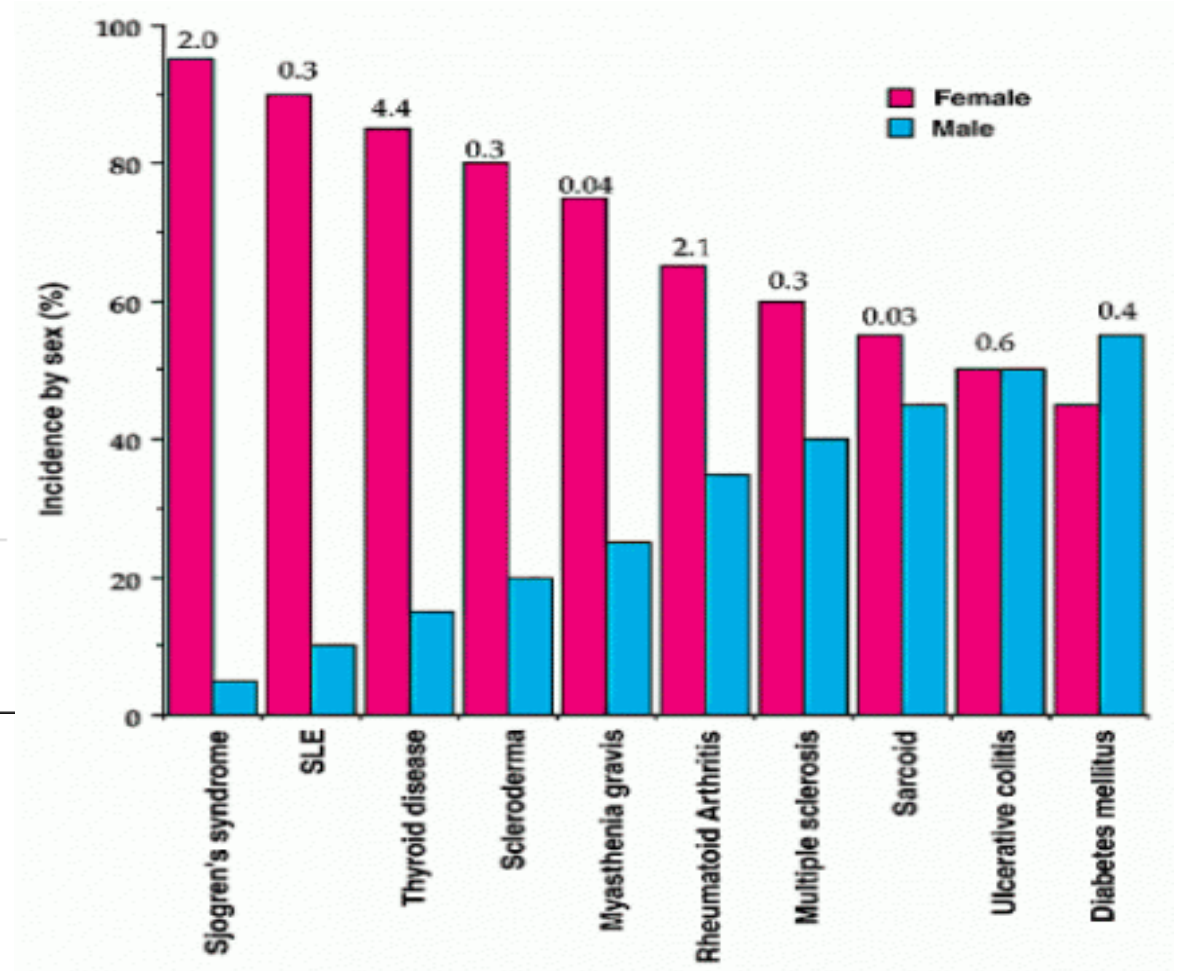
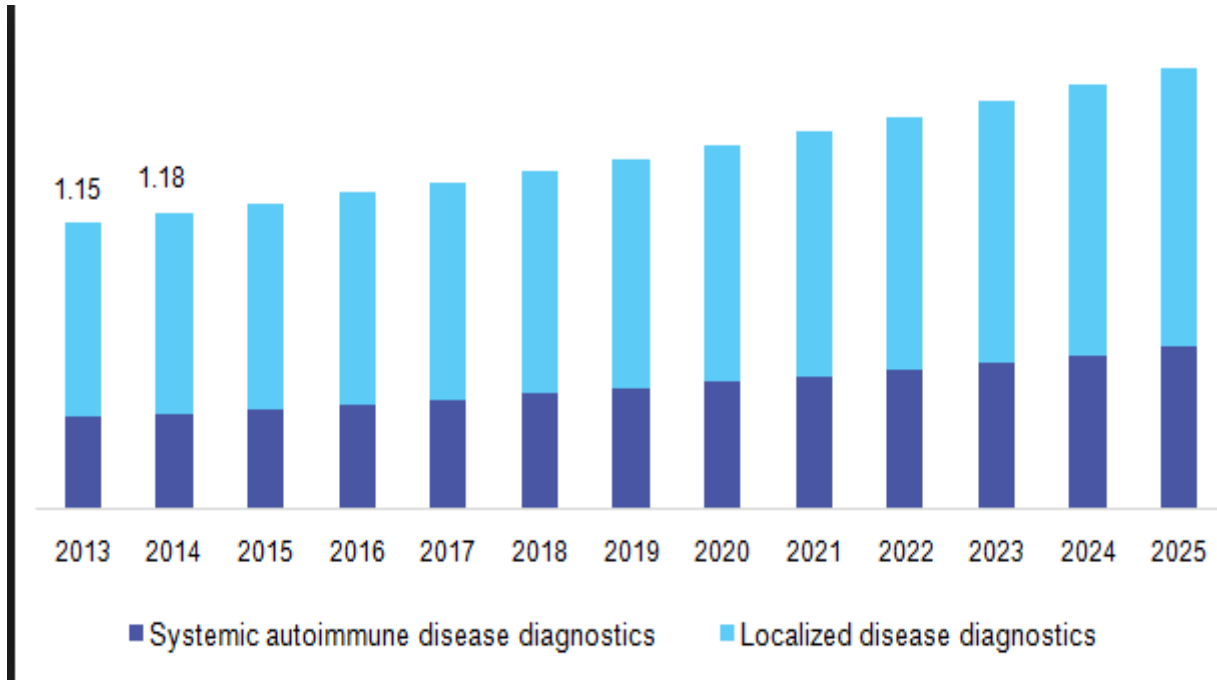




Organ-Specific	Systemic
Hashimoto thyroiditis	Systemic lupus erythematosus
Autoimmune hemolytic anemia	Rheumatoid arthritis
Autoimmune atrophic gastritis of pernicious anemia	Sjögren syndrome
Multiple sclerosis	Reiter syndrome
Autoimmune orchitis	Inflammatory myopathies*
Goodpasture syndrome	Systemic sclerosis (scleroderma)*
Autoimmune thrombocytopenia	Polyarteritis nodosa*
Insulin-dependent diabetes mellitus	
Myasthenia gravis	
Graves disease	
Primary biliary cirrhosis*	
Autoimmune (chronic active) hepatitis*	
Ulcerative colitis*	

\*The evidence supporting an autoimmune basis of these disorders is not strong.

# Recent trends in Autoimmune Diseases



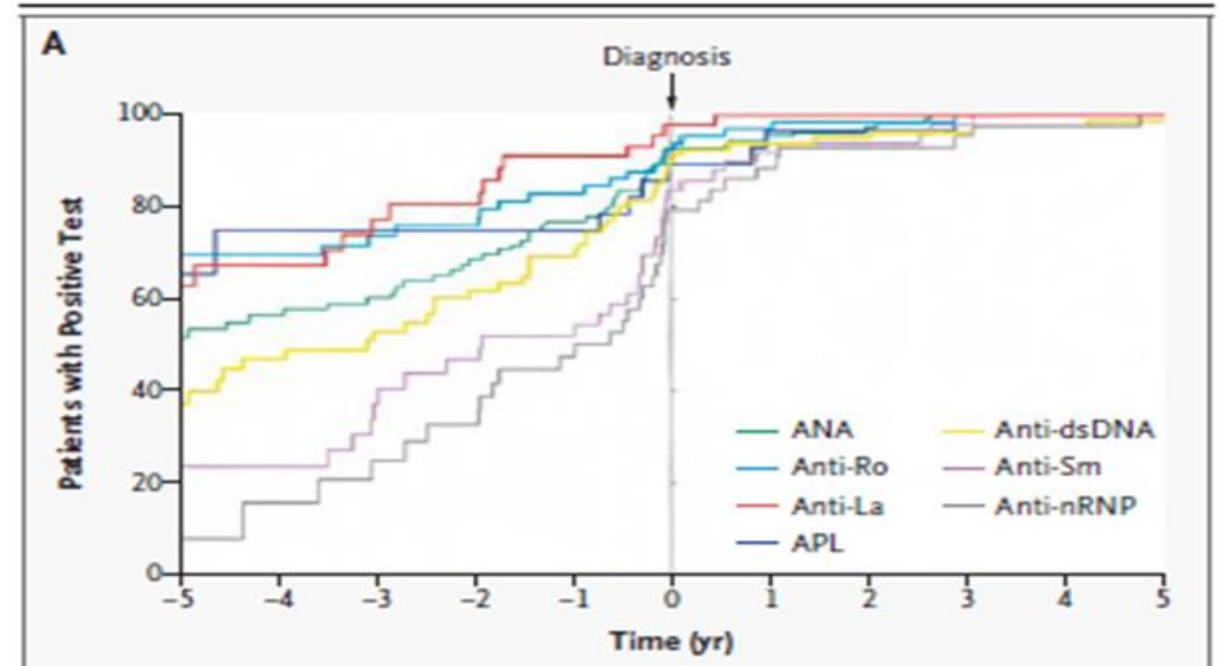
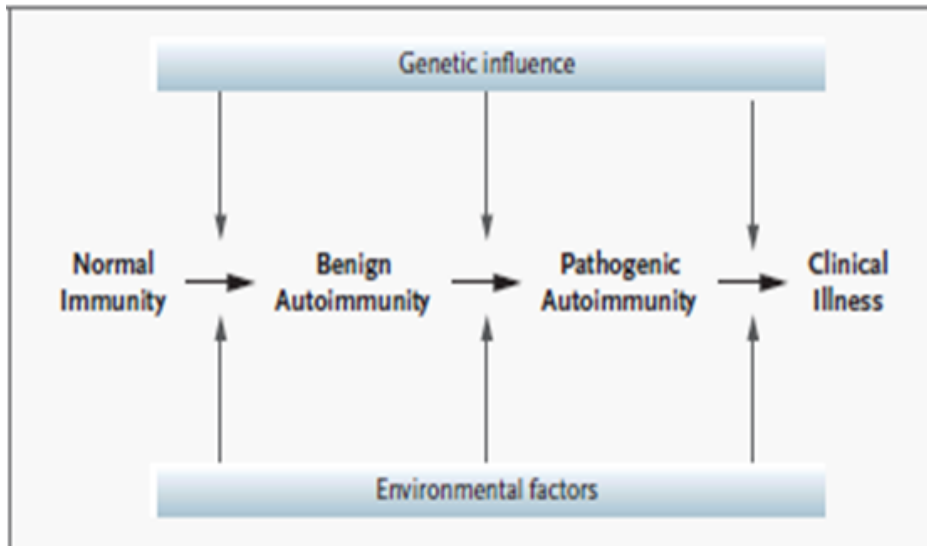


# Autoimmune disease Associated Autoantibody

Autoantibody	Disease Associated
Anti-dsDNA	Lupus
Anti-Smith	
Rheumatoid Factor (Anti-Fc portion of IgG)	Rheumatoid arthritis
Anti-Cyclic Citrullinated Peptide (Anti-CCP)	
Anti-Topoisomerase I (Anti-Scl-70)	Systemic Scleroderma
Anti-Centromere	CREST Syndrome (Scleroderma)
Anti-Basement Membrane	Goodpasture's
p-ANCA and c-ANCA	Various forms of Vasculitis

Staining	Antigen	Disease
Diffuse or homogeneous	Deoxyribonucleoprotein, histone, ds DNA	Systemic lupus erythematosus (SLE)
Peripheral or rim	ds DNA	SLE
Speckled	saline extractable antigens Sm	SLE
	<b>SS-A, SS-B</b>	<b>Sjögren's Syndrome</b>
	<b>Scl-70</b>	Progressive systemic sclerosis
	RNP	Mixed connective tissue disease, SLE
Nucleolar	Nucleolar RNA	SLE, scleroderma
Centromere	Centromere/kinetochore region of chromosome	Crest subset of systemic sclerosis

# Autoantibodies are typically present years before onset of symptoms or diagnosis of Autoimmune disease



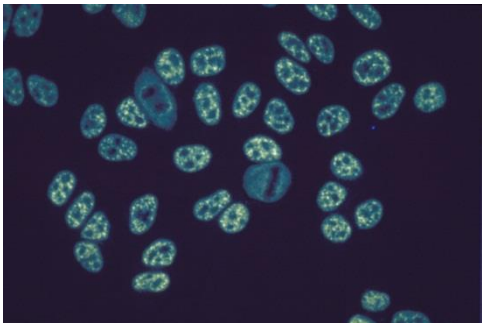
# Antinuclear Antibody Testing (ANA)

## Connective Tissue Disease

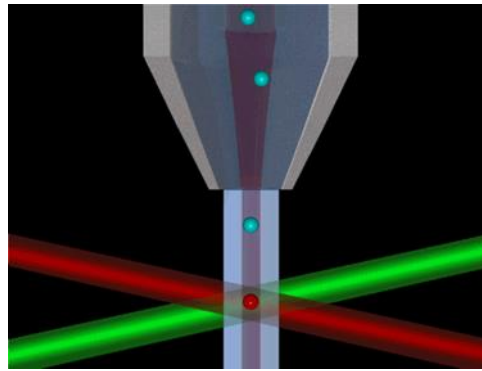
### Methods

- Indirect Immunofluorescent Method (IFA)
- Multiplex Bead Assay-EIA (BioPlex)
- Double Immunodiffusion Assay (Ouchterlony)
- Line Immunoassay (Immunoblot)

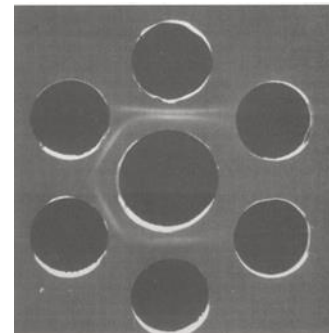
IFA



BioPlex



Ouchterlony



Immunoblot



# ANA by EIA

## ANA by Multiplex methodology

### BioPlex



### ENA EXTRACTABLE NUCLEAR ANTIGENS

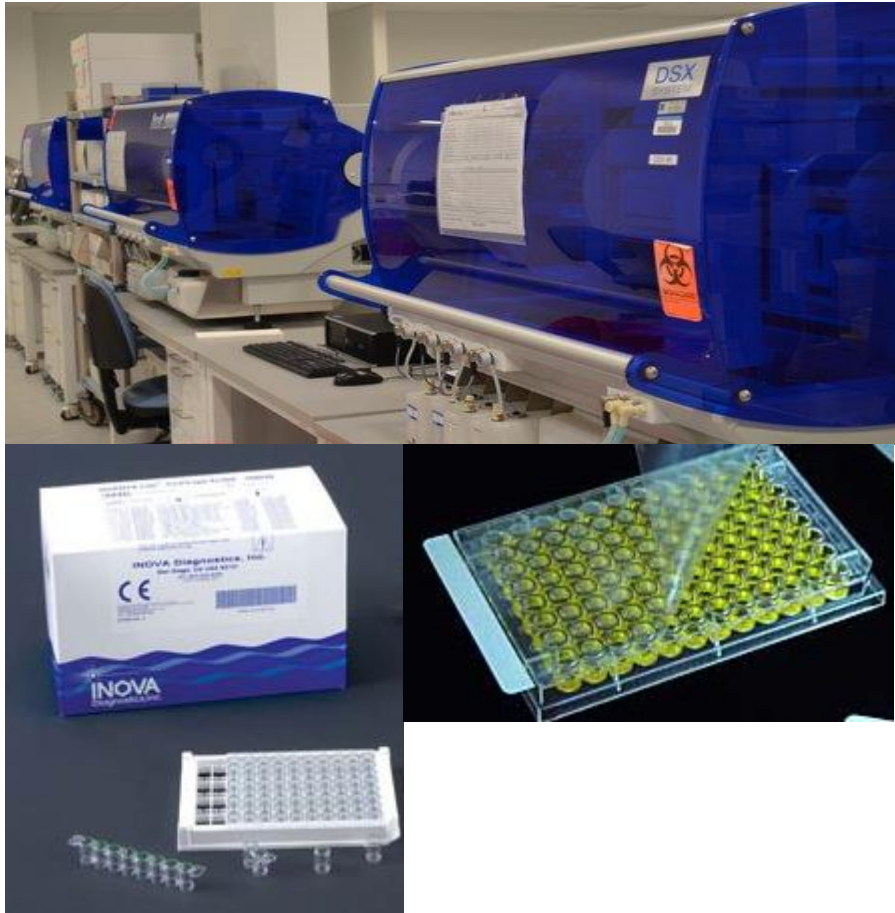
Smith (Sm)  
SSa (Ro)  
SSb (La)  
RNP  
Scl-70  
Jo-1  
Ribo P  
Centromere  
Chromatin

## dsDNA Methods of Testing





# DNA by Enzyme Immunoassay (ELISA)

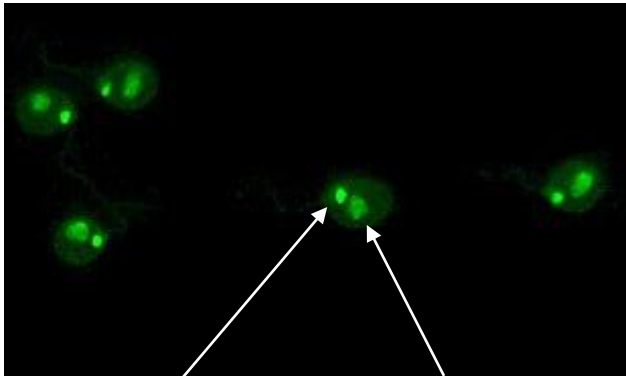
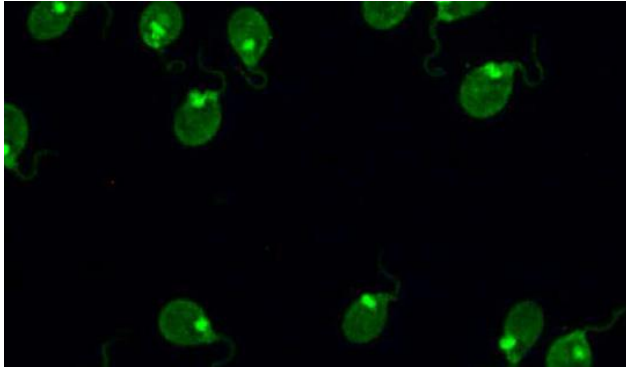


- $<30$  IU/mL = Negative
- $35 - 75$  IU/mL = Borderline
- $>75$  IU/mL = Positive

Positives if part of the panel reflex to Crithidia test.

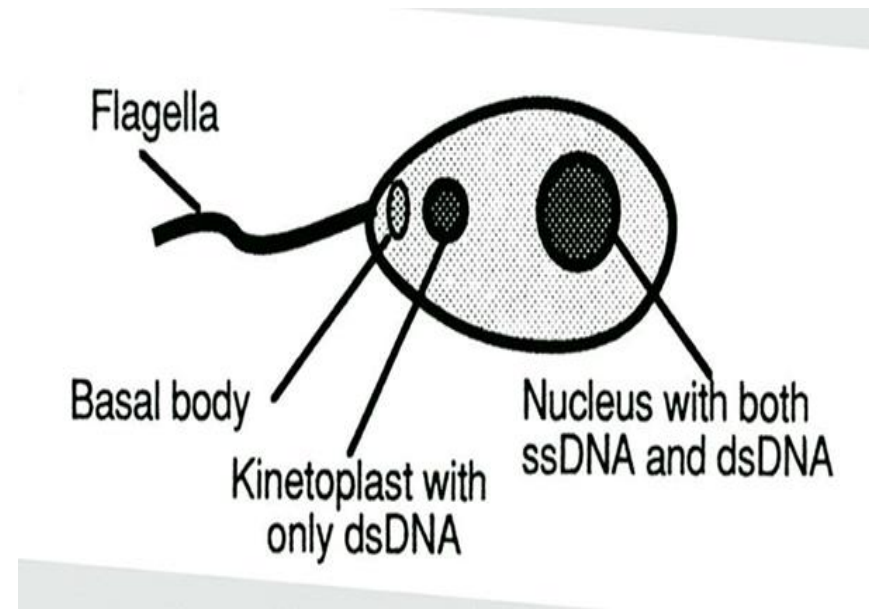
Some Lupus patients at the height of their symptoms may have DNA value  $>1000$  IU/mL.

# Crithidia luciliae

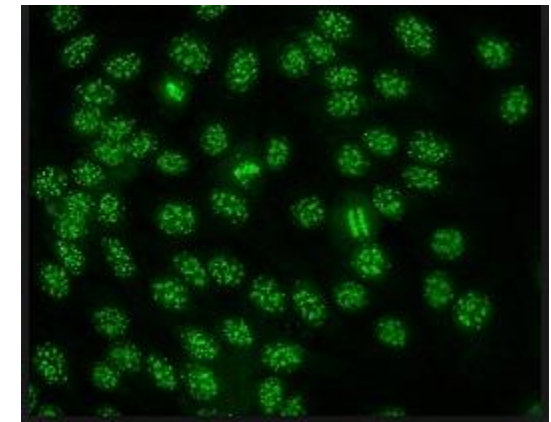
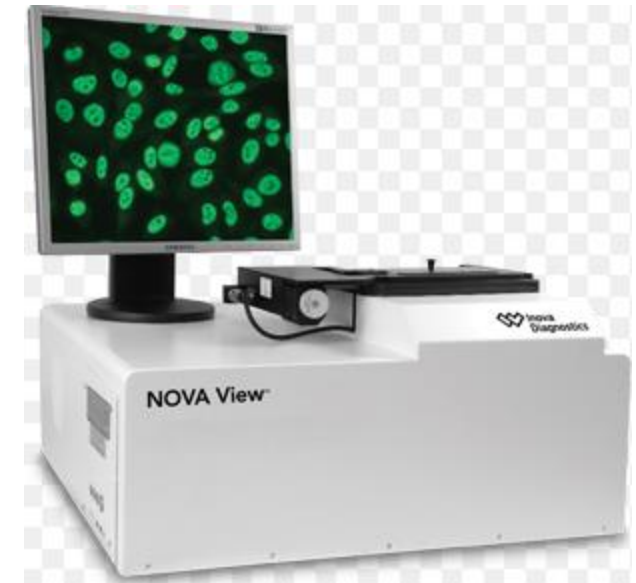
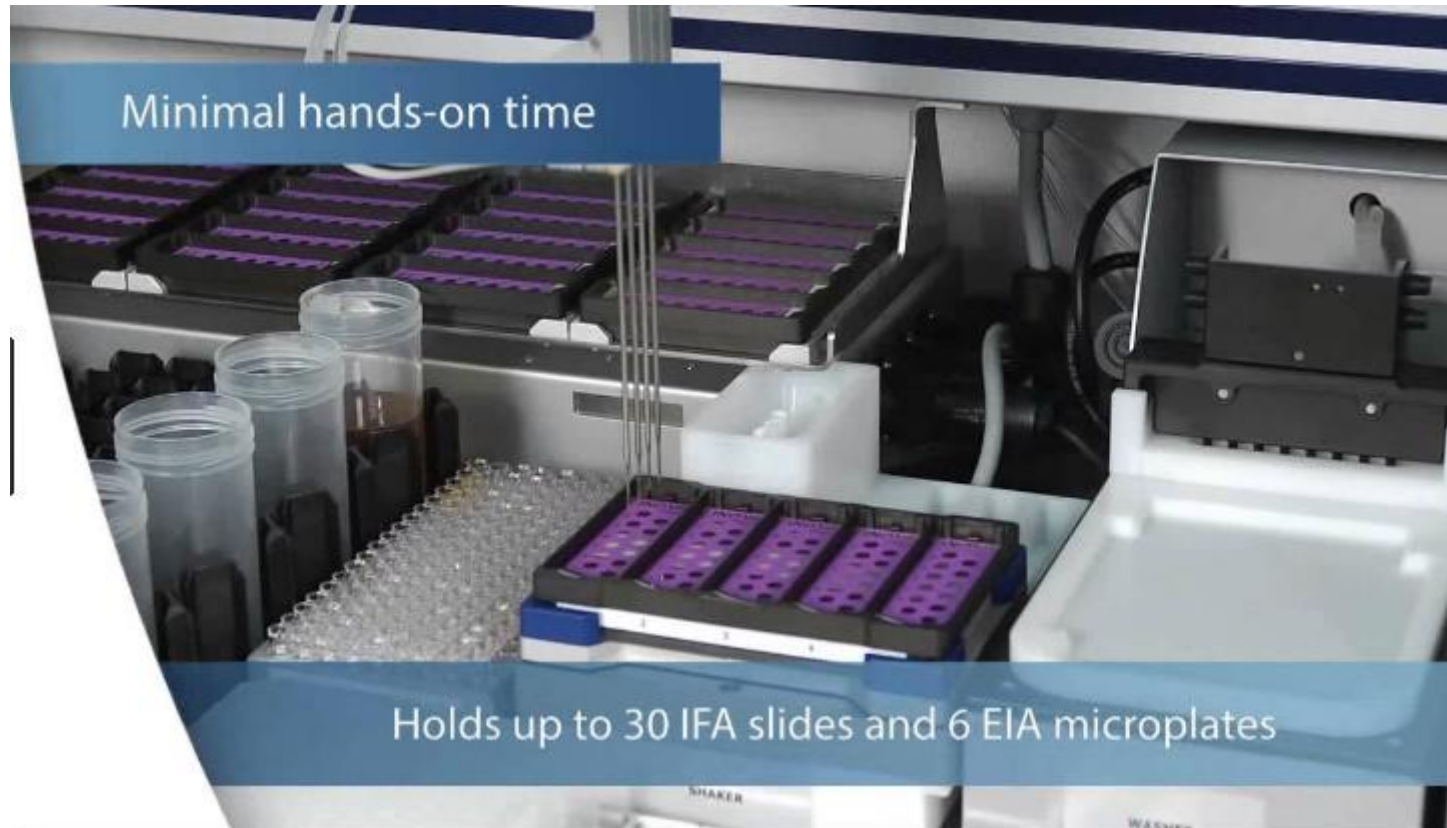


Kinetoplast

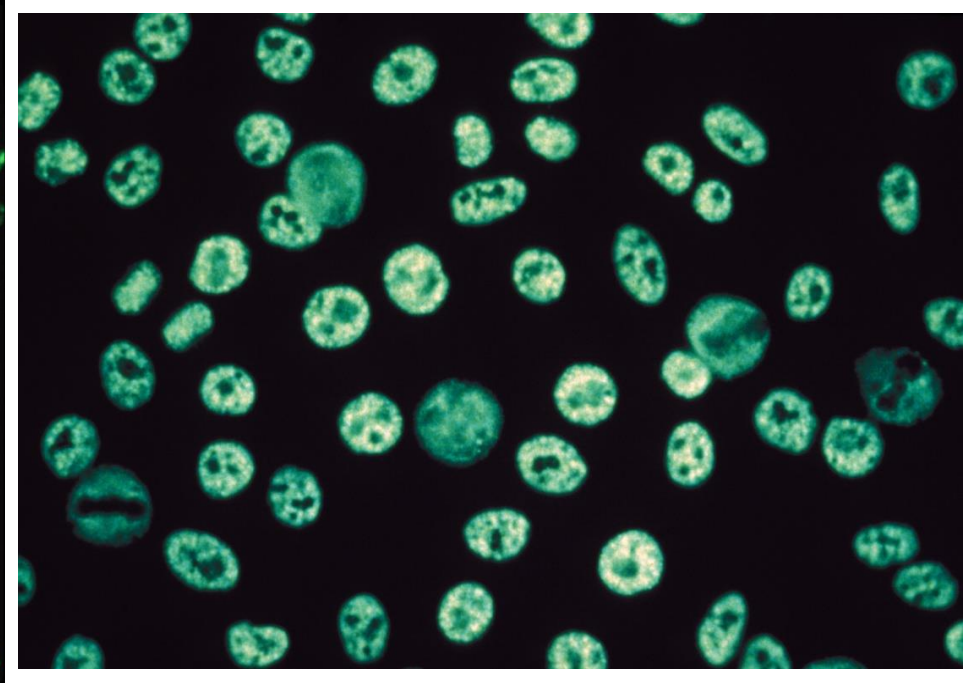
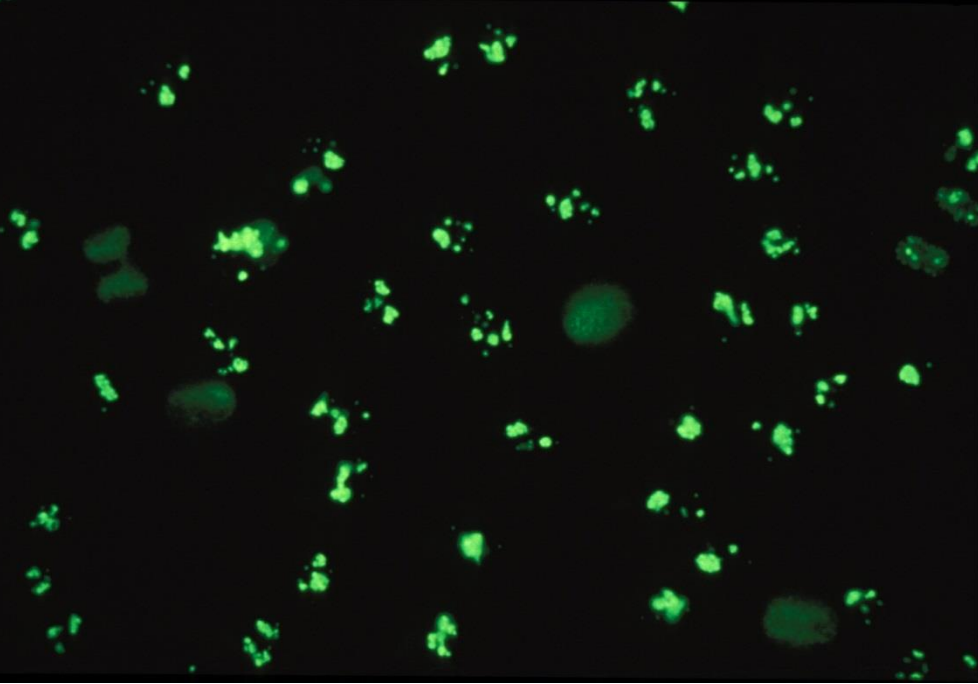
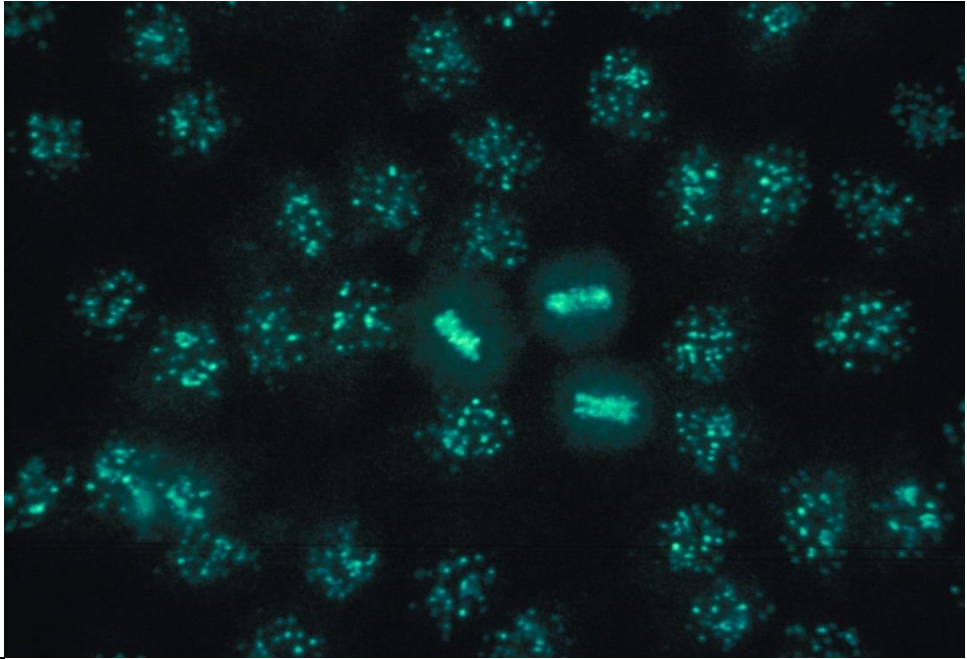
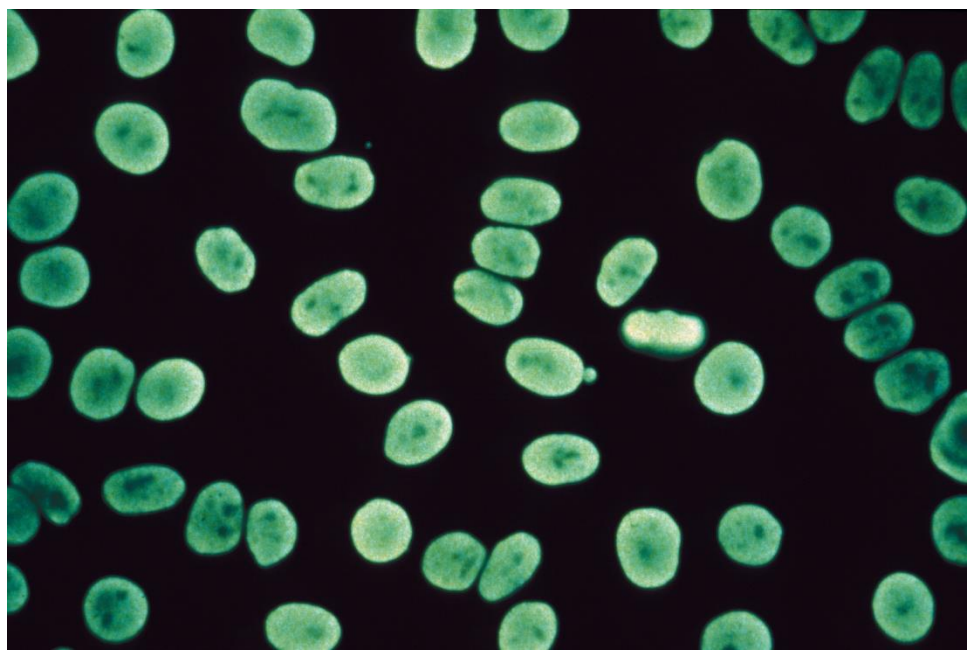
Nucleus



# ANA by Indirect Immunofluorescence Assay (IFA)









# Clinical manifestations of the Connective Tissue diseases



Lacrimal and salivary malfunction and swelling of salivary glands.

## CREST syndrome

This is a variant of systemic sclerosis  
Relatively good prognosis

**Fluorescent ANA test (indirect IF) :** nuclear centromeres

The mnemonic stands for **C**alcinosis, **R**aynaud's phenomenon  
**E**sophageal dysmotility, **S**clerodactyly and **T**elangiectasia.

Raynaud's phenomenon –  
antibodies to **nRNP**



# Anti-Neutrophil Cytoplasmic Antibodies (ANCA) Vasculitis

## Granulomatosis with Polyangitis (Wegener's Disease)

C-ANCA

P-ANCA



C-ANCA = PR3  
P-ANCA = MPO

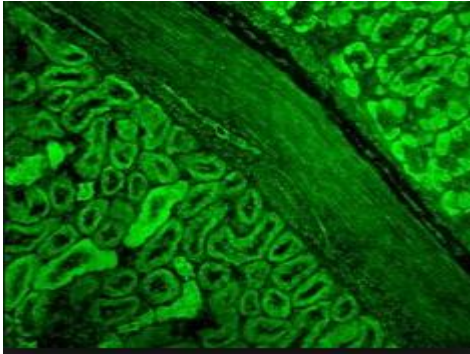


# Algorithm for ANCA testing

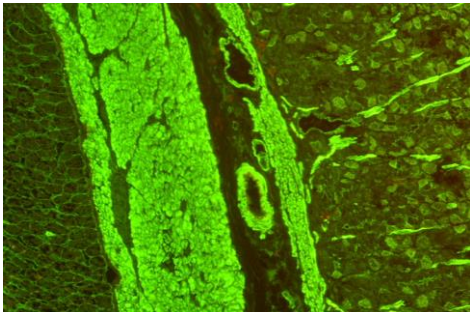
Antibody	Ethanol Substrate	Formalin Substrate	Confirmatory ELISA Test
cANCA	Cytoplasmic pattern	Cytoplasmic pattern	Anti-Proteinase 3 (PR3)
pANCA	Perinuclear pattern	Cytoplasmic pattern	Anti-Myeloperoxidase (MPO)
ANA	Perinuclear/nuclear	Nuclear/Negative	Negative for PR3 and MPO, ANA on HEp2 cells
Atypical P-ANCA	Very Perinuclear with no nuclear extension	Negative or Perinuclear	Negative for PR3 or MPO. Lysozyme, elastase, lactoferrin, cathepsin G, 50 kD Nuc. Env protein

# Autoimmune Liver Disease

## Testing for AMA, ASMA



**AMA- Positive seen in Primary Biliary Cirrhosis**

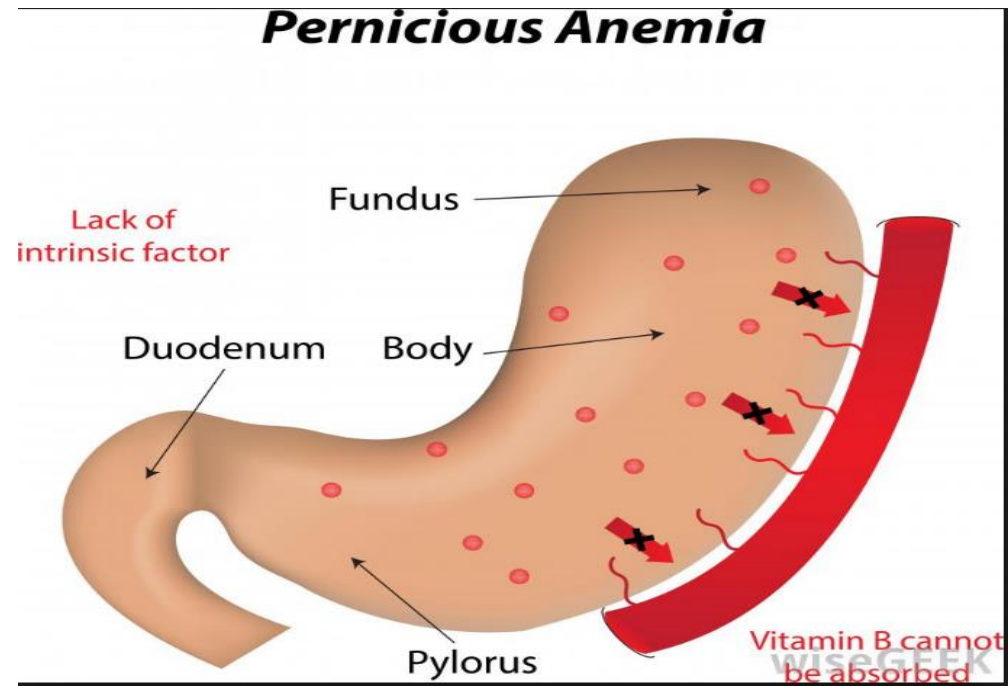
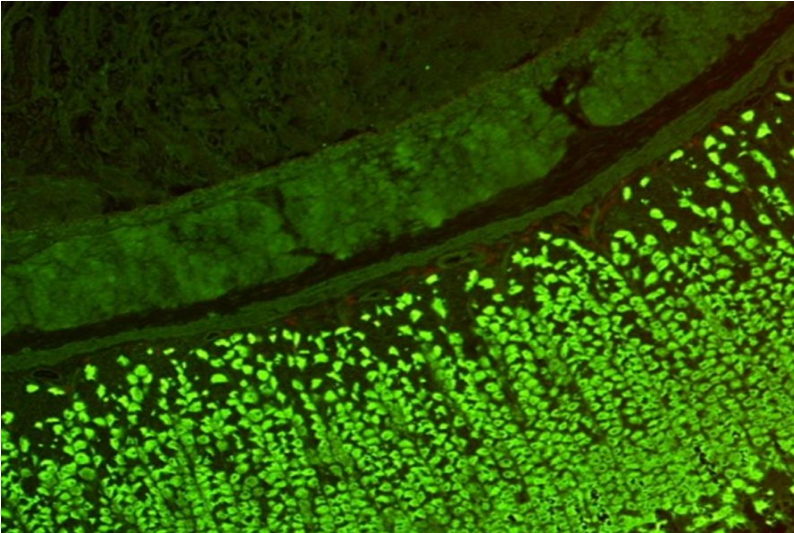


**ASMA – Positive seen in Autoimmune Hepatitis**





# Testing for APCA



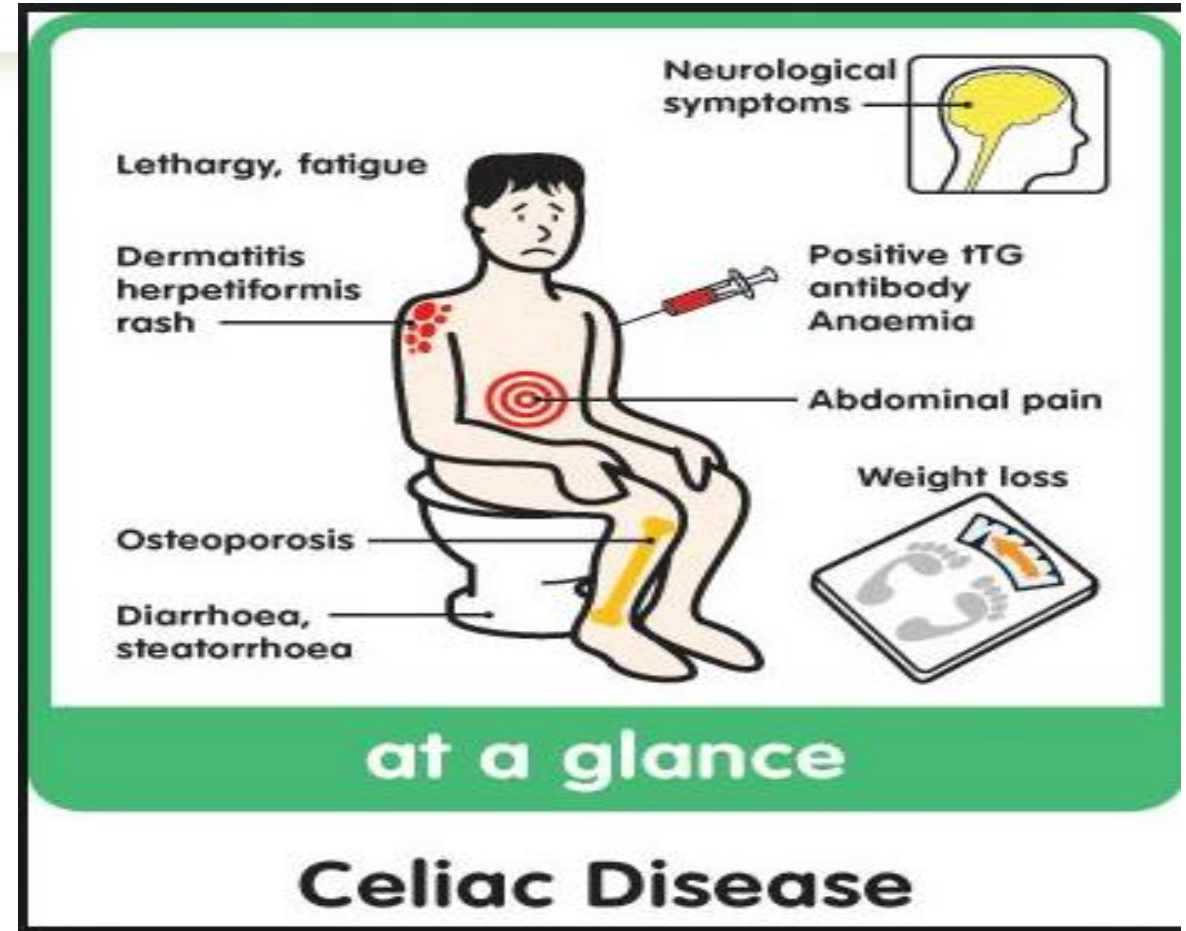
**APCA – Positive seen in pernicious anemia**

# Celiac Disease

## Symptoms



With time intestinal villi are damaged due to abnormal immune reaction to gluten consequently resulting in malabsorption of nutrients.



# Testing for Celiac Disease



Testing for selective IgA deficiency  
IgA low ( $<7$  mg/dl)

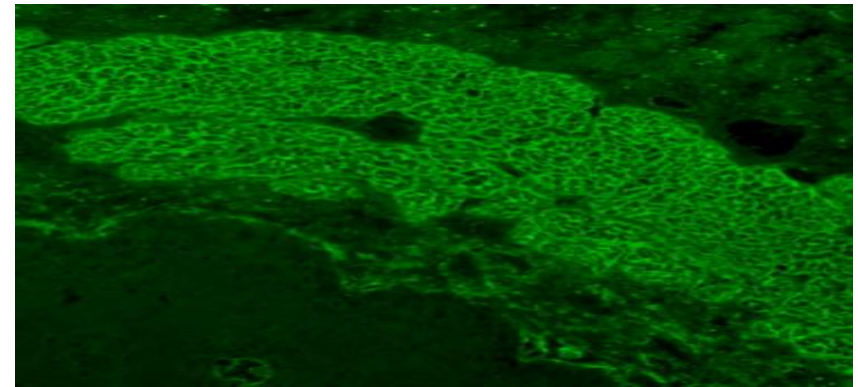


**TTGA**

Gliadin IgA  
Gliadin IgG  
TTGG



**Endomysial Antibody**



# Rheumatoid Arthritis (RA)



Autoimmune disease that attacks joint linings (synovial membranes).

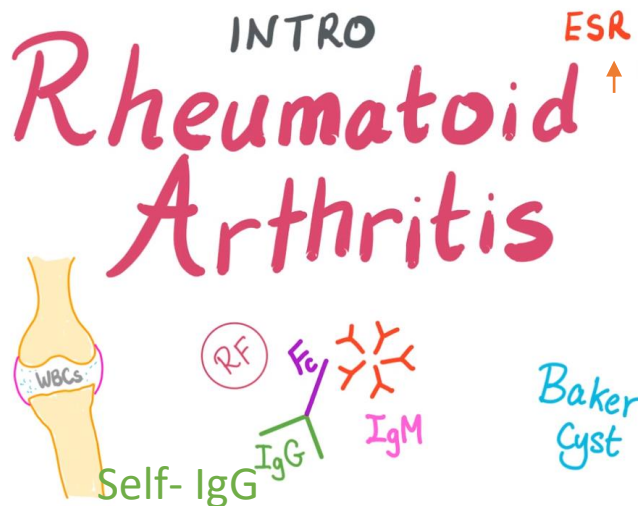
The inflammation associated with rheumatoid arthritis can damage other parts of the body as well.

## Testing

**CCP** – Cyclic Citrullinated Peptide Antibodies highly specific and more sensitive than RF

**RF** – Rheumatoid Factor (could be of any class but most laboratory tests reflect the amount of IgM).

**CRP** - C Reactive Protein (Inflammatory marker)





# Summary

- Reviewed Autoimmune Diseases and the ANA testing by different methods.
- Discussed ANCA testing and algorithm followed in our lab.
- Discussed Autoimmune Liver disease testing, Celiac disease and Rheumatoid Arthritis.