
In the Name of God

Pearls and Pitfalls of Rheumatologic Lab Investigations

Indication and Preparation

NA by Nahid Aslani MD

No screening test is ideal for detecting rheumatic diseases

Diagnosis depends on appropriate **history** and thorough **physical examination**

Laboratory investigations may be useful in confirming or ruling out rheumatic disease after a clinical diagnosis is considered.

Rheumatologic lab investigations as part of a 'panel' of tests??

Diagnosis → **Prognosis** → **Monitoring**

DIAGNOSIS

PROGNOSIS

MONITORING

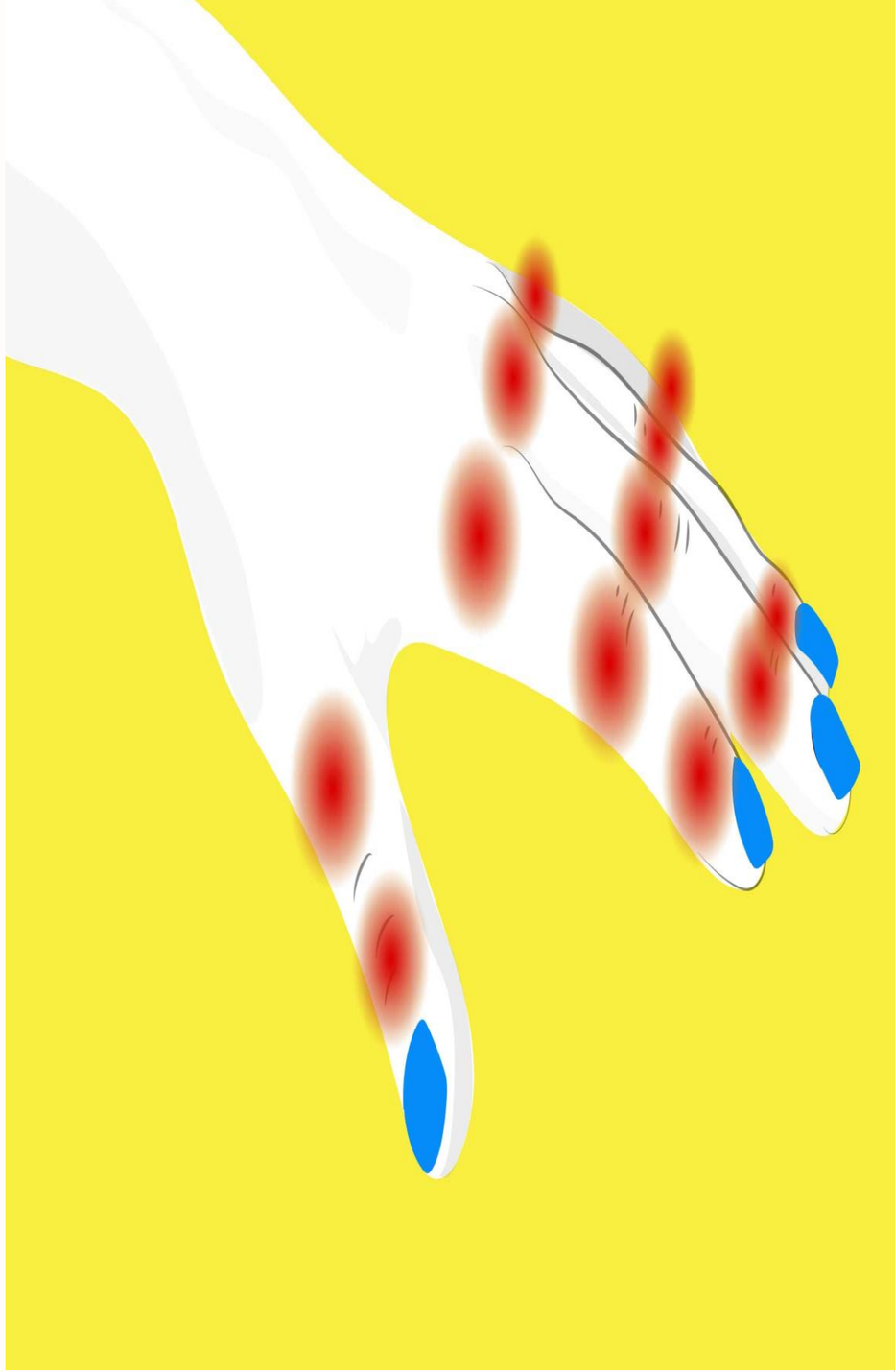
BIOMARKERS

in Rheumatic Diseases

DRUG DEVELOPMENT

- *Identify responders*
- *Assess patient response*

Bay-Jensen AC, Reker D, Kjelgaard-Petersen CF, et al. Osteoarth and Cart 2016; 24: 9-20. Karsdal MA, Henriksen K, Leeming DJ, et al. Biomarkers 2009; 14: 181-202. Bauer DC, Hunter DJ, Abramson SB, et al. Osteoarth and Cart 2006; 14: 723-7.



Case 1

A 13 years girl presented with for routine checkup.

Examination is normal

Patients' mother has had a rheumatologic disorder.

What laboratory investigations you prefer to order for this patient?

FANA(IIF ANA)?

Enzyme-linked immunosorbent assay (ELISA)?

Anti-CCP?

RF (Rheumatoid Factor) ?

Anti ds DNA ?

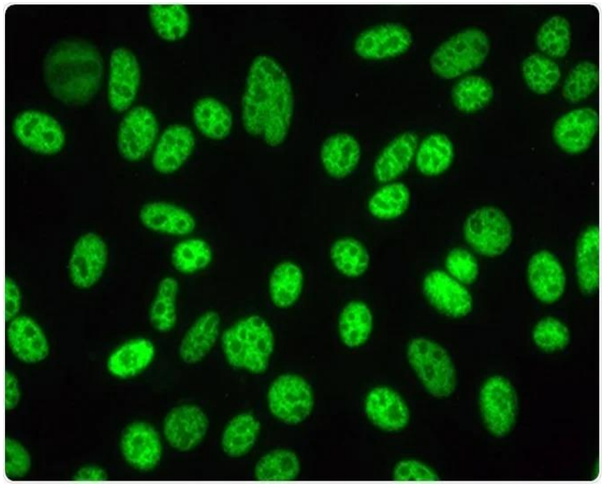
FANA(IIF ANA): 1/80 DFS70

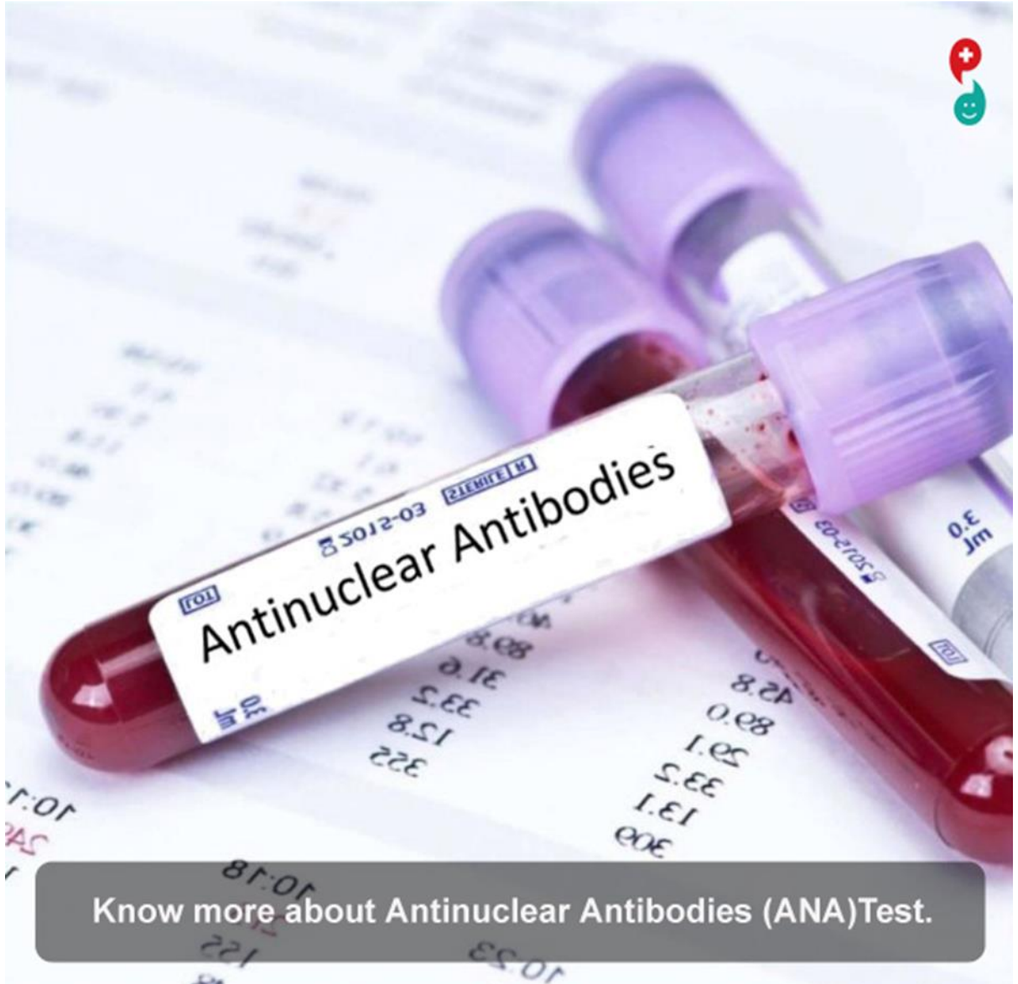
RF (Rheumatoid Factor) : -ve

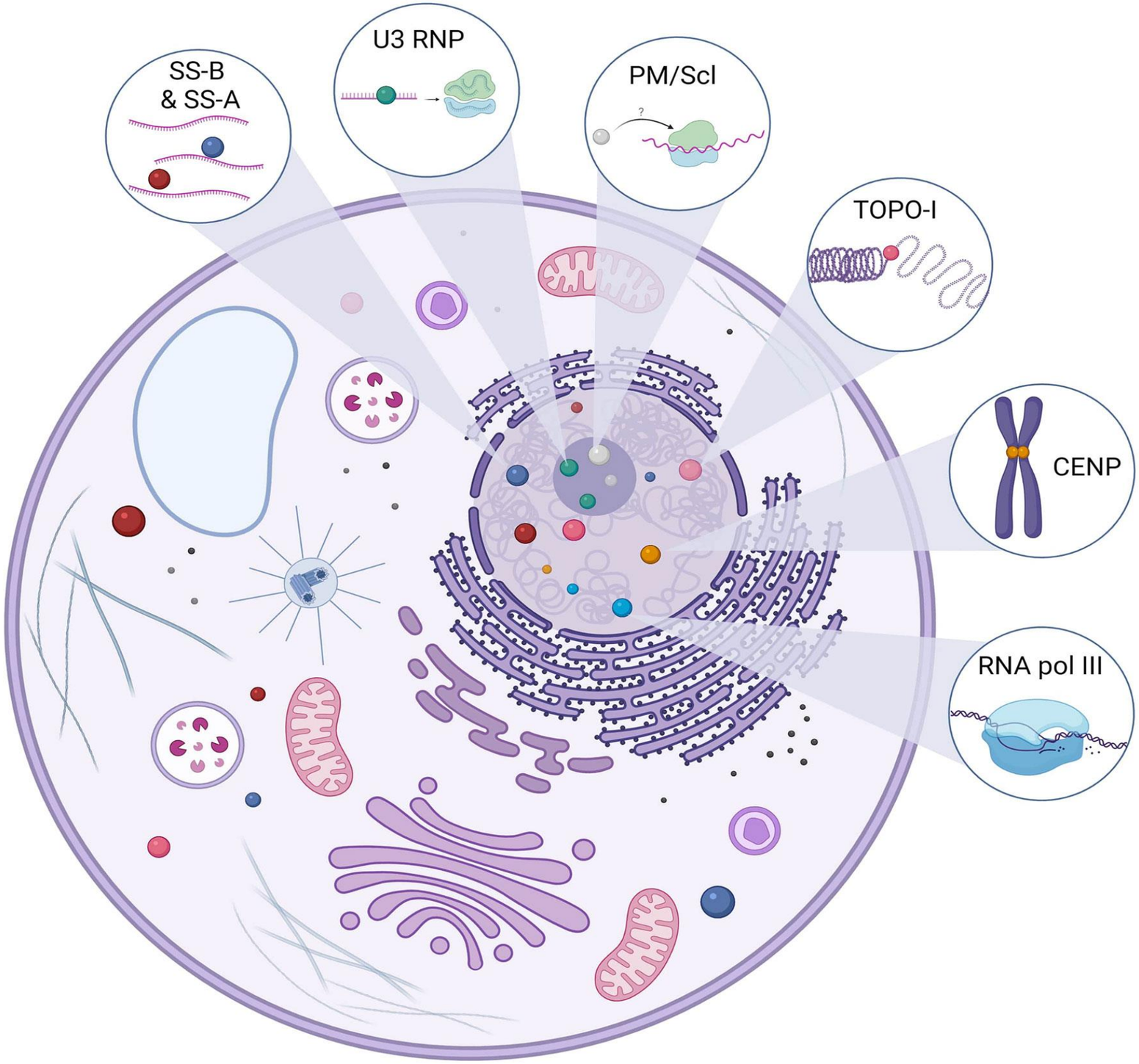
Anti-CCP: -ve

Anti ds DNA : -ve

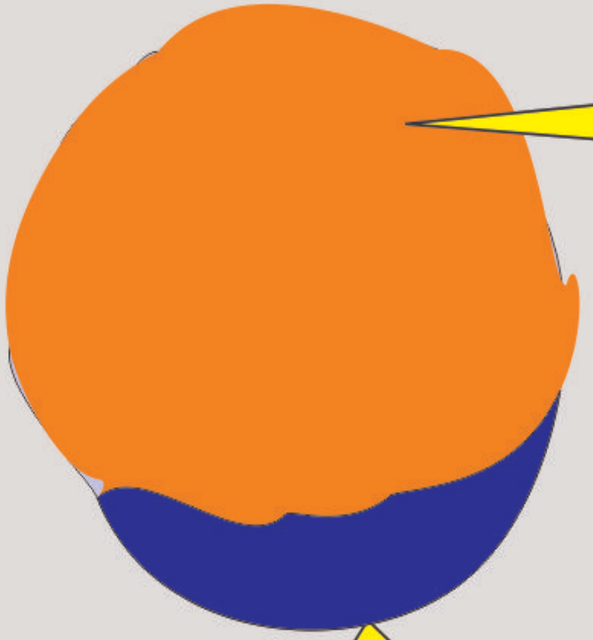
Anti Nuclear Antibody(ANA)






SS-B
& SS-A
U3 RNP
PM/Scl
?
TOPO-I
CENP
RNA pol III

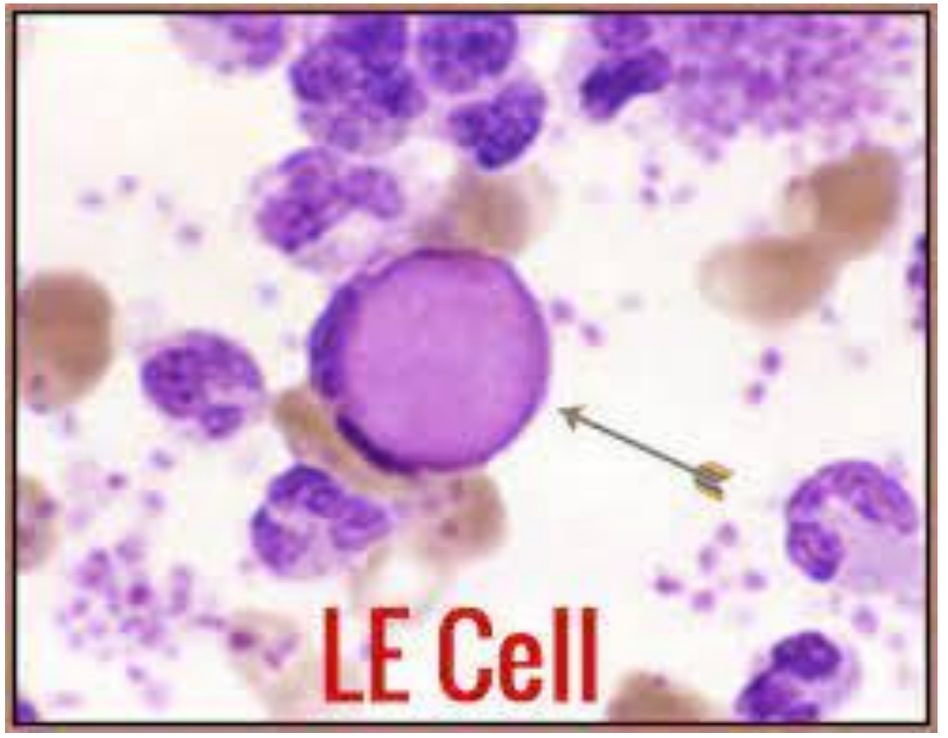
LE cell phenomenon



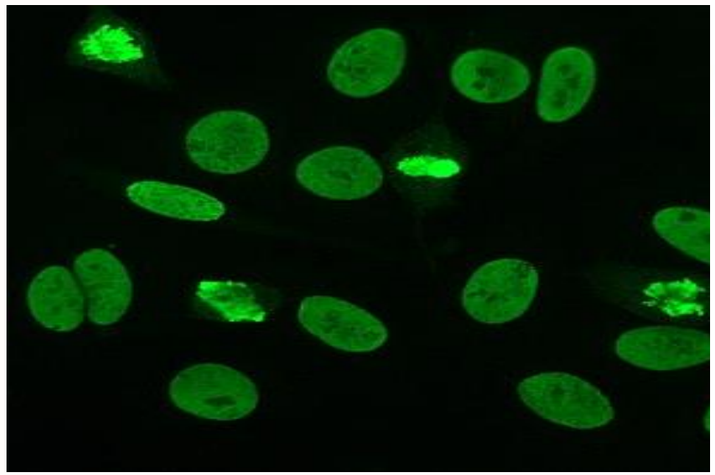
Antinuclear antibody

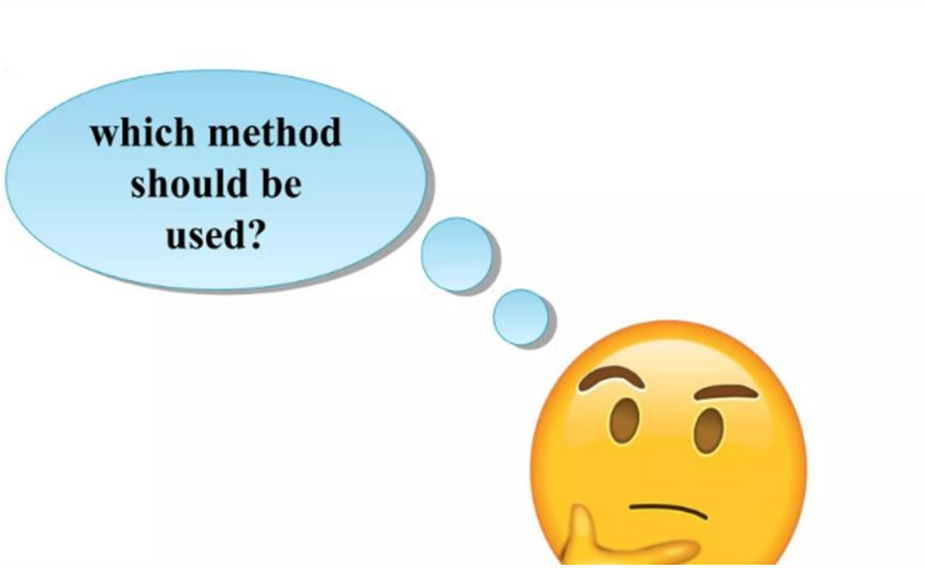
Nucleus pushed to periphery

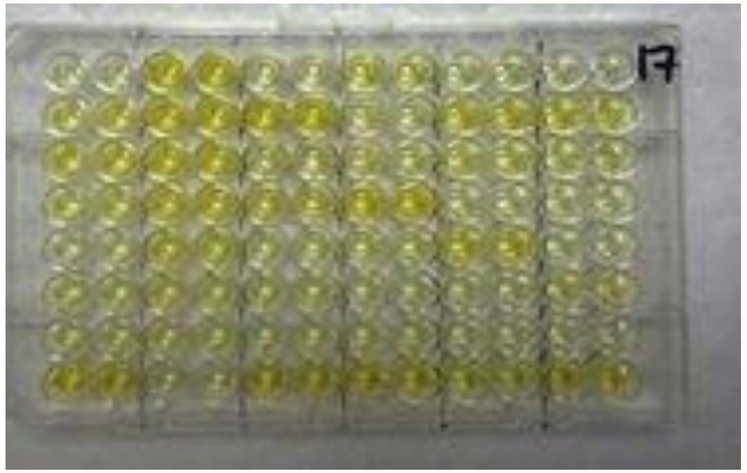
labpedia.net



How to detect ANA?



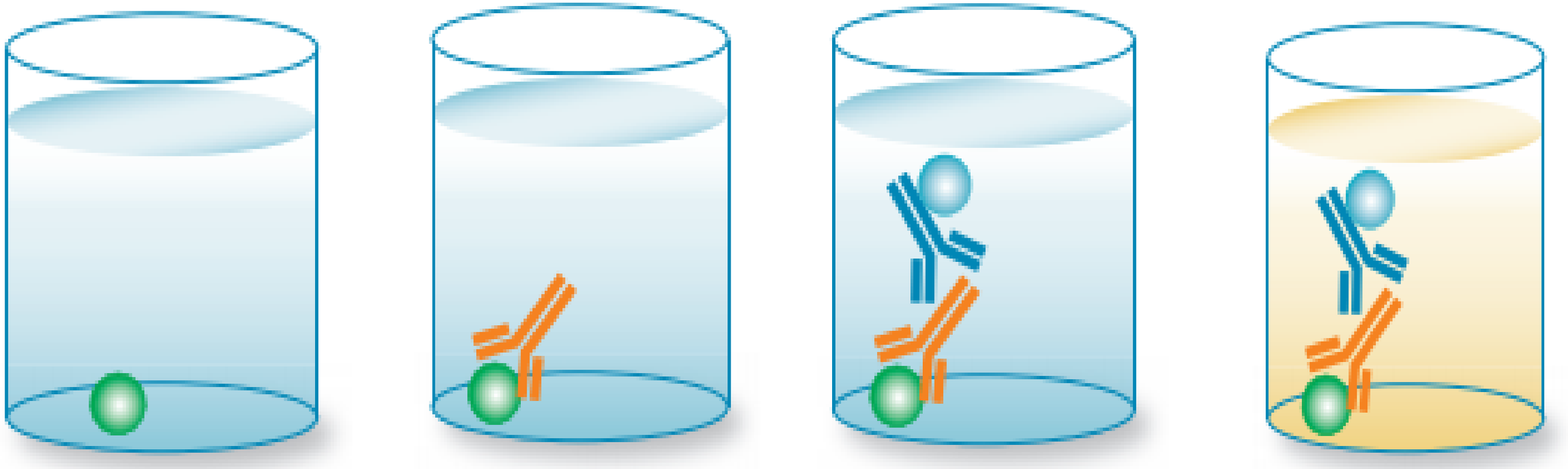




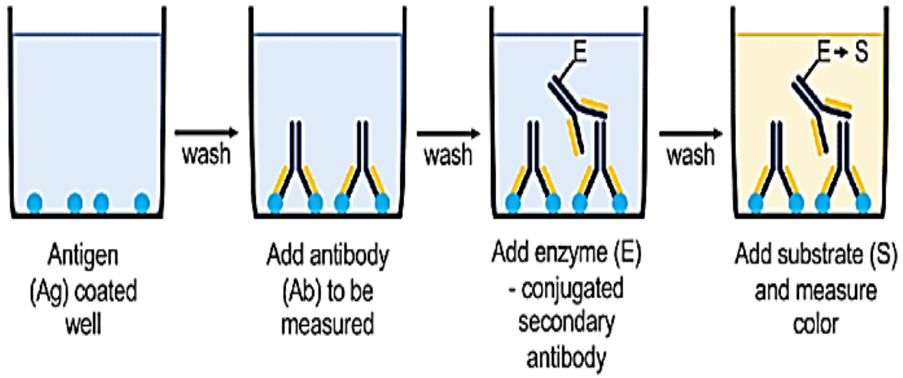
Indirect Immunofluorescence (IIF)

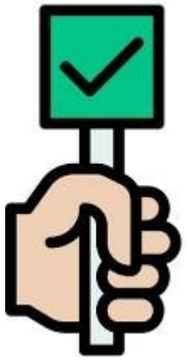
Enzyme-linked immunosorbent assay (ELISA)

INDIRECT EL ISA TEST



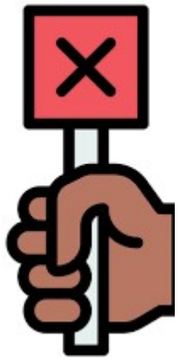
EL ISA TEST





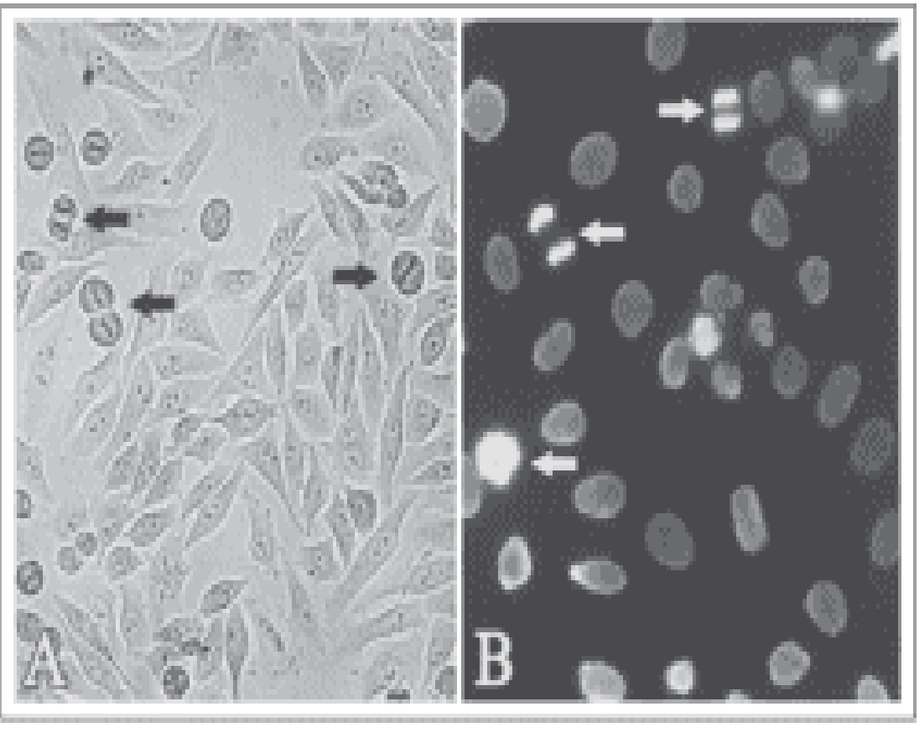
Suitability for high-throughput testing

The semi-quantification of test results

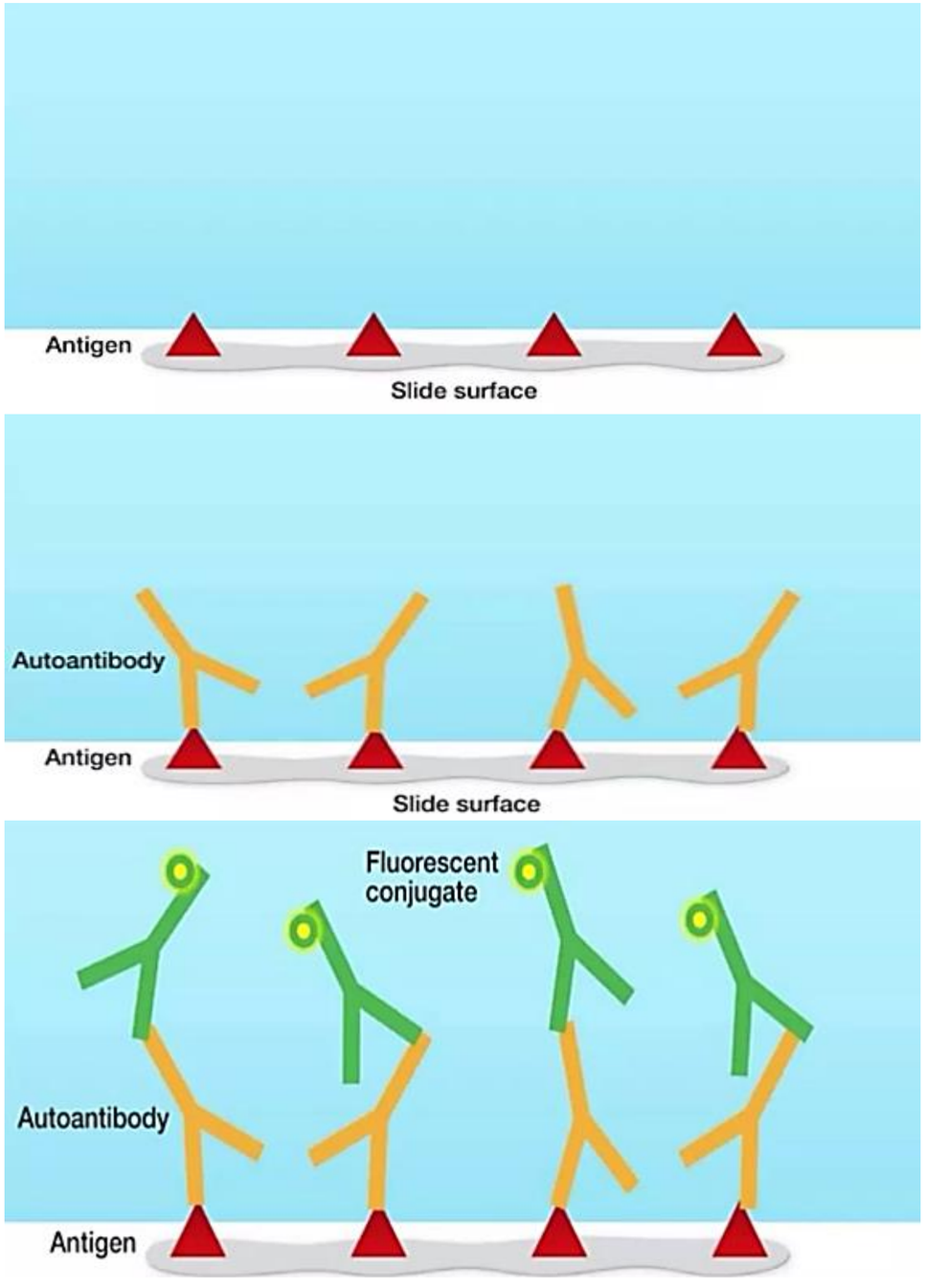


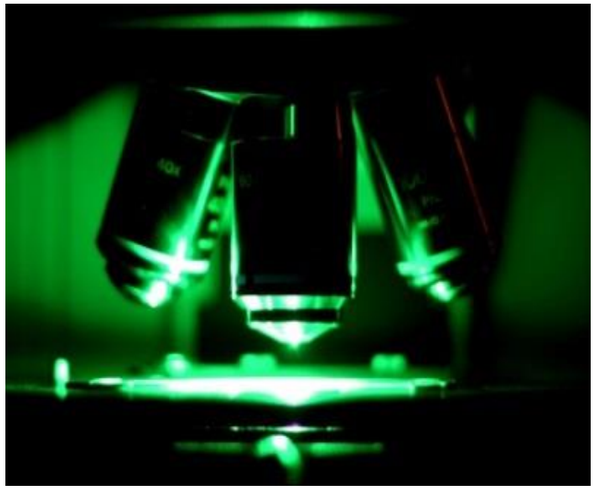
Less sensitive than IIF

INDIRECT IMMUNOFLUORESCENCE (IIF)

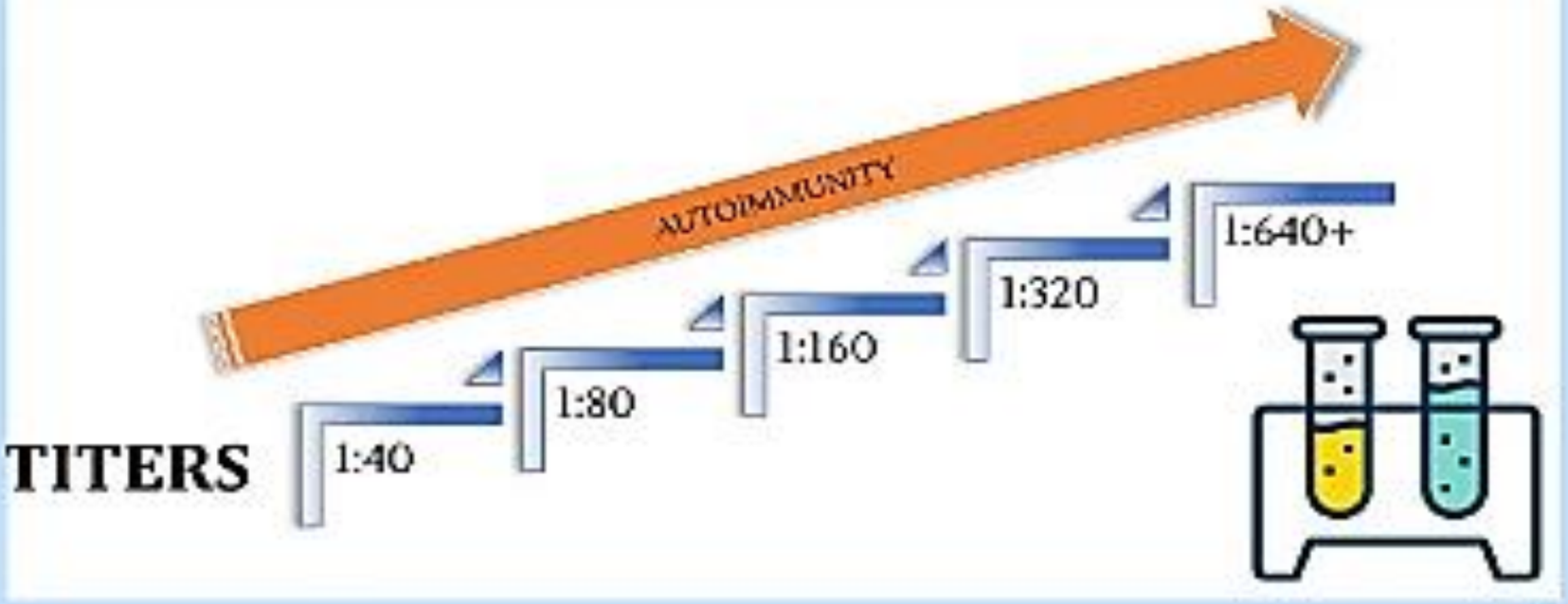


HEP-2 CELLS

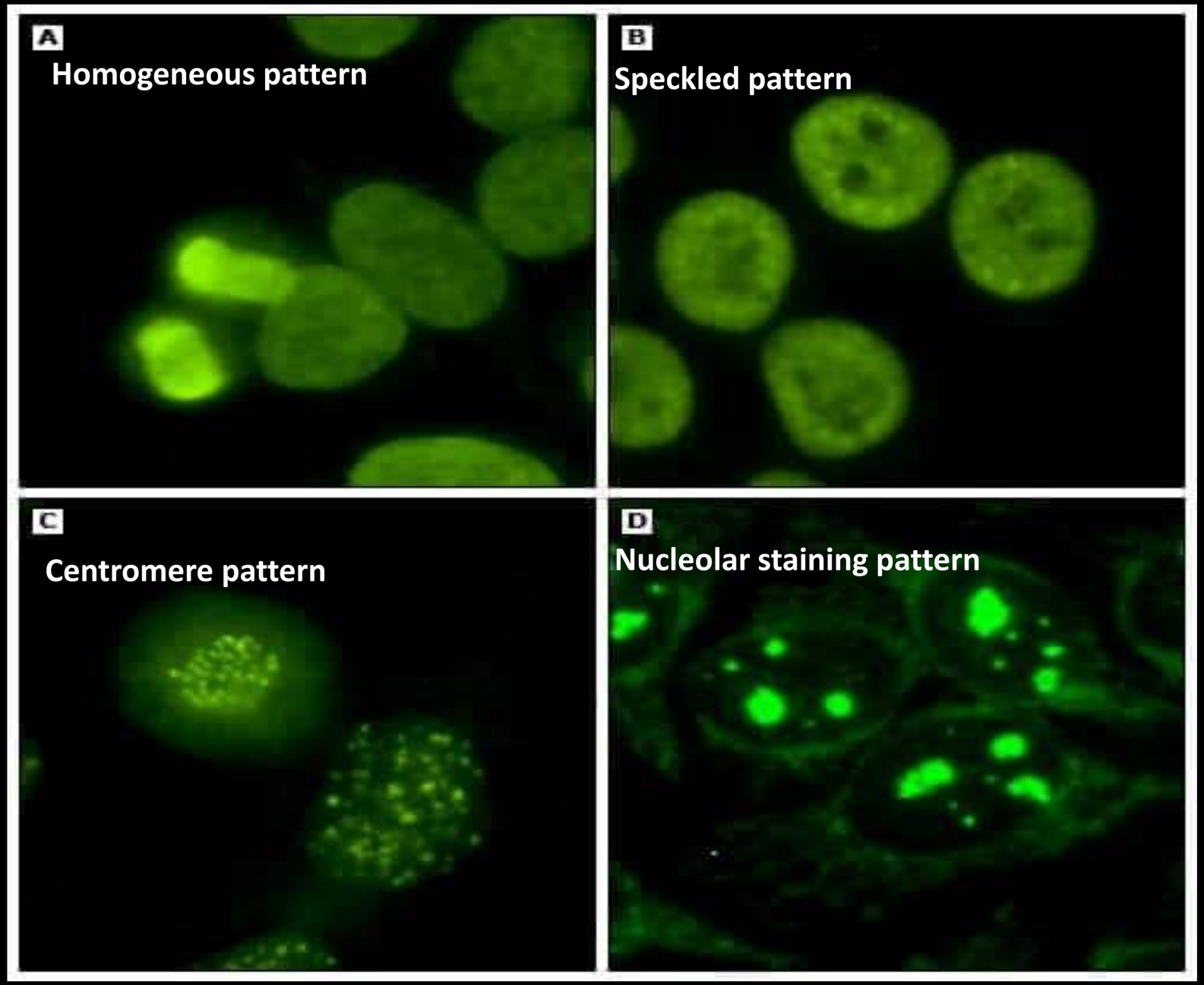




ANA "titer" -What does it mean?



Four common ANA staining patterns



Positive ANA

Homogeneous/Rim

SLE

Drug induced SLE

Speckled

SLE/MCTD

SS/SSC/PM

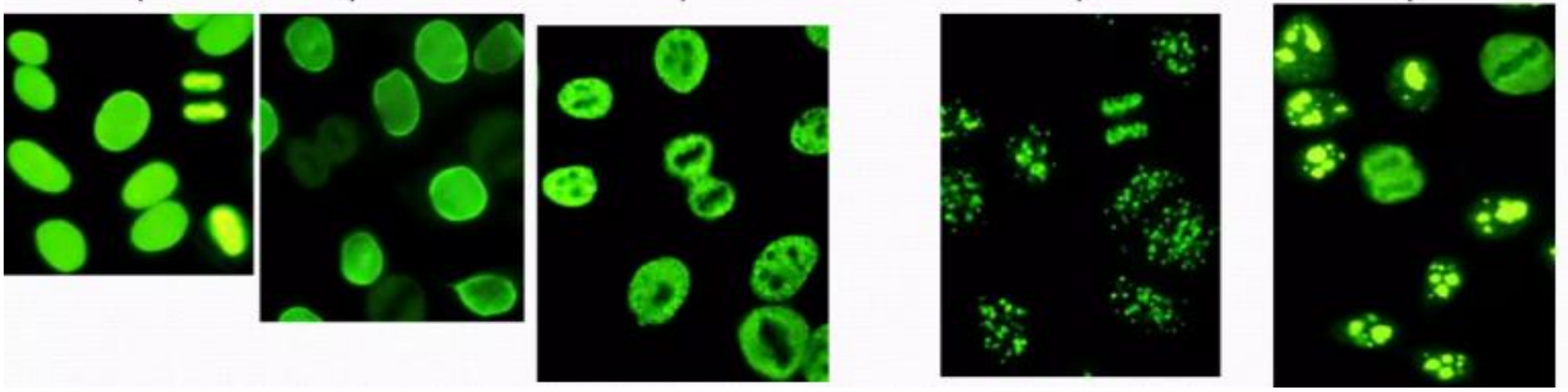
Centromere

Scleroderma

Limited

Nucleolar

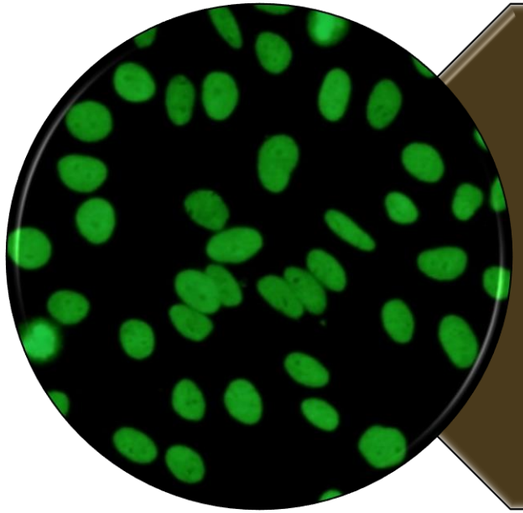
Systemic Sclerosis



FANA

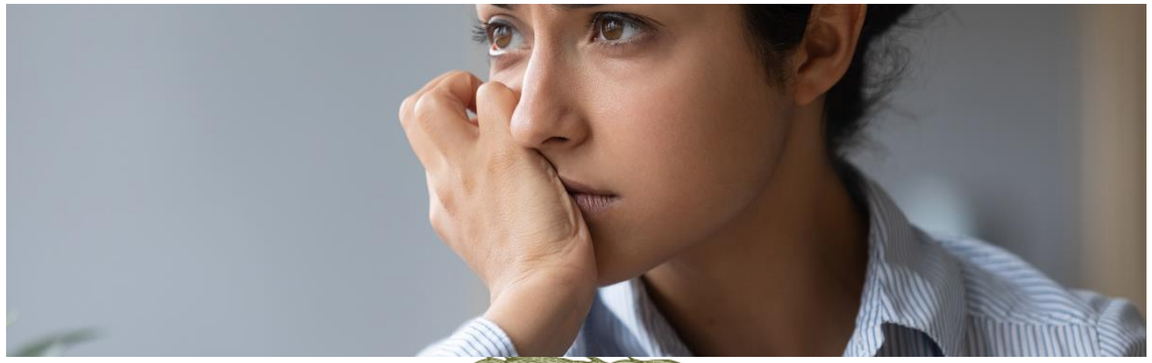
Large number of autoantibodies that can be detected using the HEp-2 cells

- Requirement for well-trained technicians
- Ro60 antigen(-)
- Ribosomal P antigens(-)



The gold standard method for ANA detection is indirect immunofluorescence (IIF) using HEp-2 cells

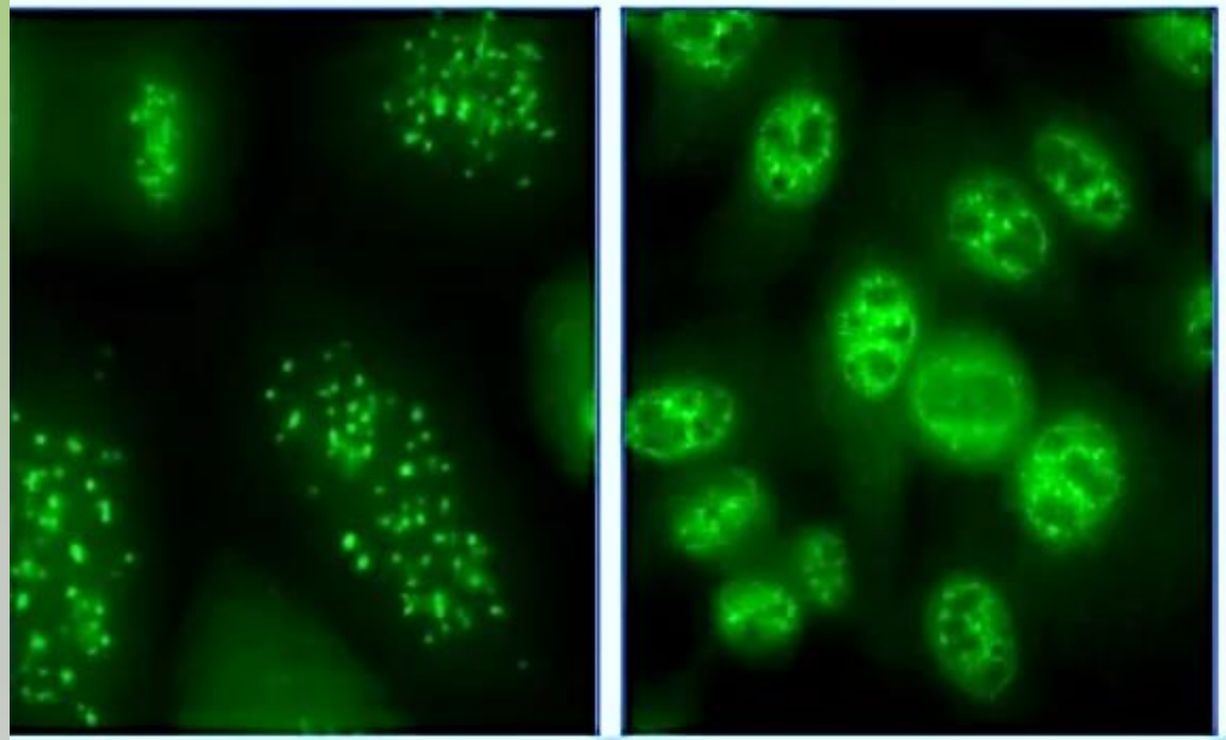
I don’t have any symptoms of lupus, but a positive ANA test. Can I have lupus?



CLINICAL LIMITATIONS OF ANA TESTING

- False positive results and may potentially distract the clinician from the correct diagnosis.
- If a test for ANA is ordered indiscriminately, 5 percent will have a positive result at the predetermined screening dilution (usually 1:160

ANA Titers **Healthy people**



- ANA 1:40: 20% to 30%
- ANA 1:80: 10% to 15%
- ANA 1:160: 5%
- ANA 1:320: 3%

- Elderly (>age 70): up to 70% positive at ANA titer 1:40
- Small % of elderly with autoimmune disease

Systemic autoimmune diseases

Infectious diseases*
Viral:
EBV
HIV
HCV
Parvovirus 19
Bacterial:
SBE
Syphilis

Malignancies*
Lymphoproliferative diseases
Paraneoplastic syndromes

Miscellaneous diseases*
Inflammatory bowel disease
Interstitial pulmonary fibrosis

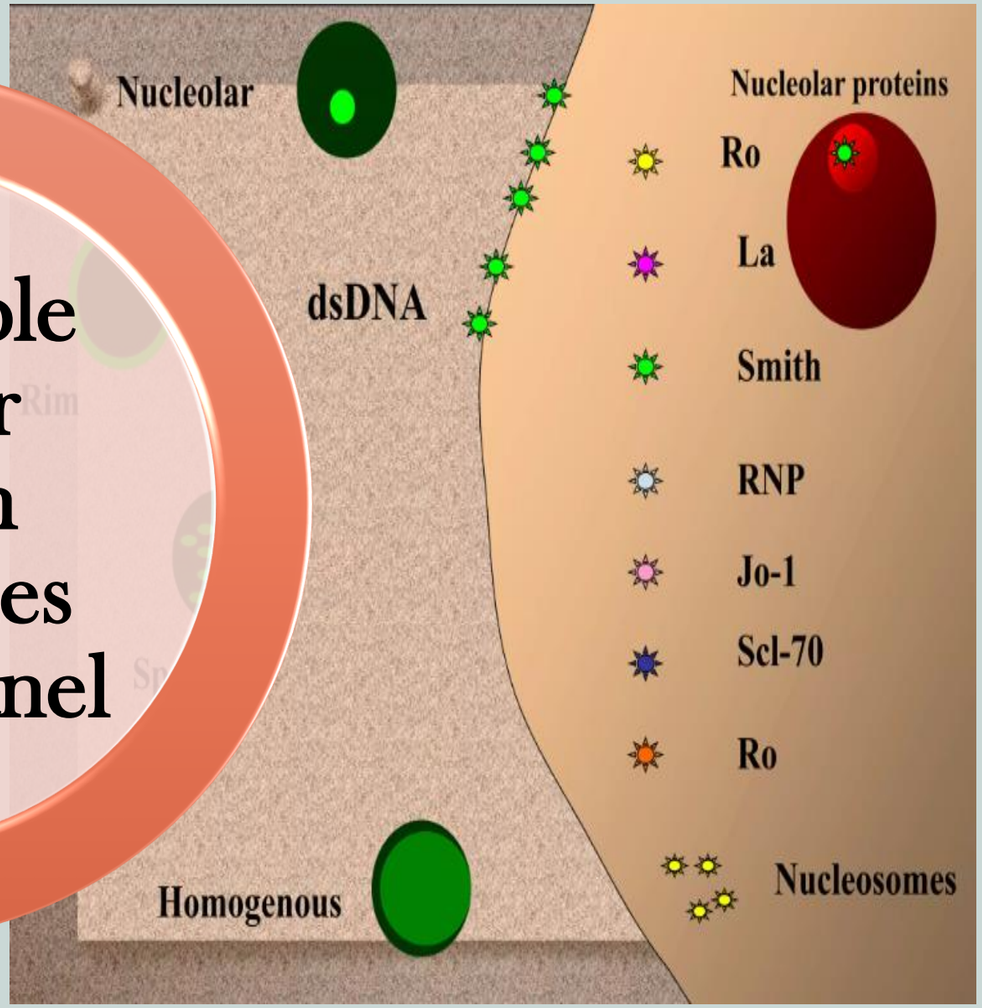
Organ-specific autoimmune diseases
Autoimmune hepatitis
Primary biliary cholangitis
Hashimoto's thyroiditis

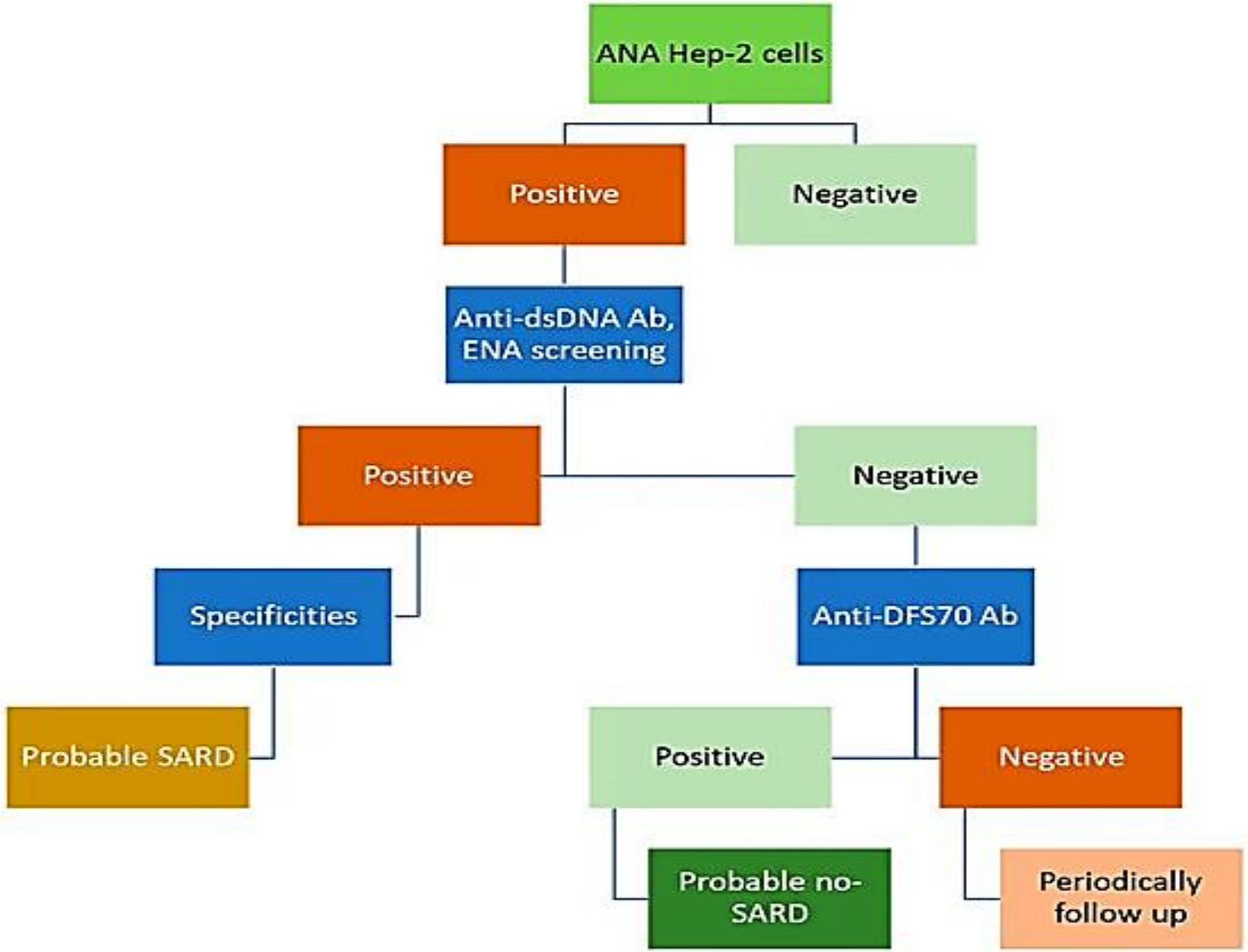
Diseases associated with a positive ANA

“I have symptoms of lupus, but a negative ANA test. Can I still have lupus?”

- anti-Ro antibodies
- anti-La antibodies
- anti-Sm antibodies
- anti-nRNP antibodies
- anti-Scl-70 antibodies
- anti-dsDNA antibodies
- anti-histone antibodies
- antibodies to nuclear pore comple
- anti-centromere antibodies
- anti-sp100 antibodies

Extractable Nuclear Antigen Antibodies (ENA) Panel





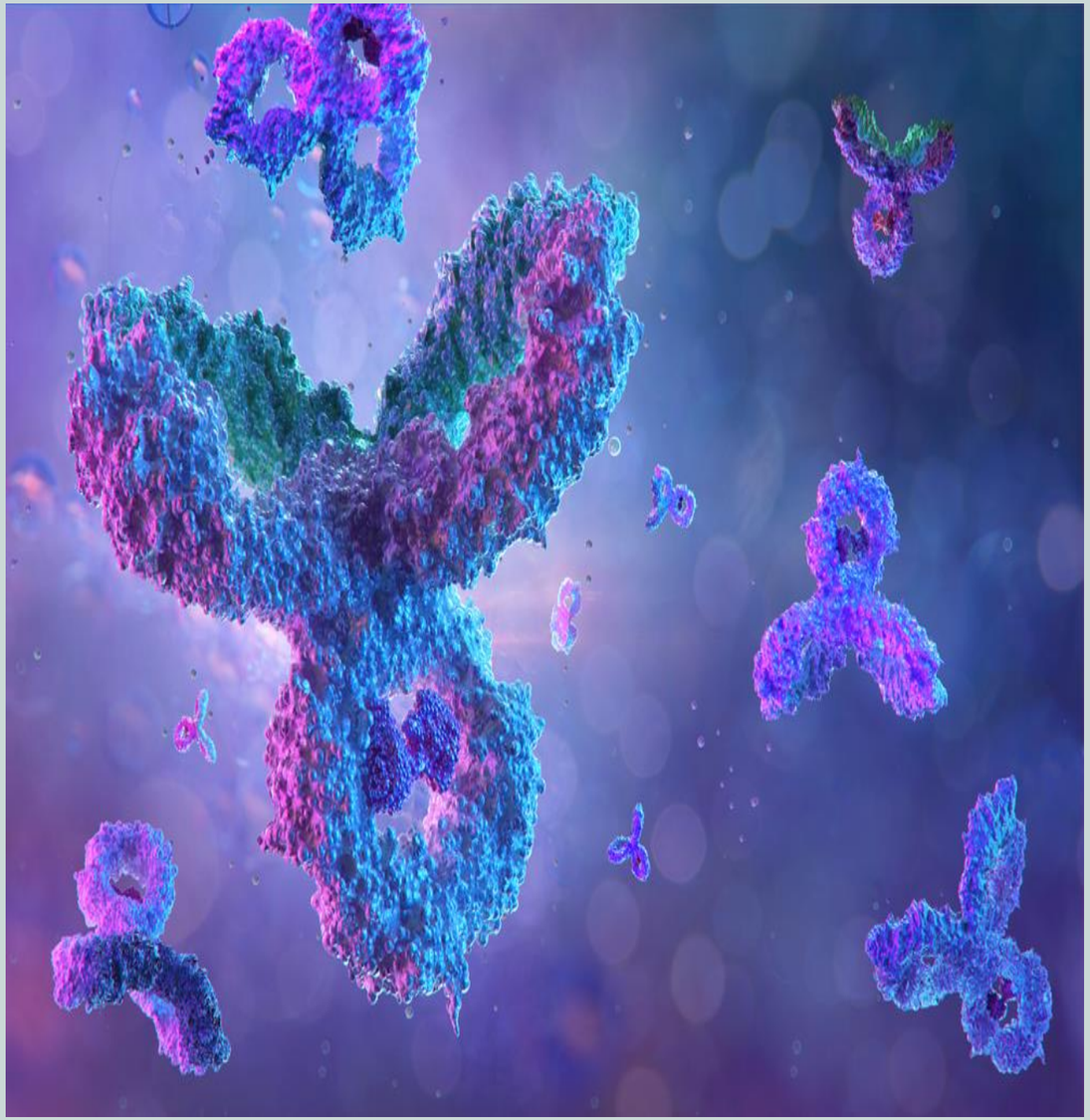
Diagnostics 2022, 12(3), 647; https://doi.org/10.3390/diagnostics12030647

Case 2

A 2-year boy presented with physiologic GERD feature and a positive Rheumatoid Factor with normal HX & Ph/EX

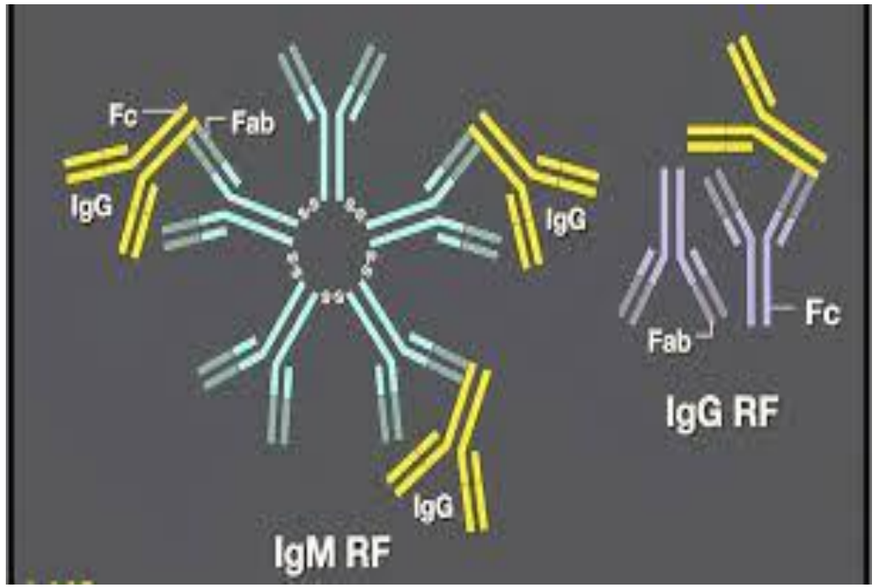
Case 3

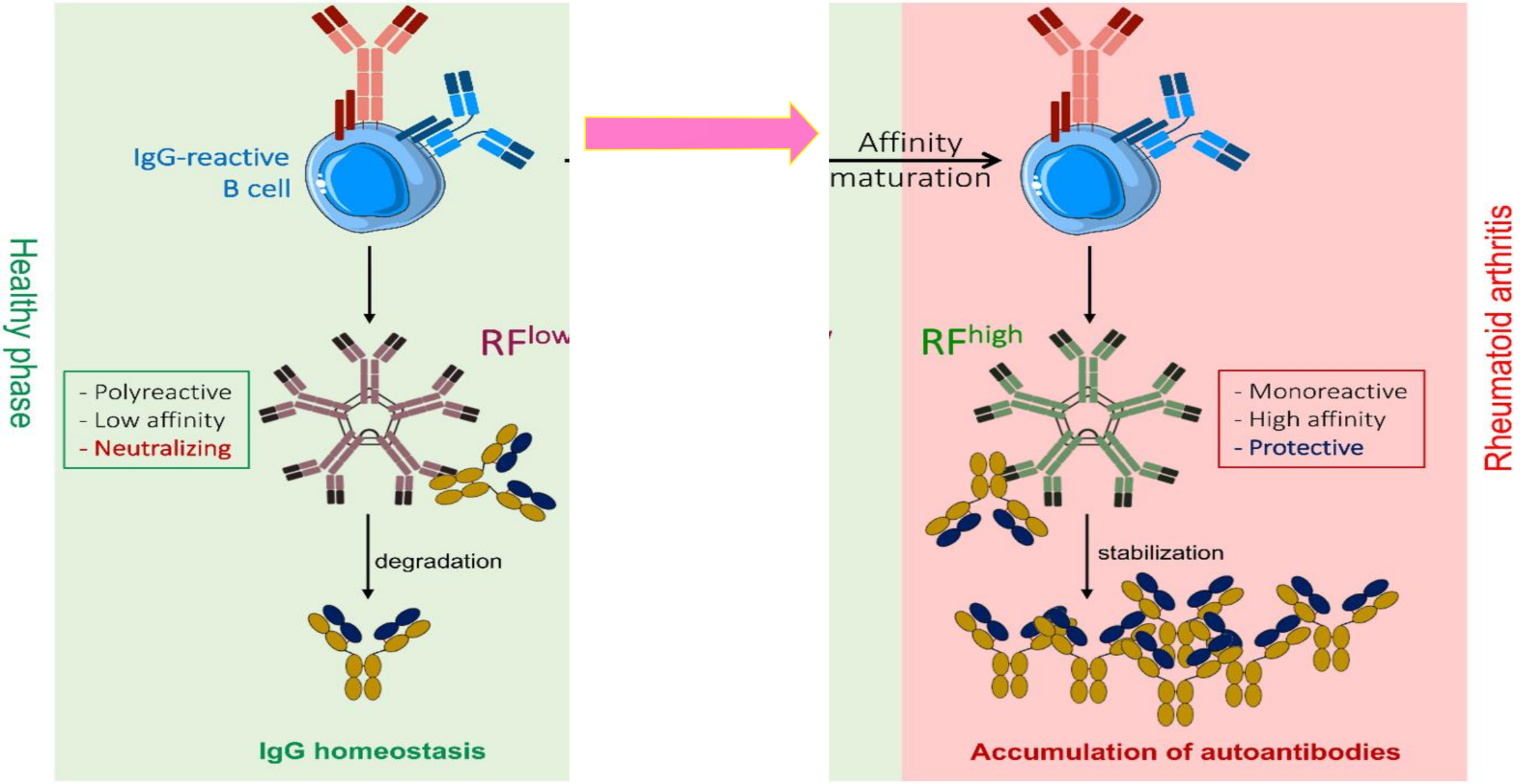
An 10-year-old boy with inflammatory polyarthritis was reported to be negative for RF, ANA, and anti-CCP in the investigations, and he was assured that he does not have a rheumatological disease.



Rheumatoid factors (RFs)

MISNOMER

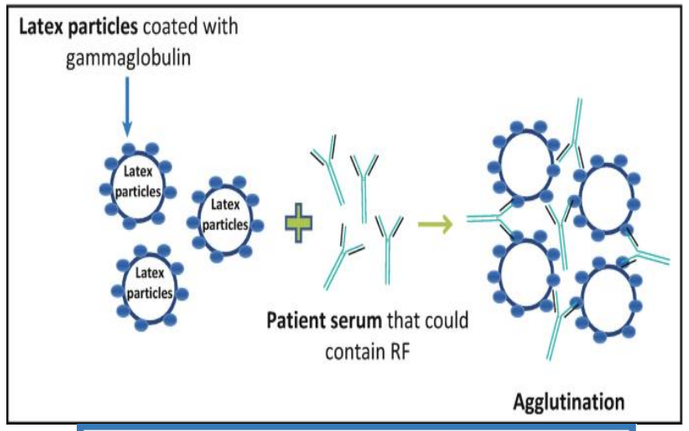


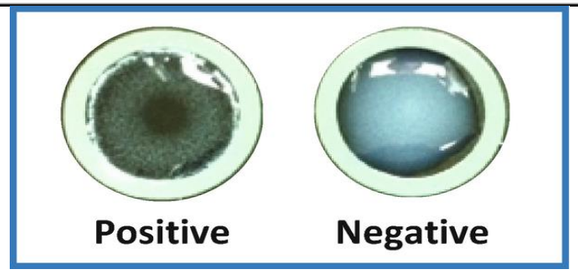


Front. Immunol., 21 October 2022, https://doi.org/10.3389/fimmu.2022.1016263

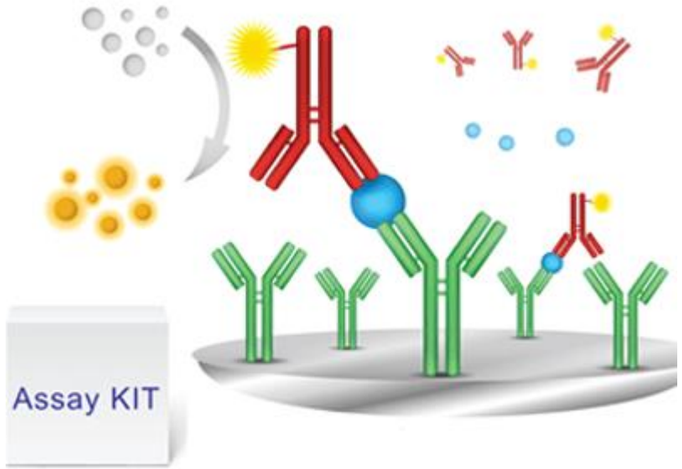
RF Assay

Latex Agglutination

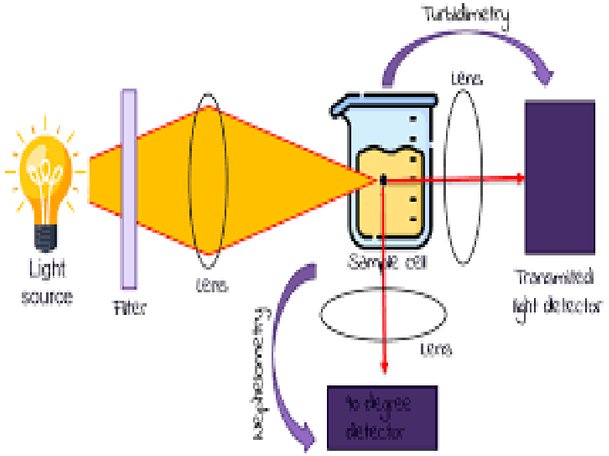


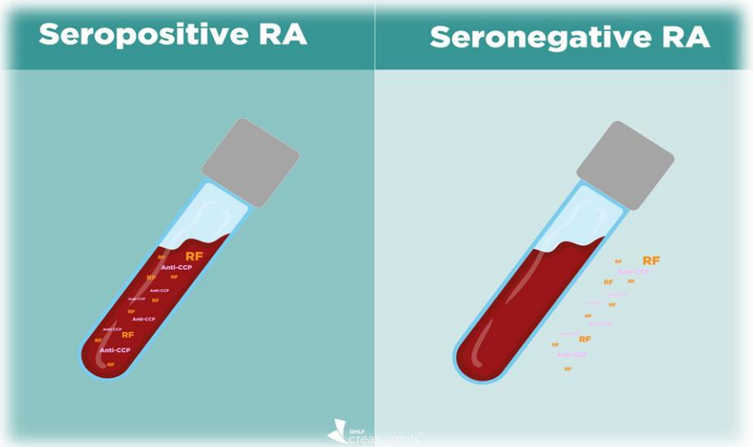


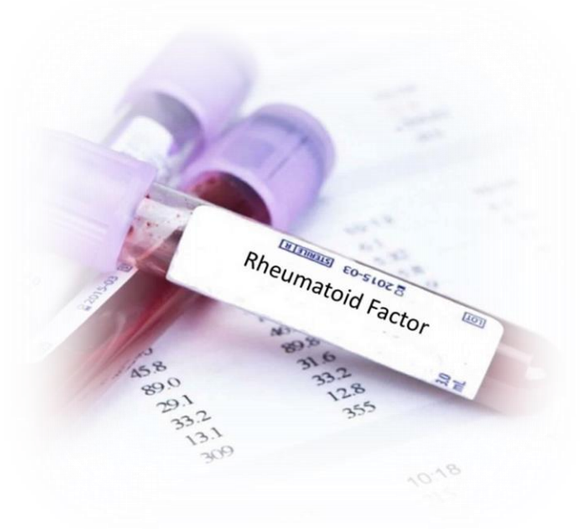
ELISA



Nephelometry or turbidimetry





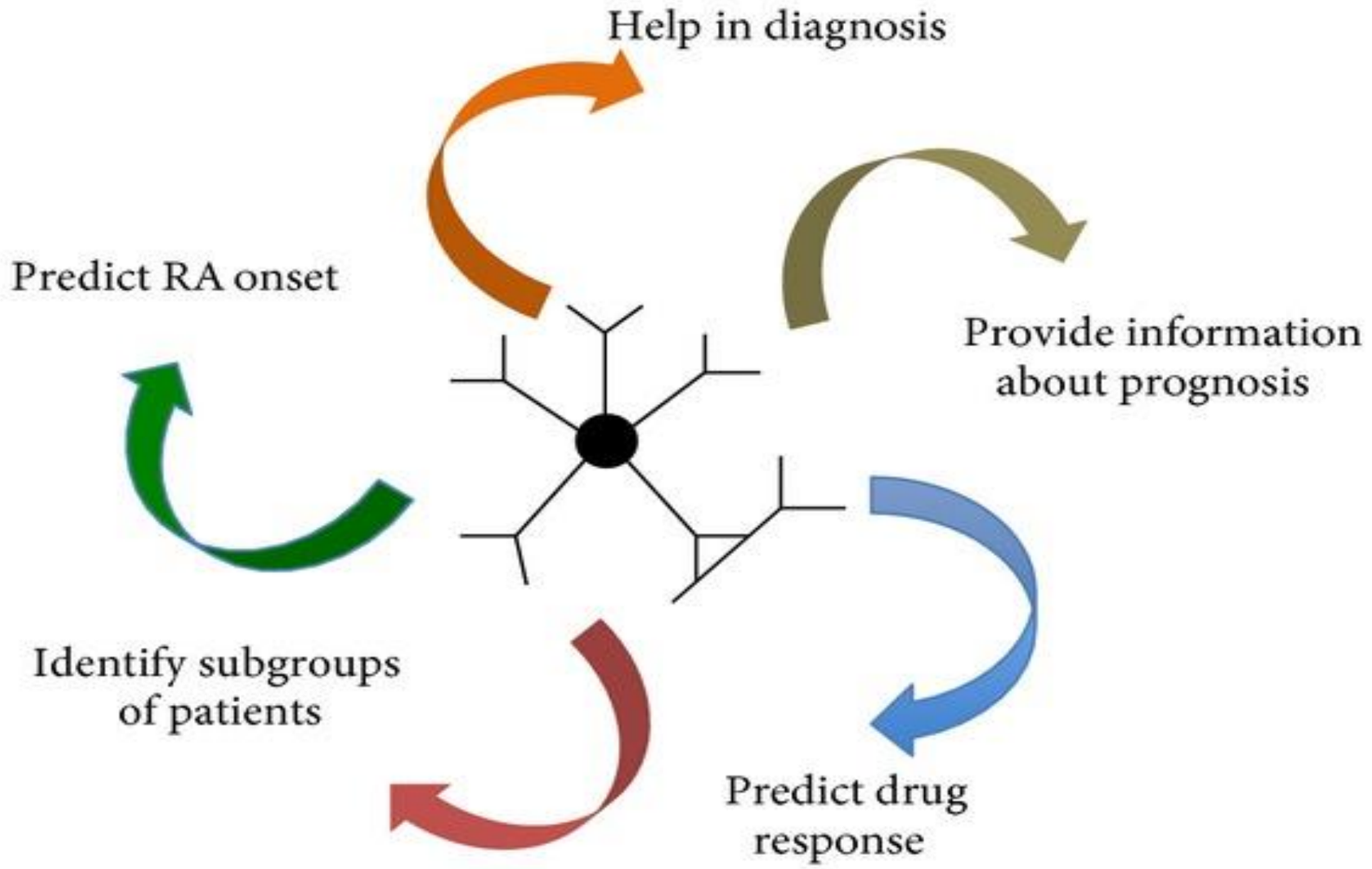


Conditions associated with rheumatoid factor

Condition	Frequency
Rheumatoid arthritis	70%
Other autoimmune rheumatic conditions	
Primary Sjögren syndrome	75%–95%
Systemic lupus erythematosus	15%–35%
Systemic sclerosis	20%–35%
Systemic vasculitis	5%–20%
Infections[a]	
Infective endocarditis	40%
Syphilis	8%–37%
Hepatitis B	25%
Hepatitis C	76%
Human immunodeficiency virus infection	10%–20%
Tuberculosis	15%
Other diseases	
Liver cirrhosis	25%
Mixed cryoglobulinemia	100%
Primary biliary cirrhosis	45%–70%
Healthy people	5%–25%[b]

Disease Markers
Volume 35 (2013), Issue 6, Pages 727–734
http://dx.doi.org/10.1155/2013/726598

Rf Prognostic Value



Case 4

A 5 year-old girl presented with intermittent Limping

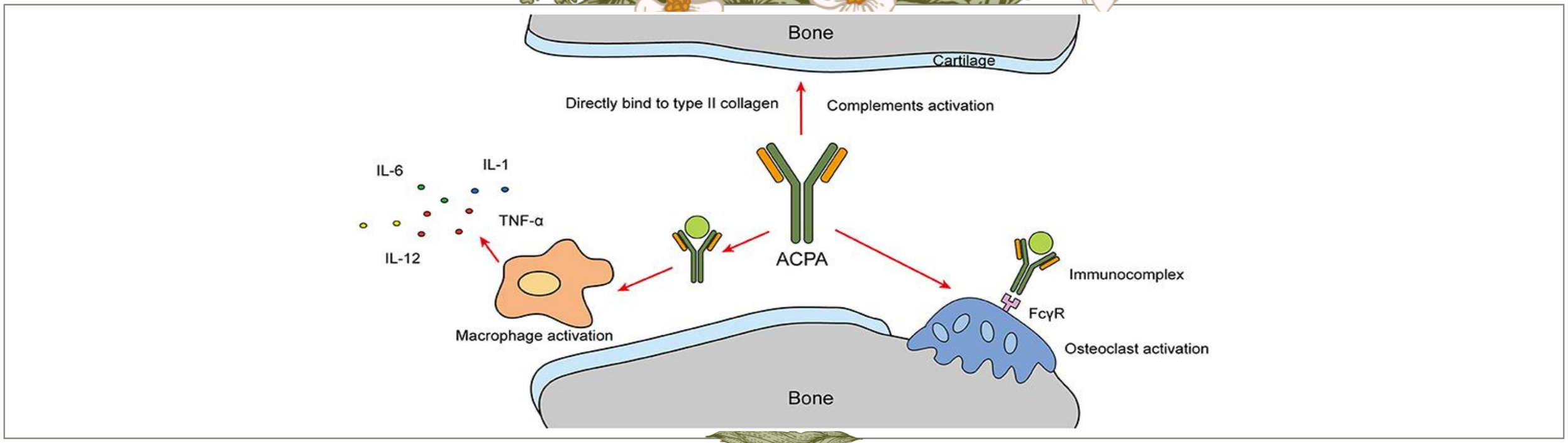
ACCP positive

MRI revealed SI

What's your DX?

Anticitrullinated Peptide Antibodies

ACPA


Bone
Cartilage
Directly bind to type II collagen
Complements activation
IL-6
IL-1
TNF-α
IL-12
ACPA
Immunocomplex
FcγR
Macrophage activation
Osteoclast activation
Bone

	Sensitivity	Specificity
Anti-CCP	62.5%	89.1%
RF	85.3%	64.7%
CCP or RF	86.7%	61.5%
CCP and RF	62.9%	92.3%

Anti-CCP+, anti-cyclic citrullinated peptide antibody positive; RF, rheumatoid factor.

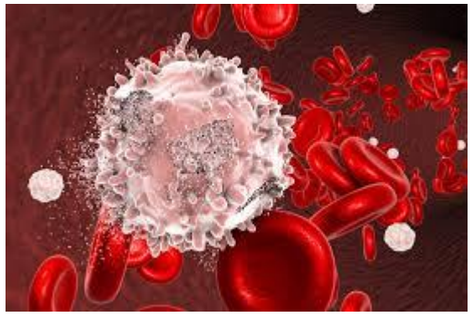
APLAR Journal of Rheumatology 2007; 10: 121–124

Detection of ACPA in other diseases

	n	ACPA+ (n, %)
Psoriatic arthritis	1343	115 (8.6%)
SLE	1078	84 (7.8%)
Sjögren's syndrome	609	35 (5.7%)
Spondyloarthropathy	431	10 (2.3%)
Scleroderma/CREST	380	26 (6.8%)
Hepatitis C/cryoglobulinemia	285	10 (3.5%)
Osteoarthritis	182	4 (2.2%)
Hepatitis B	176	1 (0.6%)
Juvenile idiopathic arthritis	169	13 (7.7%)
Polymyalgia rheumatica	146	0 (0%)
Vasculitis/ Wegener's granulomatosis	107	5 (4.7%)
Tuberculosis	96	33 (34.3%)
Polymyositis/dermatomyositis	75	0 (0%)

Arthritis Rheum. 2009 November 15; 61(11): 1472–1483. doi:10.1002/art.24827.

CASE REPORT
published: 24 May 2021
doi: 10.3389/fmed.2021.627004



Gastric Adenocarcinoma Presenting as a Rheumatoid Factor and Anti-cyclic Citrullinated Protein Antibody-Positive Polyarthritis: A Case Report and Review of Literature

Manuel Silvério-António [1,2], Federica Parlato [3], Patrícia Martins [1,2], Nikita Khmelinskii [1,2], Sandra Braz [3], João Eurico Fonseca [1,2] and Joaquim Polido-Pereira [1,2]*

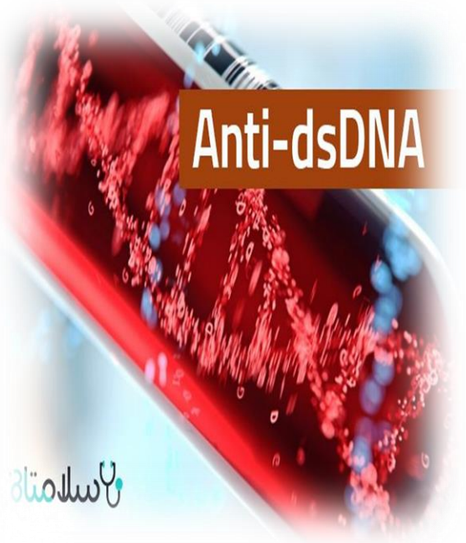
[1] Rheumatology Department, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon Academic Medical Centre, Lisbon, Portugal, [2] Rheumatology Research Unit, Faculdade de Medicina, Instituto de Medicina Molecular, Universidade de Lisboa, Lisbon, Portugal, [3] Medicina 2 Department, University Hospital Center of Lisbon North, Lisbon, Portugal

FOLLOW UP

Neuroblastoma with positive Anti-CCP

Case 5

A 13-year-old girl with suspected to SLE with a negative ANA and high titer of anti ds DNA(IgG)
What is your interpretation?



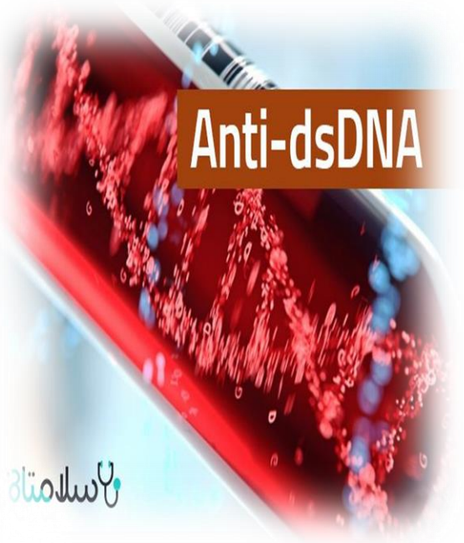
Case 6

A 18-year-old girl presented with raised LFT with a positive **Anti ds DNA(IgM)**

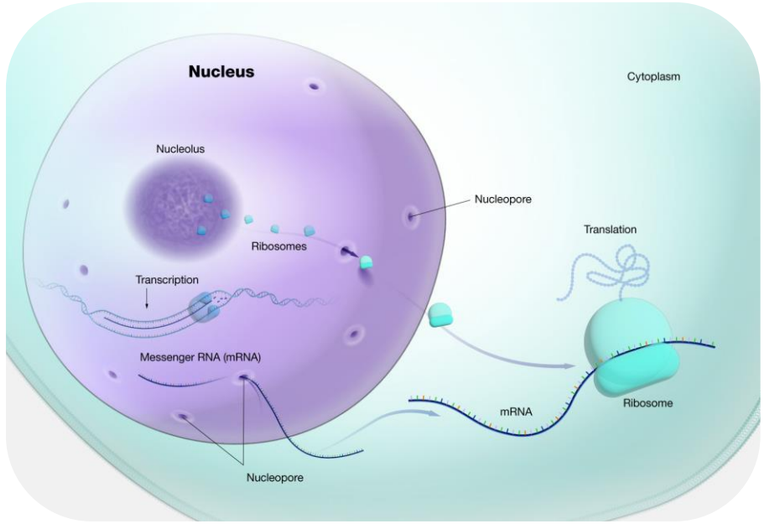
What is your interpretation?

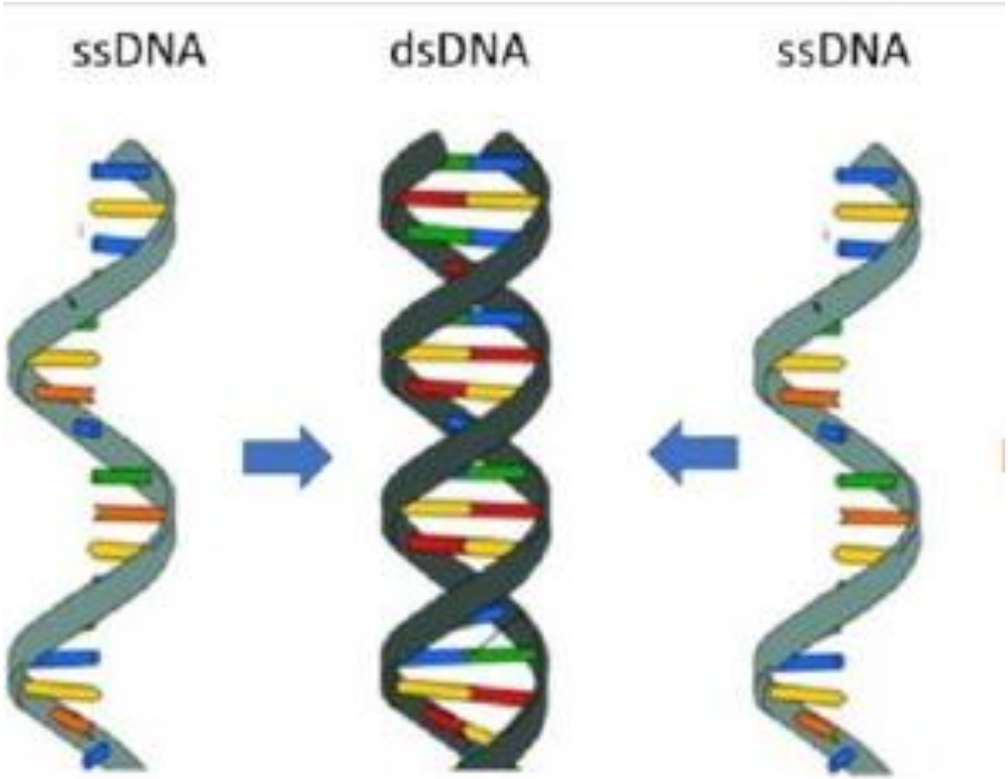
Case 7

A 10-year-old girl with known case of SLE with clinical remission and **positive anti-ds DNA**
What is your plan?

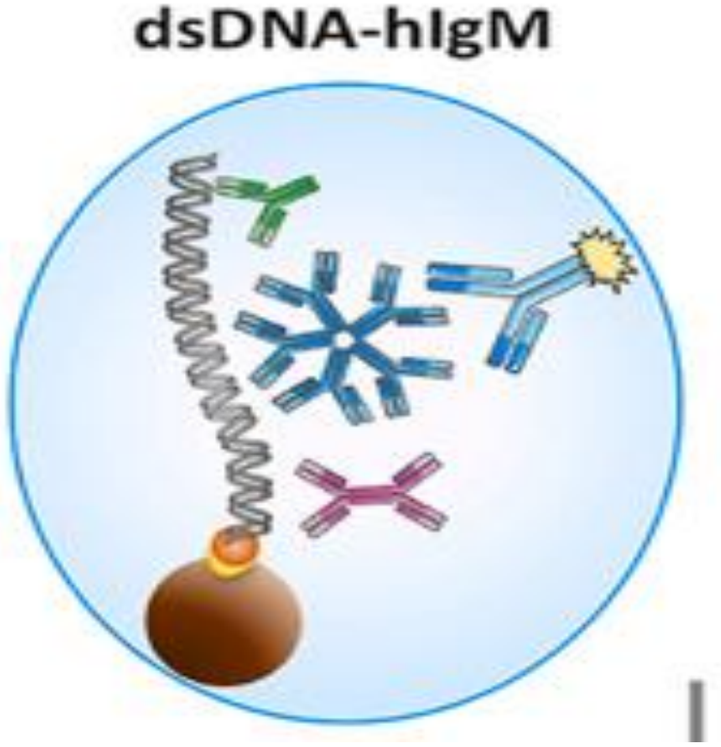


Anti-dsDNA antibodies


Nucleus
Cytoplasm
Nucleolus
Nucleopore
Translation
Ribosomes
Transcription
Messenger RNA (mRNA)
mRNA
Ribosome
Nucleopore


ssDNA
dsDNA
ssDNA

High Avidity Ig G



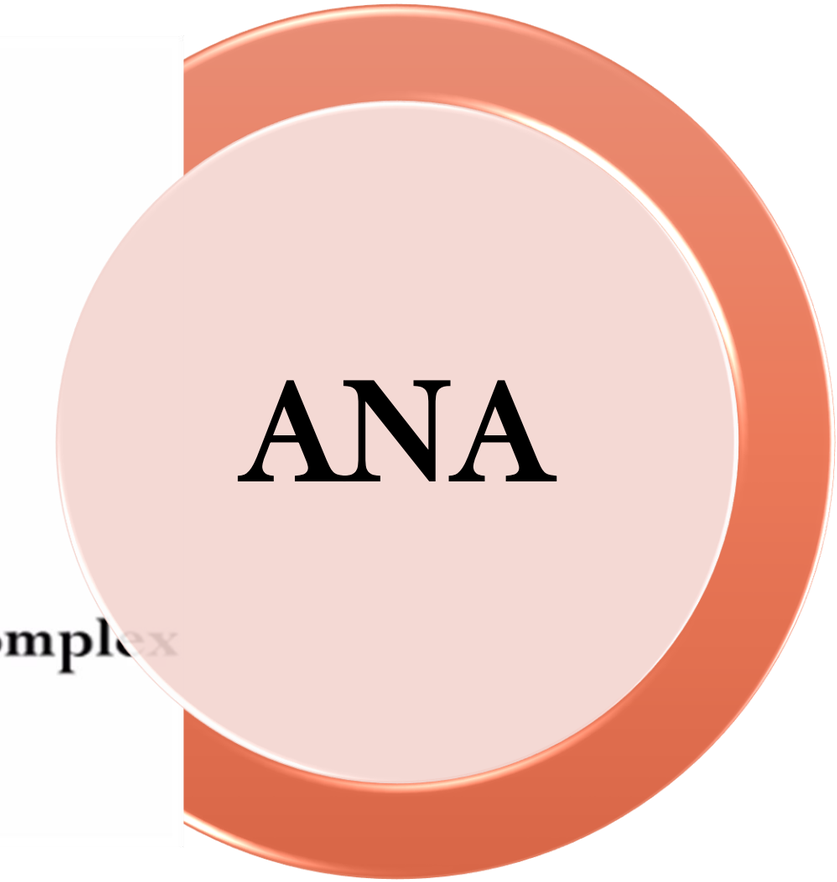
	Anti-dsDNA ELISA	Anti-dsDNA Farr RIA	Anti-dsDNA Crithidia IIFT
DNA	purified	purified	In situ
Antigen	Solid phase fixed	Liquid phase fixed	In situ fixed
Anti-dsDNA dsDNA			

- SLE
- Other autoimmune diseases (Sjogren, Autoimmune Hepatitis)
- Biliary Cirrhosis
- Chronic Hepatitis
- Infectious mononucleosis

Arthritis & Rheumatism (Arthritis Care & Research)
Vol. 47, No. 5, October 15, 2002, pp 546–555
DOI 10.1002/art.10558

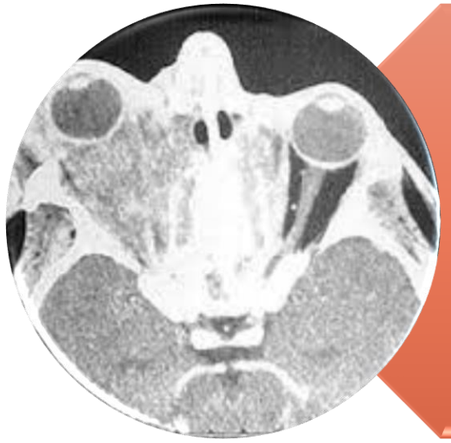
Anti ds-DNA as a part of ANA

- anti-Ro antibodies
- anti-La antibodies
- anti-Sm antibodies
- anti-nRNP antibodies
- anti-Scl-70 antibodies
- anti-dsDNA antibodies
- anti-histone antibodies
- antibodies to nuclear pore complex
- anti-centromere antibodies
- anti-sp100 antibodies



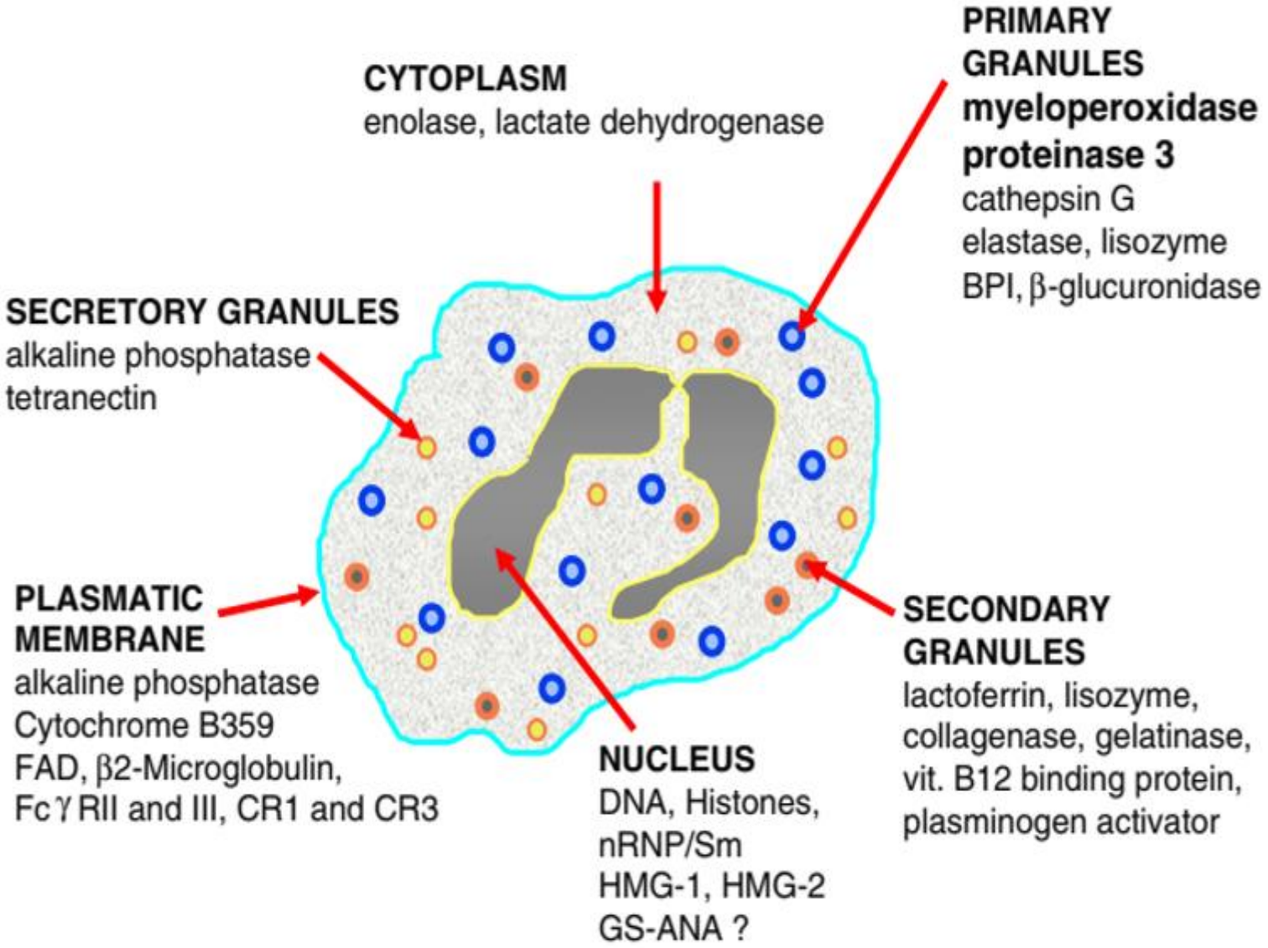
Medicine (Baltimore). 1981 May;60(3):208-17. doi: 10.1097/00005792-198105000-00004

Case 8



10-year-old girl with chronic sinusitis, retro-orbital mass, glomerulonephritis and pulmonary nodule and negative ANCA test. What is your plan?

Antineutrophil cytoplasmic autoantibodies (ANCA)



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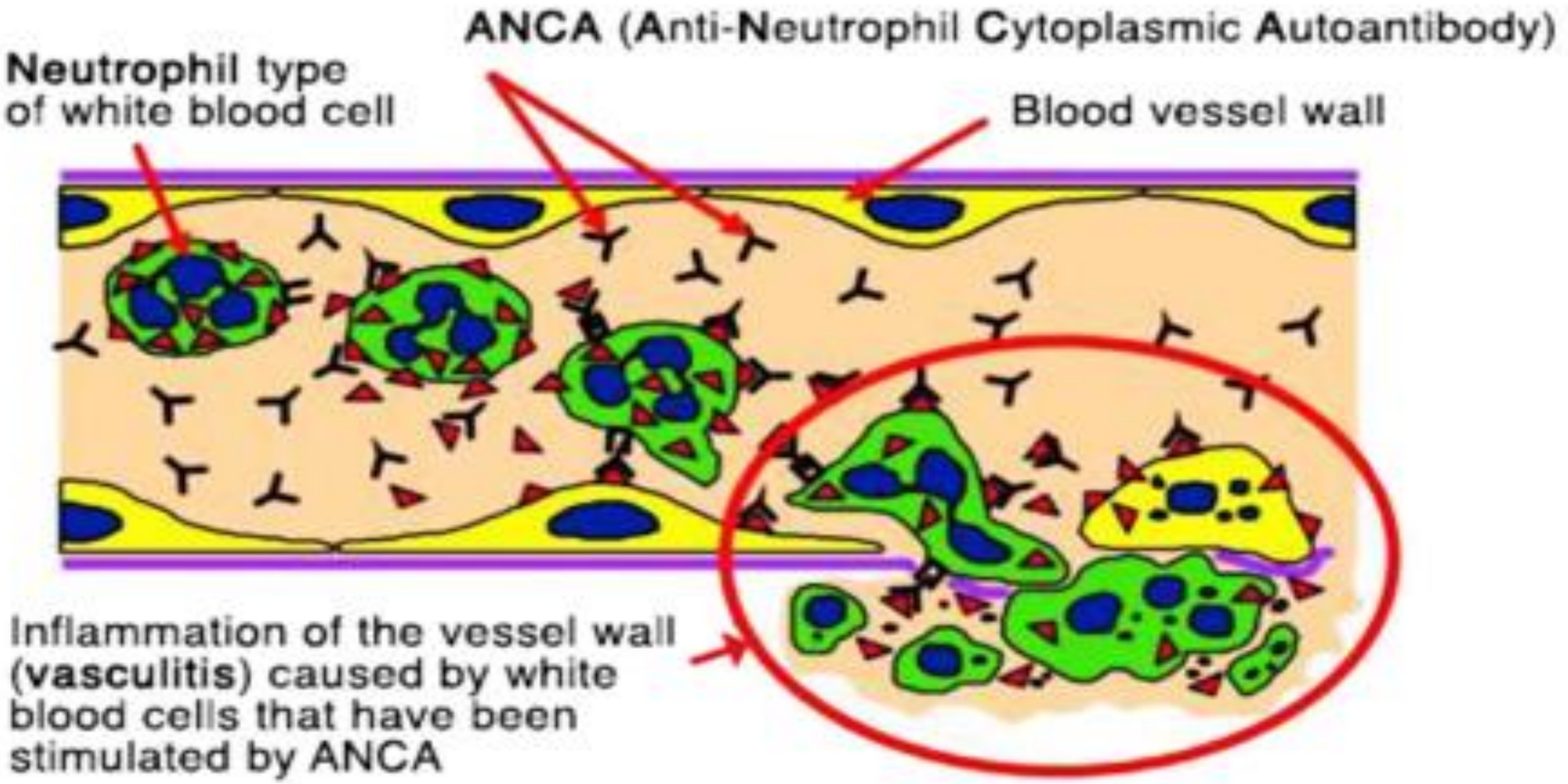
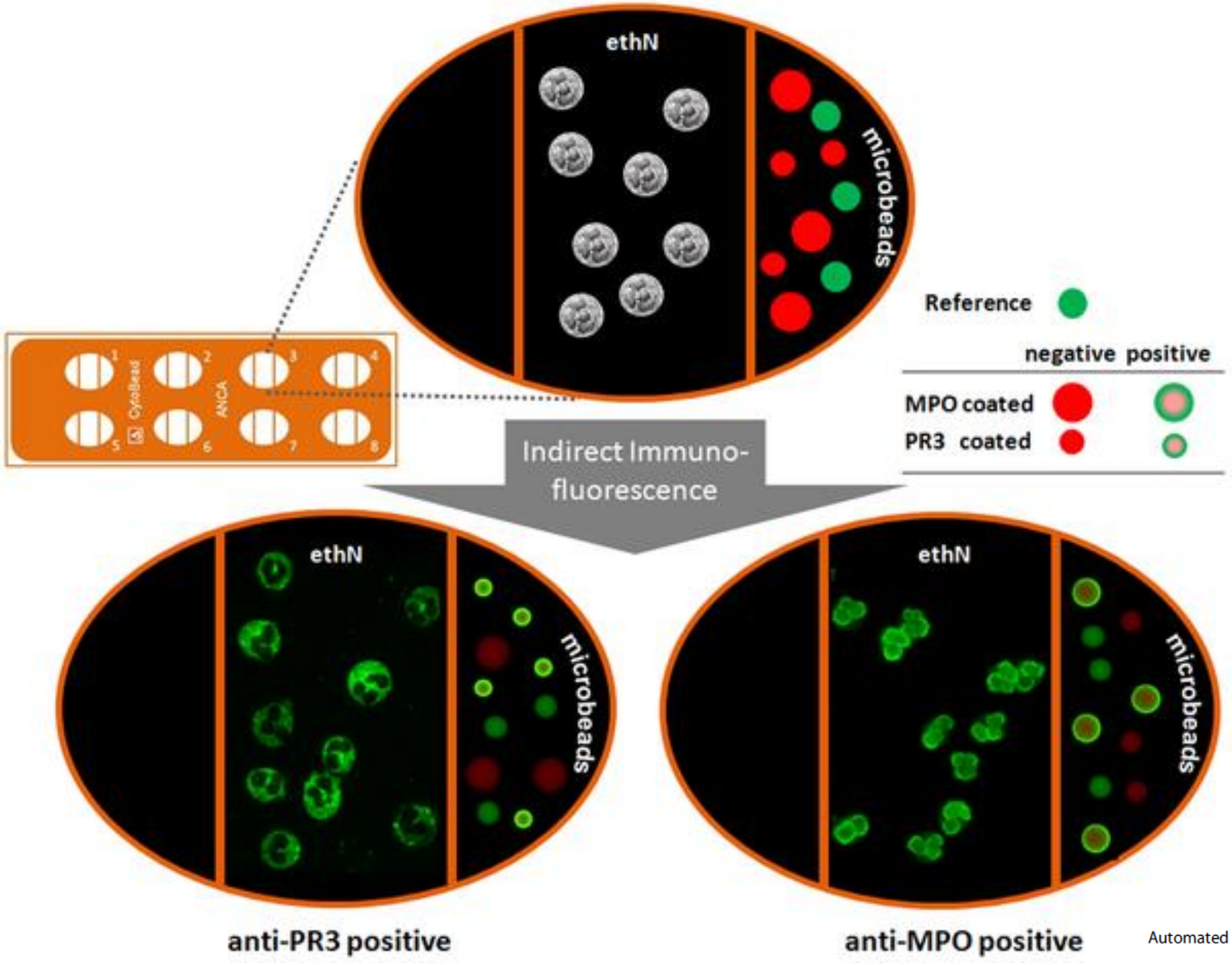


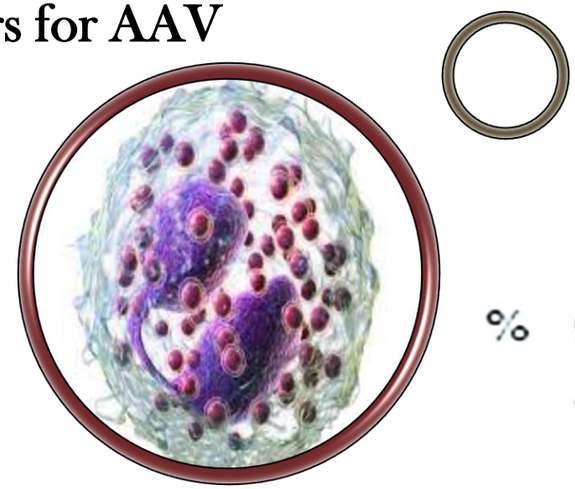
VASCULITIS UK

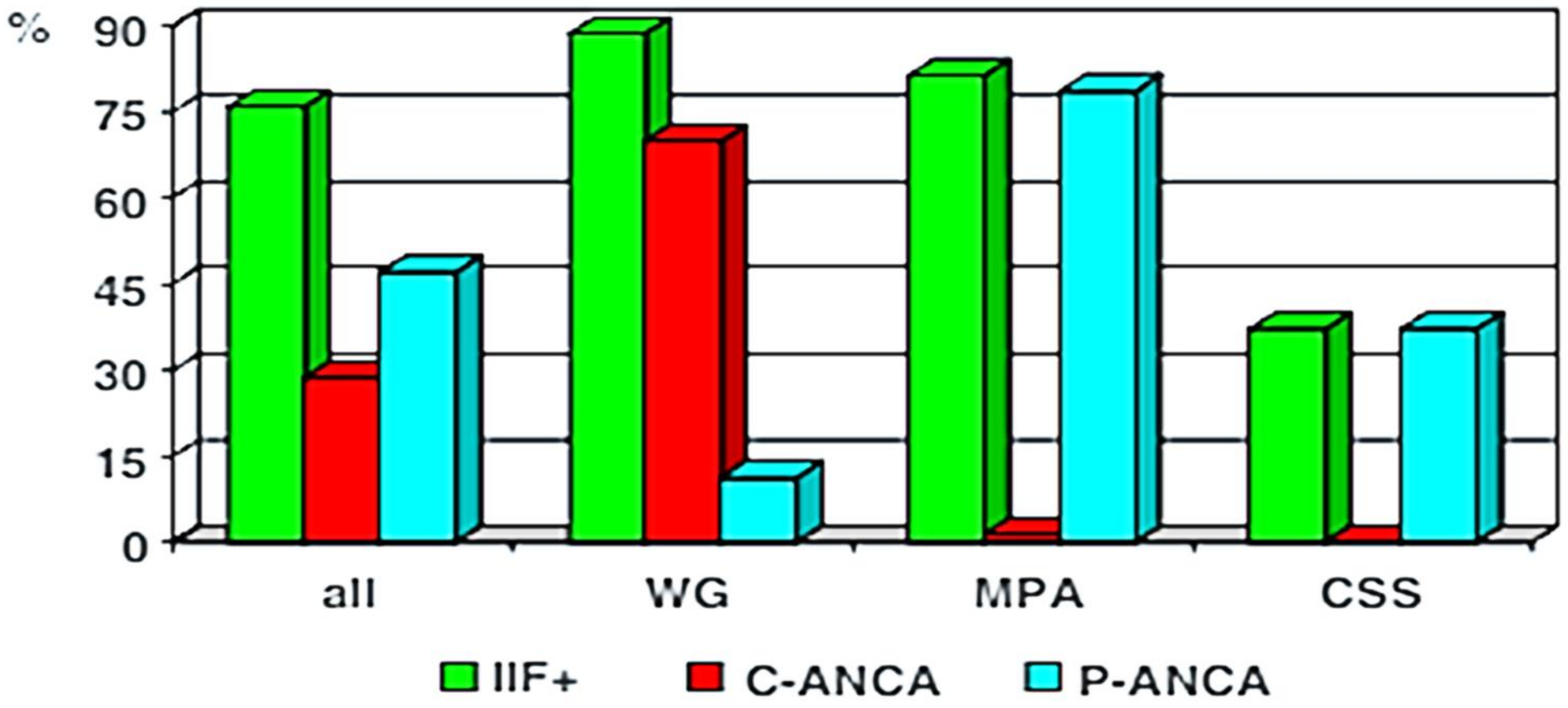


Automated Screening and Confirmatory ANCA Testing

doi:10.1371/journal.pone.0107743

ANCA are extremely important serological markers for AAV





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Clinical indication for ANCA testing
Glomerulonephritis, especially rapidly progressive glomerulonephritis
Pulmonary hemorrage
Cutaneous vasculitis
Multiple lung nodules
Chronic destructive disease of the upper airways
Long standing sinusitis or otitis
Subglottic tracheal stenosis
Peripheral neuropathy
Retro-orbital mass
Other possible indications for ANCA testing
Pulmonary fibrosis, with systemic features
Episcleritis, uveitis, retinal vasculitis, with systemic features

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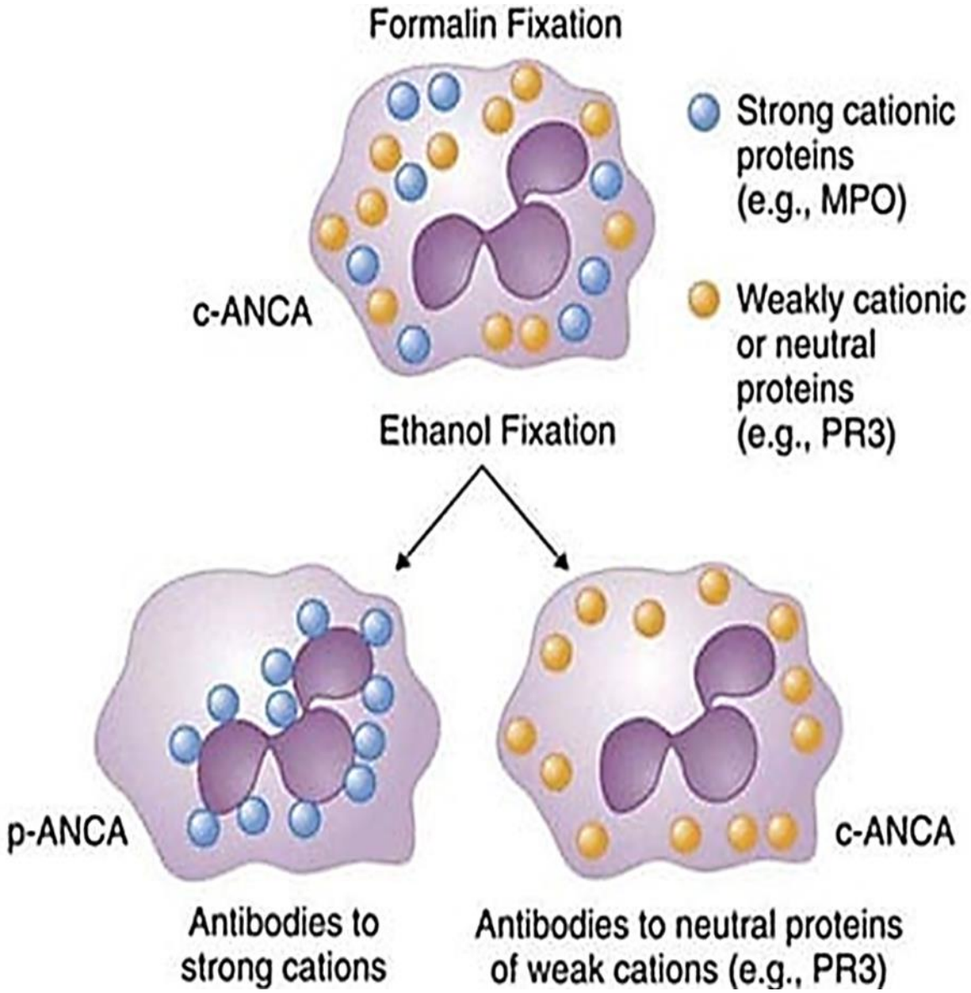
Category	Condition
Vasculitic	Cryoglobulinemia
	Leukocytoclastic cutaneous vasculitis
	Henoch-Schönlein purpura
	Polyarteritis nodosa
	Giant cell arteritis
	Takayasu's arteritis
	Behçet's syndrome
Other autoimmune	Systemic lupus erythematosus
	Goodpasture's disease
	Sjögren's syndrome
	Polymyositis and dermatomyositis
	Scleroderma
	Mixed-connective tissue disease
	Rheumatoid arthritis
	Spondyloarthropathies
	Inflammatory bowel disease (ulcerative colitis and Crohn's disease)
	Psoriatic arthritis
	Hashimoto's disease
	Multiple sclerosis
	Postinfectious glomerulonephritis
Infectious	Mycobacterium tuberculosis
	Human immunodeficiency virus
	Hepatitis C
	Pneumocystis carinii
	Poliomyelitis
	Endocarditis
Miscellaneous	Sarcoidosis
	Interstitial pulmonary fibrosis
	Myocardial infarction
	Cystic fibrosis
	Cocaine abuse
	Alport's syndrome

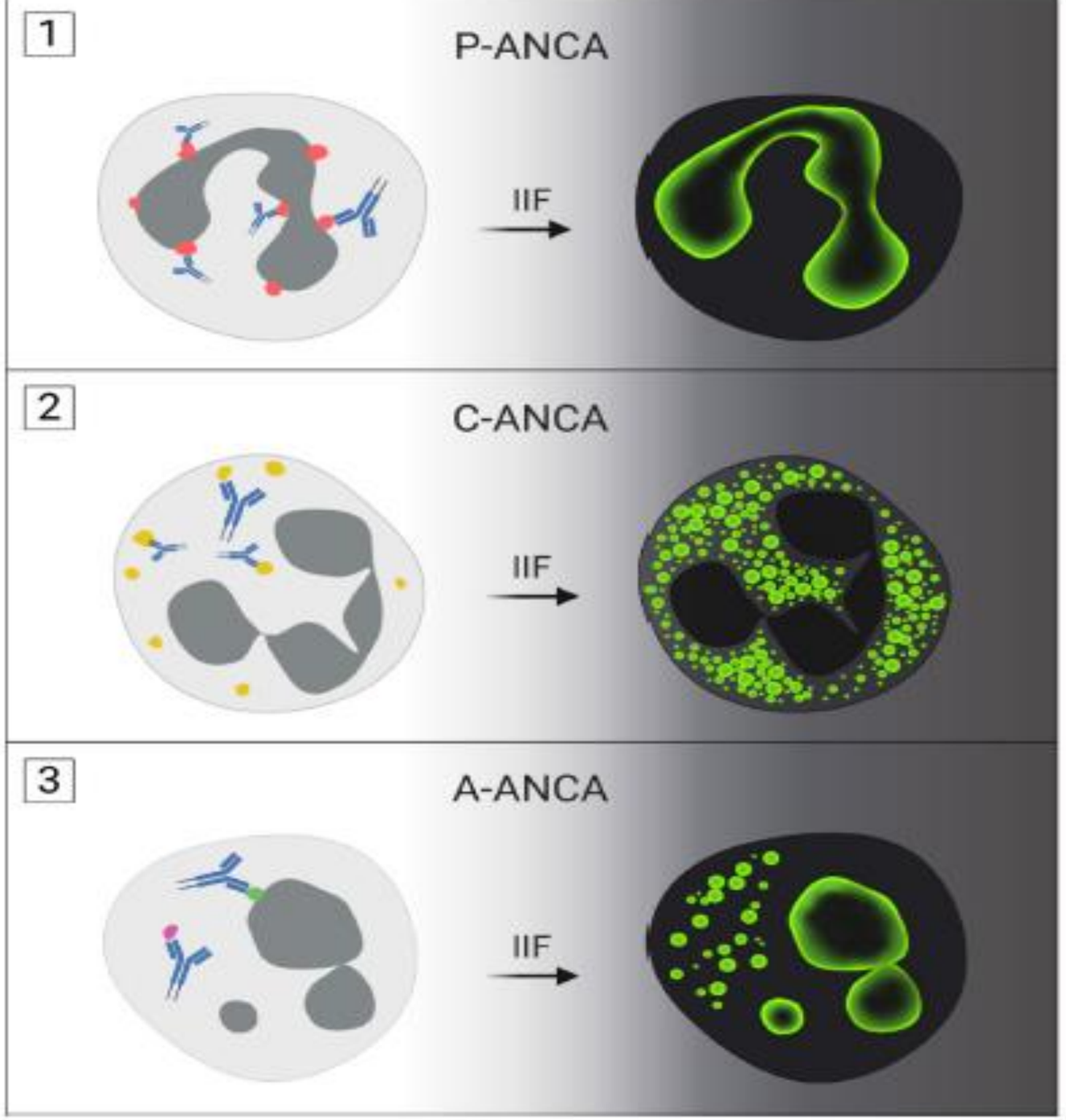
Abbreviations: ANCA, antineutrophil cytoplasmic antibodies; ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence.

Data from Refs. [48,49,53,90]

IIF ANCA

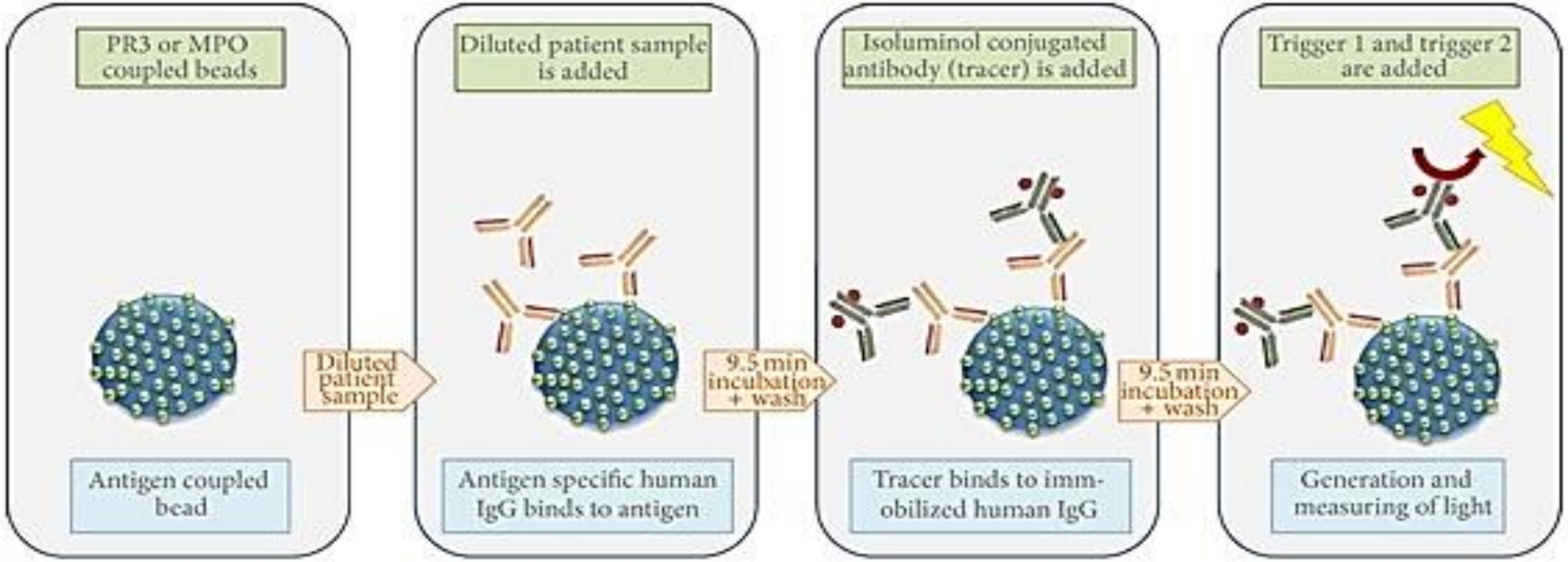

Formalin Fixation
c-ANCA
Strong cationic proteins (e.g., MPO)
Weakly cationic or neutral proteins (e.g., PR3)
Ethanol Fixation
p-ANCA
c-ANCA
Antibodies to strong cations
Antibodies to neutral proteins of weak cations (e.g., PR3)

IIF ANCA

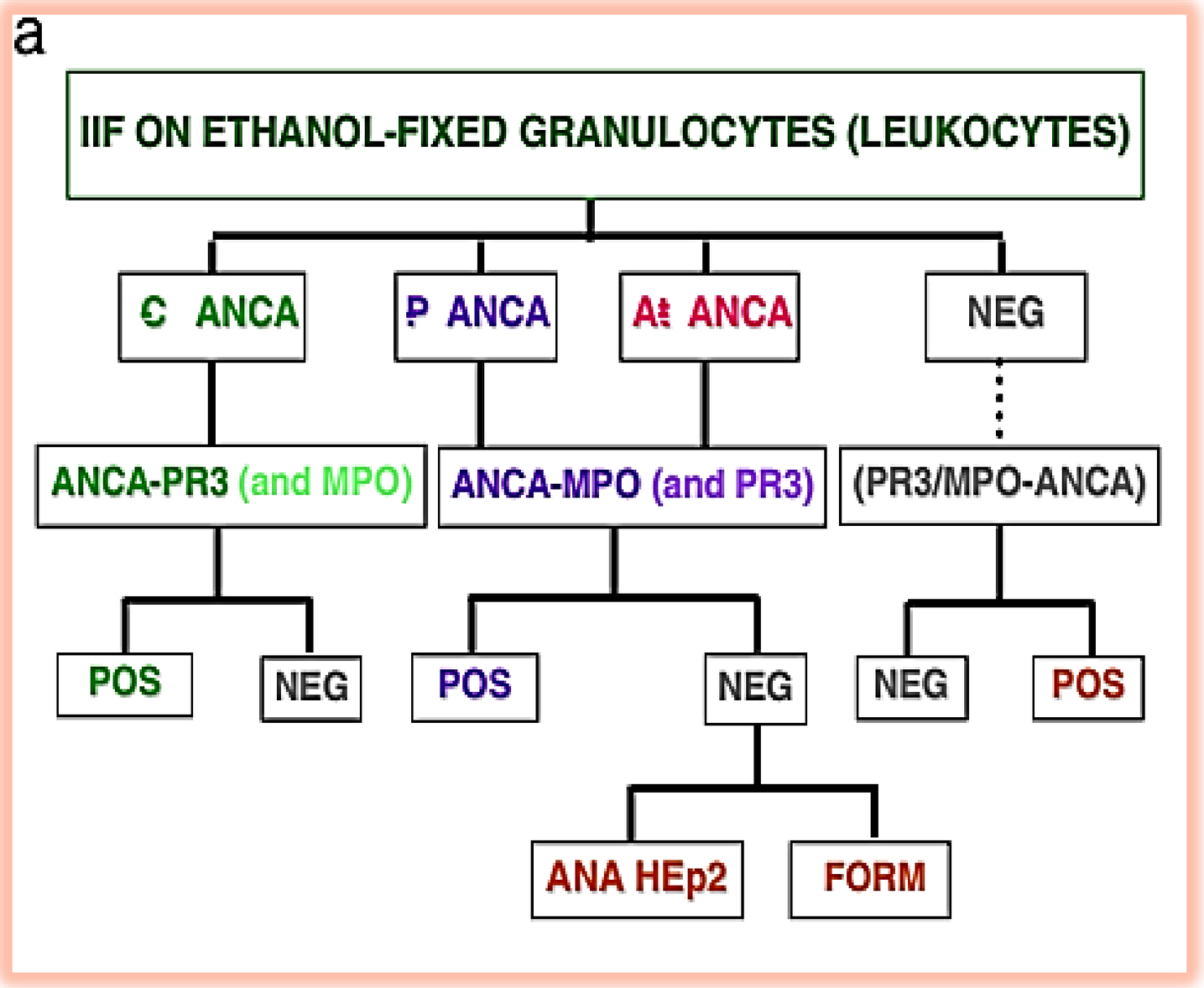


Autoimmunity Reviewshttps://doi.org/10.1016/j.autrev.2021.102759,

Antigen-specific immunometric assays



ANCA ELISA
Anti-PR3
Anti-MPO



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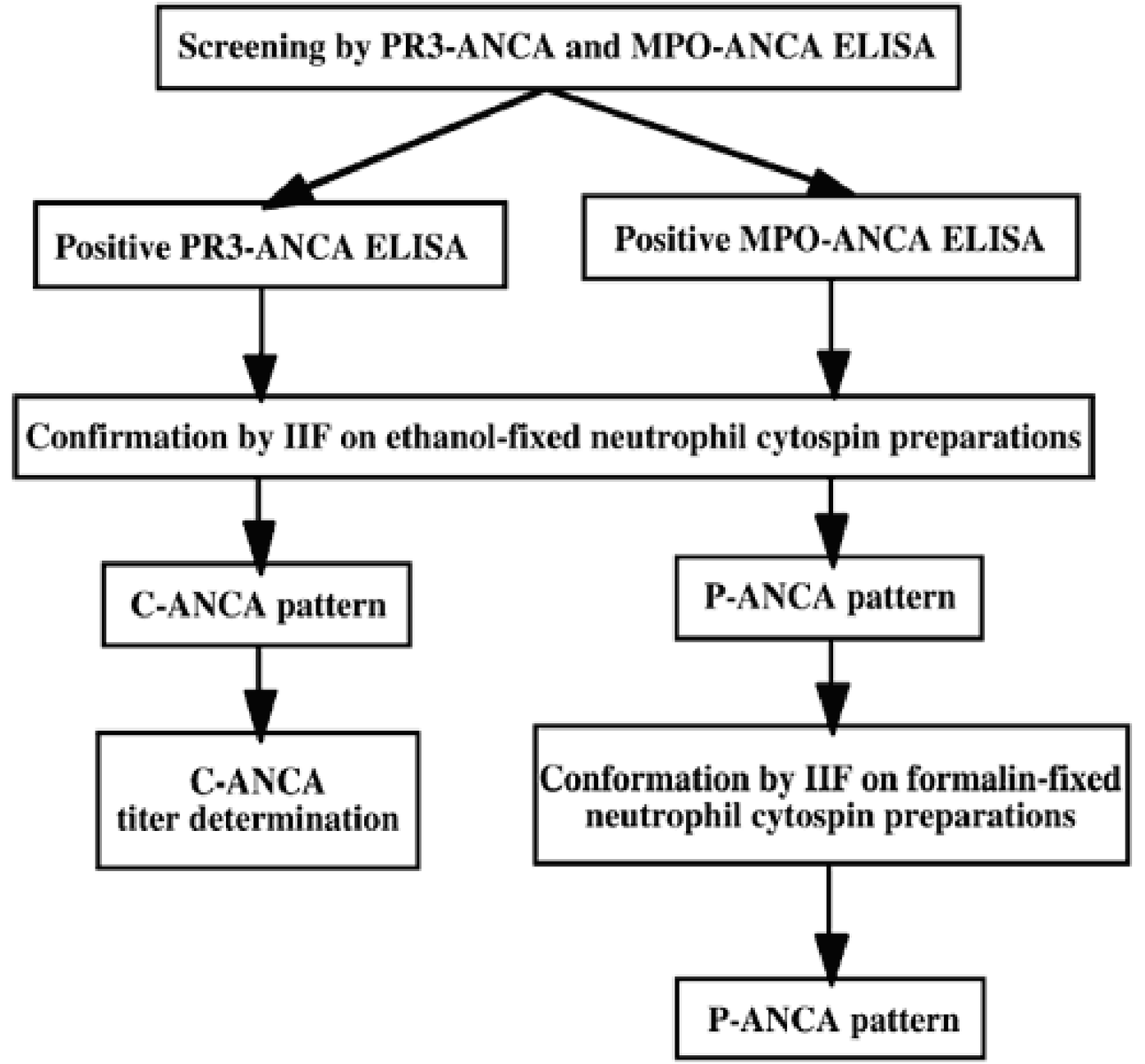
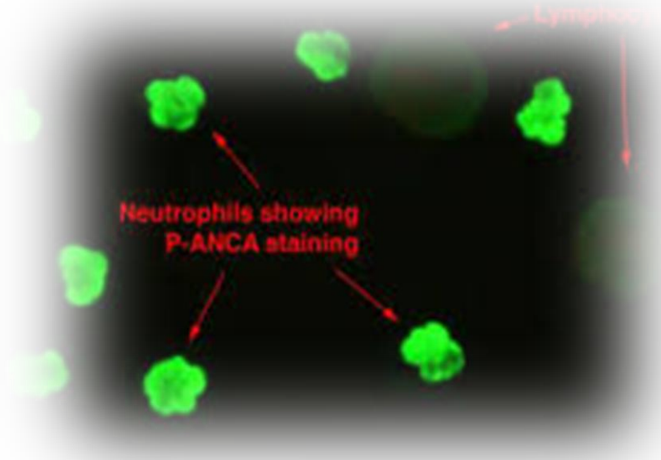
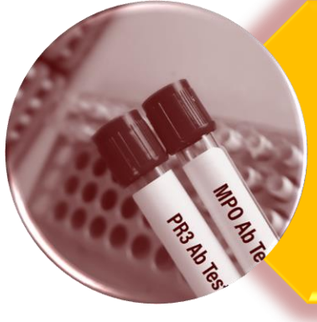


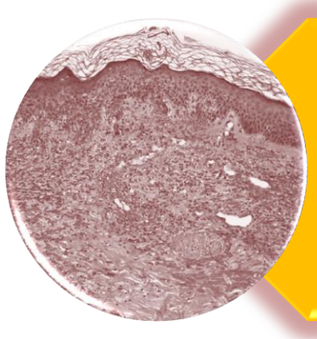


Consensus recommendations suggest combining IIFT and Ag-specific assays MPO/PR3-ANCA

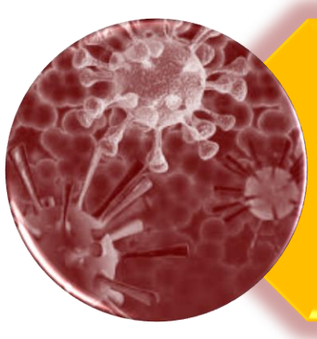
Highlights of ANCA



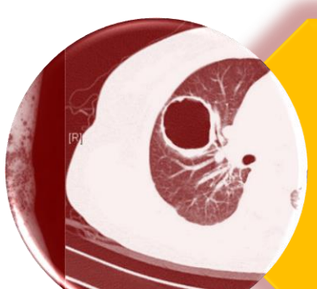
Only ANCA directed towards the PR3 and MPO are clinically relevant for AAV



ANCA are not diagnostic for AAV, clinical/histological confirmation should be valued



In differential diagnosis, infectious diseases must be considered



ANCA titer often correlates with disease activity, but exceptions are well documented.

THE TAKE-HOME MESSAGE

No lab test is as good as your history and physical exam

No lab test "screens" for CTD's Disease pattern recognition is far more helpful than any serology or test

Know the SENS and SPEC of lab tests for different diseases Say "NO" to laboratory panels


THANK YOU
FOR YOUR
ATTENTION