



ANA – understanding the misunderstood

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Conflict of Interest

Received research support from Amgen and Novartis (over 5 years ago)

Received education support from Amgen, AbbVie

Have spoken at conferences for Amgen, Merck, Aventis, Novartis, Eli Lilly

Advisory Boards: Amgen, Roche, Sanofi, Celgene, AbbVie, Merck, Novartis, Eli Lilly, Janssen, UCB

Member of the CRA Choosing Wisely Committee – some slides shared by CRA Choosing Wisely committee

By the end of this presentation, you will:

Know	Know when to order an ANA
Understand	Understand the implications of ANA positivity
Know	Know what additional investigations are helpful when the ANA is positive

ANA testing

Not a single antibody

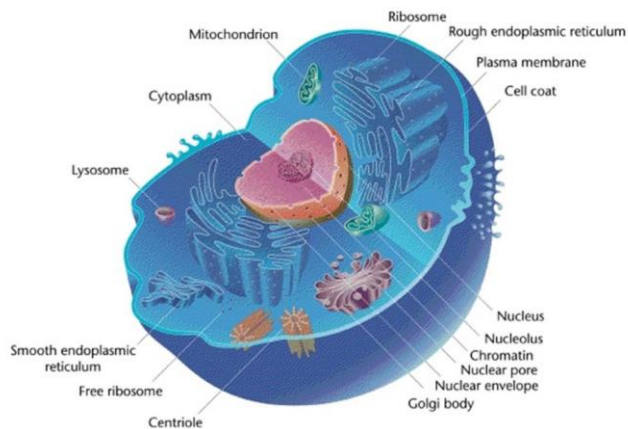
Family of antibodies directed against **nuclear constituents**

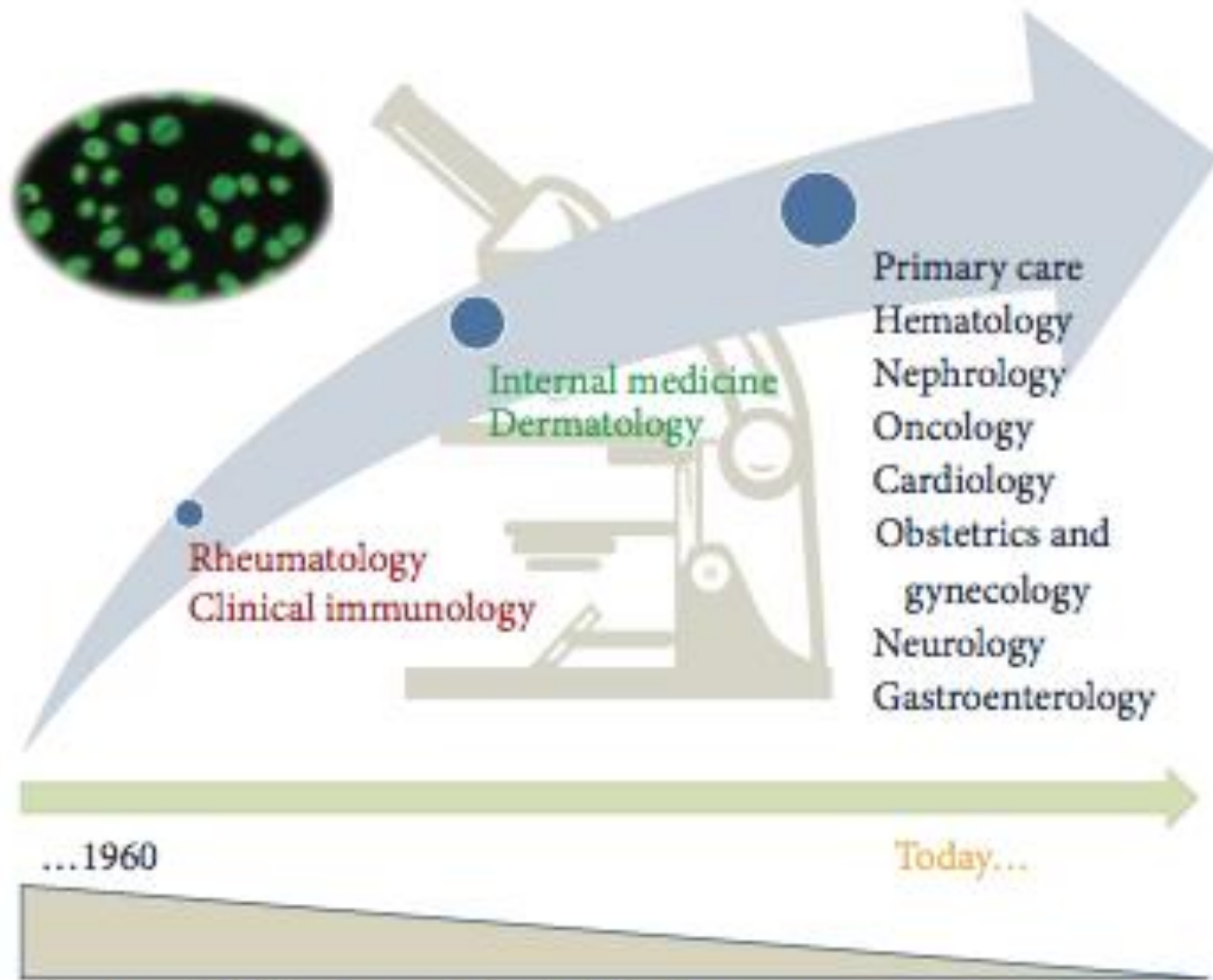
Protein antigens complexed with RNA and some enzymes in the nucleus

ssDNA, dsDNA, histones, centromeres, proteins complexed with RNA, topoisomerase

Extractable-nuclear antigens (ENAs)

- Ro, La, Sm, Jo-1, RNP, Scl-70





Changes in ANA referral patterns

Pretest probability decreased → significant consequences on posttest probability



ANA testing in Canada

- In BC, more than 94,000 ANA tests were performed in 2011/12
 - (\$2.24 million, annually)
- In one teaching hospital, ANA positive **15% of the time**
- 1500 repeat tests –
 - the majority within 3 months of a previously negative result
 - Less than 1% became significantly positive



BC guidelines, Antinuclear Antibody (ANA) Testing Protocol, June/01/2013
Fitch-Rogalsky, PLOS online 2014

ANA testing in Canada



- In Alberta, 60,000 ANA done annually
- Central triage
 - 26% of referrals for + ANA found NO evidence of disease



ANA in New Brunswick - 2014

4525 ANA processed in Fredericton

6977 ANA processed in Moncton, Saint John, Miramichi

- Cost \$23/positive test
- ~ 265,000\$



Prevalence and Sociodemographic Correlates of Antinuclear Antibodies in the United States

Table 1. Estimated US prevalence of ANAs and estimated PORs for ANA associations with selected sociodemographic variables*

Characteristic	No.†	No. ANA positive†	% ANA positive (95% CI)	Age-adjusted POR (95% CI)
Total	4,754	670	13.8 (12.2–15.5)	
Age, years‡				
12–19	1,190	146	11.2 (7.8–14.6)	1.00 (reference)
20–29	686	90	13.1 (9.6–16.7)	1.20 (0.74–1.93)
30–39	642	93	13.4 (9.5–17.3)	1.23 (0.75–2.02)
40–49	581	66	11.5 (8.5–14.4)	1.03 (0.72–1.46)
50–59	478	87	17.4 (13.2–21.7)	1.68 (1.13–2.48)
60–69	525	68	13.8 (8.7–18.9)	1.27 (0.77–2.08)
70+	652	120	19.2 (15.0–23.4)	3.02
Sex§				
Male	2,285	244	9.6 (7.6–11.6)	1.00 (reference)
Female	2,469	426	17.8 (15.5–20.1)	3.60
Race/ethnicity				
Non-Hispanic white	2,118	293	13.7 (11.7–15.7)	1.00 (reference)
Non-Hispanic black	994	155	16.5 (13.5–19.4)	1.30 (1.00–1.70)
Mexican American	1,246	168	12.8 (10.3–15.3)	1.00 (0.78–1.29)
Other	396	54	12.8 (8.5–17.2)	0.96 (0.65–1.42)
Education				
0–8 years	697	106	13.6 (9.6–17.6)	1.00 (reference)
9–11 years	848	104	13.2 (10.5–15.9)	1.01 (0.65–1.56)
High school diploma/GED	1,068	141	13.1 (10.4–15.7)	1.02 (0.74–1.41)
Some college	1,152	171	14.7 (12.0–17.4)	1.19 (0.81–1.74)
College or postgraduate	815	112	13.0 (10.1–16.0)	1.01 (0.65–1.57)
Family income-to-poverty level ratio				
At or above poverty	3,370	477	13.7 (11.9–15.4)	1.00 (reference)
Below poverty	982	125	13.9 (10.7–17.2)	1.08 (0.84–1.39)

* ANAs = antinuclear antibodies; PORs = prevalence odds ratios; 95% CI = 95% confidence interval; GED = General Educational Development.

† Reflects the number of subjects within the sample, not an estimated count for the US population, whereas the percent ANA positive is a prevalence estimate for the US population.

‡ $P = 0.01$.

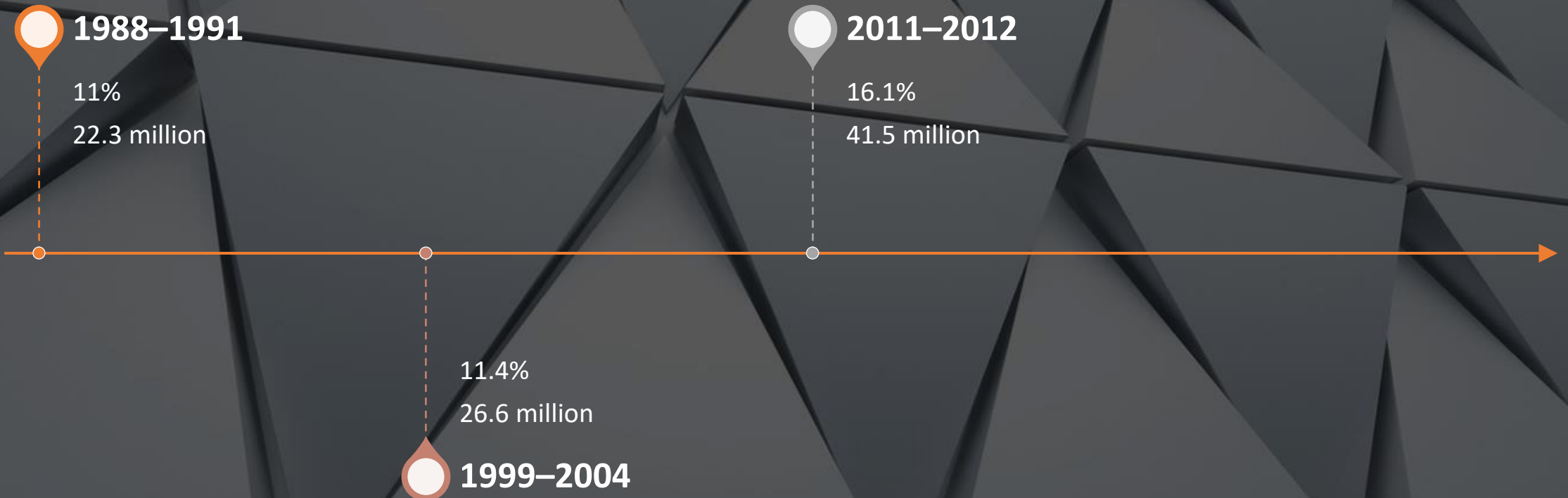
§ $P < 0.001$ by Wald's chi-square test.

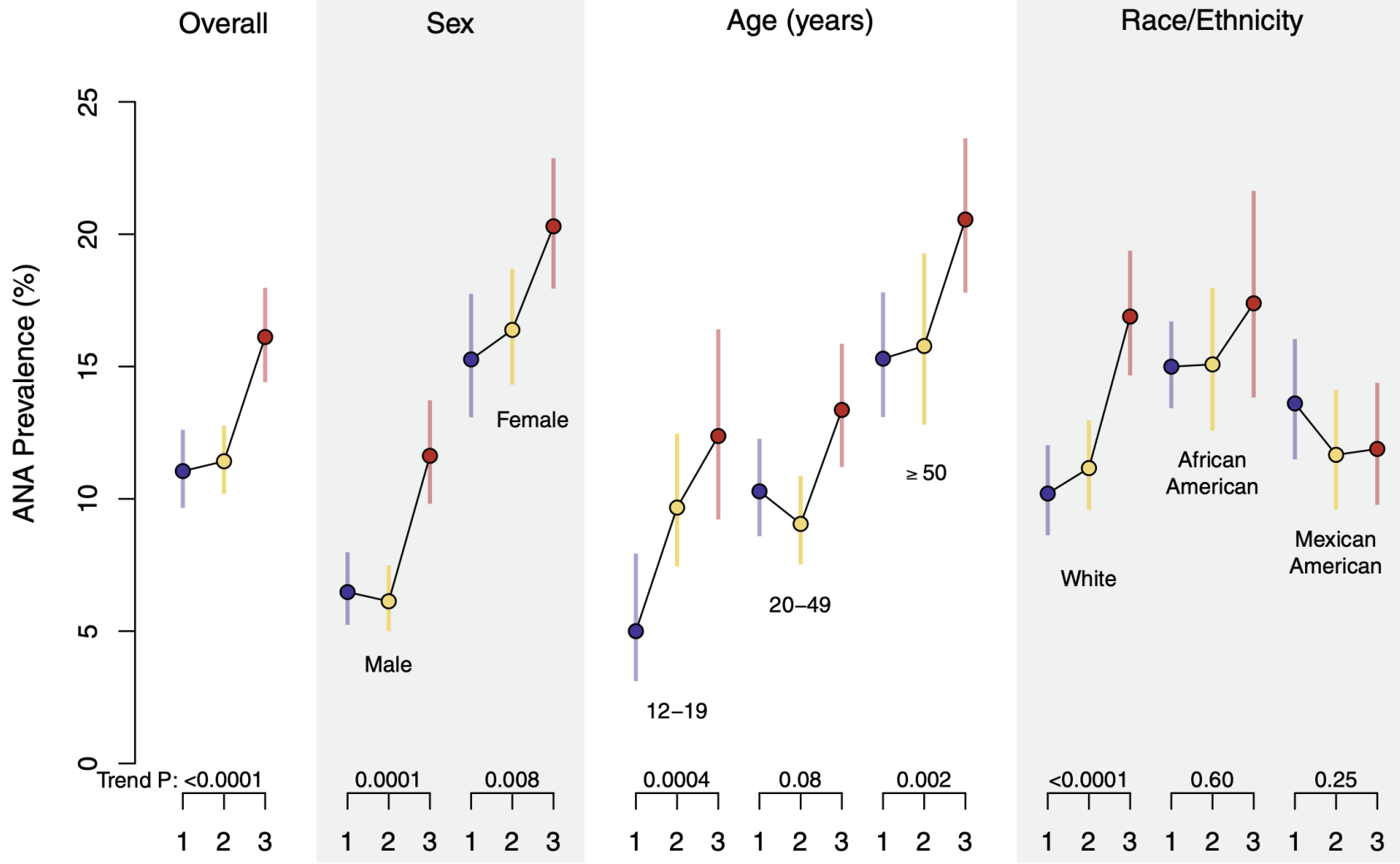
19.2% aged 70+

17.8% females

Increasing ANA?

- NHANES survey
- ANA performed on 13,519





ANA-associated diseases

Rheumatic diseases

- Systemic lupus erythematosus
- Mixed connective tissue disease
- Scleroderma
- Sjögren's syndrome
- Rheumatoid arthritis
- Polymyositis
- Dermatomyositis
- Discoid lupus

Organ-specific autoimmune diseases

- Autoimmune thyroid disease
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Autoimmune cholangitis

Other

- Drug-induced lupus
- Asymptomatic drug-induced ANA
- Chronic infections (TB, endocarditis, EBV)
- Idiopathic pulmonary fibrosis
- Primary pulmonary hypertension
- Lymphoproliferative disorders

Recommendation 1

- **1. Don't order ANA as a screening test in patients without specific signs or symptoms of systemic lupus erythematosus (SLE) or another connective tissue disease (CTD).**

Guidelines:

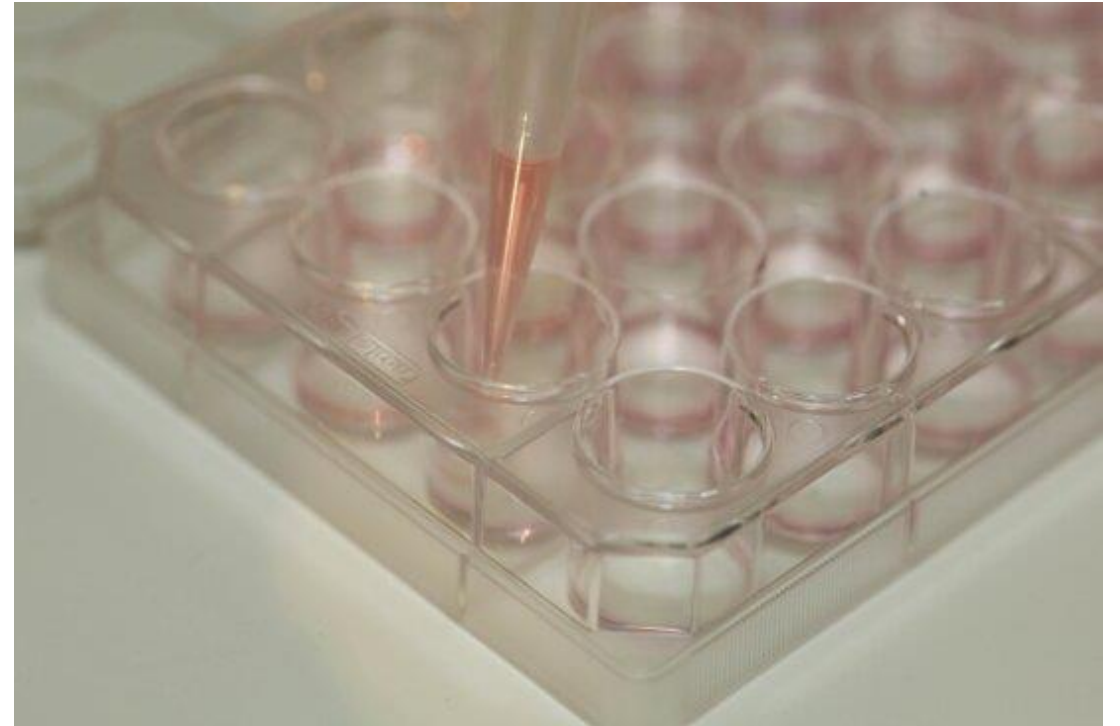
- **American College of Pathologists**
- **British Columbia Ministry of Health**
- **American College of Rheumatology**
- **Italian Society of Laboratory Medicine Guidelines**

ANA Reports

Immunofluorescence



ELISA



Range of ANA in “healthy” Individuals

Titre	Percent affected
1/40	32%
1/80	15%
1/160	7%
1/320	3%

Immunofluorescence

Pattern	Significance (disease association)	Further testing
Homogeneous	SLE, drug-induced lupus, JIA	ESR <u>and</u> CRP, C3/C4, dsDNA
Rim (peripheral)	SLE, myositis, PBC, autoimmune liver disease	ESR <u>and</u> CRP, C3/C4, dsDNA, CK, LFTs, liver-specific Abs (smooth muscle, mitochondrial)
Speckled	SLE, MCTD, Sjögren's, Scleroderma Lymphoproliferative ds, interstitial lung ds, neurologic ds, HEALTHY subjects	ENA, C3,C4 Quantitative Igs
Nucleolar	Scleroderma Autoimmune hepatitis, PBC	Creatinine, urinalysis, BP monitoring, PFTs, chest imaging
Centromere	Limited scleroderma (CREST), Raynaud's	Creatinine, urinalysis, OGD, echocardiogram

Procedure	Units	Ref Range	Positive
ANA			7
dsDNA I	IU/mL		7.1 H
Chromatin	AI	[<=0.9]	0.2
Ribosomal P	AI	[<=0.9]	6.4 H
SS-A/Ro	AI	[<=0.9]	>8.0 H
SS-B/La	AI	[<=0.9]	<0.2
Centromere B	AI	[<=0.9]	0.8
Sm	AI	[<=0.9]	>8.0 H
Sm/RNP	AI	[<=0.9]	3.6 H
RNP	AI	[<=0.9]	<0.2
Scl-70	AI	[<=0.9]	<0.2
Jo-1	AI	[<=0.9]	<0.2
ANA Reference Range !			See Note

2020/02/11 08:13 dsDNA:

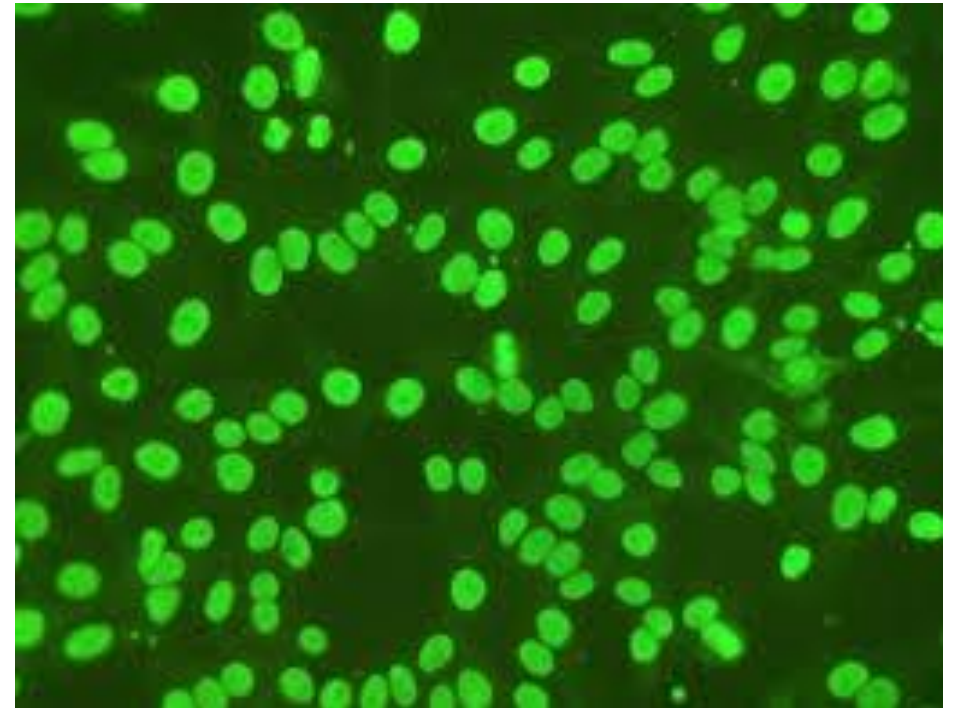
Reference Range: <= 4 IU/mL Negative
 5-9 IU/mL Indeterminate
 >=10 IU/mL Positive

2020/02/11 08:13 ANA Reference Range:

Chromatin, Ribosomal P, SS-A, SS-B, Centromere B, Sm, Sm/RNP, RNP, Scl-70, Jo-1

<1.0 AI Negative
 >=1.0 AI Positive

ELISA



Interpretation

Test	Result	Flag	Reference	Site
ENA > ENA SCREEN	NEGATIVE <i>Negative for SS-A Ro52, SS-A Ro60, SSB, SM, RNP, SCL 70 and JO-1.</i>		NEGATIVE	DEC
ANA > ANA > ANA PATTERN	POSITIVE Dense Fine Speckled <i>Anti-DFS70 if occurred exclusively, can serve as an exclusion marker for Systemic Rheumatic Autoimmune Disease. Anti-DFS70 is often present in healthy individuals but only rarely occurs in Rheumatic Disease.</i>		NEGATIVE	DEC DEC
> ANA TITRE	>1:640 <i>Please note change in Reference Range effective Dec 2/19.</i>		< 1:160	DEC
DNA	< 9.8 NEGATIVE <i>** Note: Reference Range change effective July 3, 2019 **</i>		<= 26.9 IU/mL	DEC

DEC - Dr Everett Chalmers Hospital

Testing site: Dr Everett Chalmers Regional Hospital, Fredericton, NB


- -present in **33.1%** ANA+ healthy individuals
- - **0%** of DFS70 patients with ANA associated autoimmune rheumatic diseases over 10 years follow up

Autoimmun Highlights (2016) 7:10
DOI 10.1007/s13317-016-0082-1



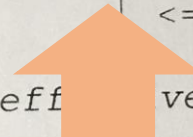
ORIGINAL ARTICLE

Measurement of anti-DFS70 antibodies in patients with ANA-associated autoimmune rheumatic diseases suspicion is cost-effective

Simón Gundín¹ · Juan Irure-Ventura¹ · Esther Asensio¹ · David Ramos¹ · Michael Mahler² · Victor Martínez-Taboada¹ · Marcos López-Hoyos¹ 

Interpretation

Test	Result	Flag	Reference	Site
ENNA ENNA SCREEN	NEGATIVE <i>Negative for SS-A Ro52, SS-A Ro60, SSB, SM, RNP, SCL 70 and JO-1.</i>		NEGATIVE	DEC
ANA > ANA	POSITIVE		NEGATIVE	DEC
> ANA PATTERN	Speckled			DEC
> ANA TITRE	1:320		< 1:160	DEC
> CYTOPLASMIC AB	Present		Negative	DEC
	<i>This pattern may be seen in Primary Biliary Cholangitis (PBC) and Autoimmune Hepatitis (AIH), rarely in Systemic Autoimmune Rheumatic Diseases (SARD). For GI conditions, may consider ordering IFA tests for AMA, ASMA & APCA.</i>			
DNA	12.0		<= 26.9 IU/mL	DEC
	NEGATIVE			
	** Note: Reference Range change effective July 3, 2019 **			



Symptom complexes



Spondyloarthritis

- Iritis
- GI/ IBD
- Psoriasis
- Recent GI/GU infections
- Urethritis



Rheumatoid arthritis

- Eye (scleritis/ episcleritis)
- Lung
- Skin (vasculitis)
- Nodules



Connective tissue disease

- Alopecia
- Sicca
- Oral ulcers
- Rash/ photo-sensitivity
- Raynaud's
- Lung
- Kidney

Investigations



Spondyloarthritis

- CBC
- CRP
- Creatinine

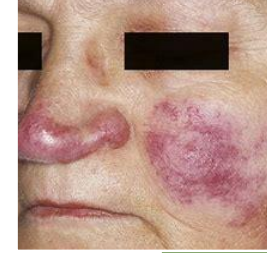
- X ray SI joints
- MRI SI joints



Rheumatoid arthritis

- CBC, CRP
- Creatinine
- ALT, albumin
- RF, anti-CCP
- Hep B/C serology

- CXR
- X ray hands, feet, other affected joints (bilat)



Connective tissue disease

- CBC, ESR, CRP
- C3/ C4
- Urinalysis
- Creatinine
- Consider CK/ LDH
- ANA
- dsDNA, ENA
- (ANCA)

- Consider CXR

ACR Criteria for SLE - 1982

- **S**erositis
- **O**ral lesions
- **A**NA
- **P**hotosensitivity

- **B**lood (cytopenias)
- **R**enal
- **A**rthritis
- **I**mmunologic (other antibodies)
- **N**eurologic

- **M**alar rash
- **D**iscoid rash



4/11 criteria

82.8% sensitivity

93.4% specificity



Entry criterion			
Antinuclear antibodies (ANA) at a titer of $\geq 1:80$ on HEp-2 cells or an equivalent positive test (ever)			
↓			
If absent, do not classify as SLE If present, <u>apply additive criteria</u>			
↓			
Additive criteria			
Do not count a criterion if there is a more likely explanation than SLE. Occurrence of a criterion on at least one occasion is sufficient. SLE classification requires at least one clinical criterion and ≥ 10 points. Criteria need not occur simultaneously.			
Within each domain, only the highest weighted criterion is counted toward the total score§.			
Clinical domains and criteria	Weight	Immunology domains and criteria	Weight
Constitutional		Antiphospholipid antibodies	
Fever	2	Anti-cardiolipin antibodies OR	
Hematologic		Anti- $\beta 2$ GP1 antibodies OR	
Leukopenia	3	Lupus anticoagulant	2
Thrombocytopenia	4	Complement proteins	
Autoimmune hemolysis	4	Low C3 OR low C4	3
Neuropsychiatric		Low C3 AND low C4	4
Delirium	2	SLE-specific antibodies	
Psychosis	3	Anti-dsDNA antibody* OR	
Seizure	5	Anti-Smith antibody	6
Mucocutaneous			
Non-scarring alopecia	2		
Oral ulcers	2		
Subacute cutaneous OR discoid lupus	4		
Acute cutaneous lupus	6		
Serosal			
Pleural or pericardial effusion	5		
Acute pericarditis	6		
Musculoskeletal			
Joint involvement	6		
Renal			
Proteinuria $>0.5\text{g}/24\text{h}$	4		
Renal biopsy Class II or V lupus nephritis	8		
Renal biopsy Class III or IV lupus nephritis	10		

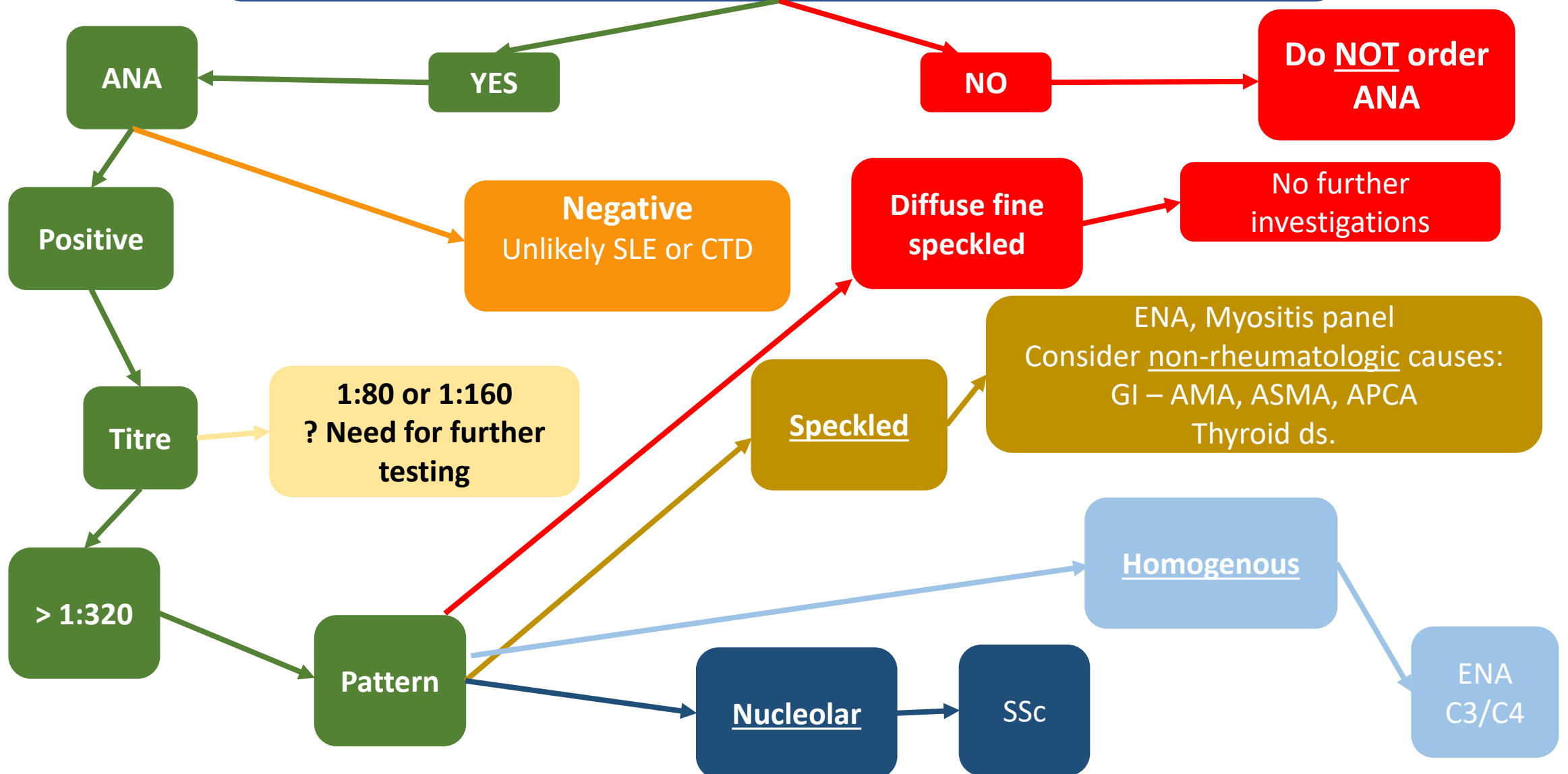
EULAR/ACR Criteria for SLE diagnosis

• Ann Rheum Dis 2019

- **Sensitivity**
96.2%
- **Specificity**
93.4%

Clinical features of connective tissue disease

2 or more organ systems affected



Key points

Consider connective tissue diseases when patients have symptoms in 2 or more organ systems

ANA should NOT be used to screen for autoimmune disease in patients with non-specific symptoms (Choosing Wisely Canada)

A Diffuse Fine Speckled pattern usually rules OUT autoimmune rheumatic disease

After a positive ANA, characterize further with:

- ENA, C3/C4 levels, CBC, Creatinine and urinalysis

