AUTOANTIBODY TESTING: PAST HISTORY, PRAGMATIC CHALLENGES AND PROMISING FUTURE: THE KODACHROME LEGACY

University of New Mexico, Division of Rheumatology
Retreat @ Santa Fe
October 19 2024
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Medical Director: MitogenDx

UNIVERSITY OF CALGARY

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- **Werfen International**
  Speaker bureau, consultant, research and diagnostic kits gifts in kind.
- **Alexion Canada**
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- Medical Director: **Mitogen Diagnostics Corporation (MitogenDx)**
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  - specific diagnostic products, kits or services are NOT promoted in this presentation
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OUTLINE & OBJECTIVES

- **Mentors make a difference**
- **Brief historical overview of ANA testing**
  - Changing bandwidth
  - Different needs by subspecialties
- **Review of key studies on population-based ANA testing**
- **Anti-dsDNA**
  - **A Puzzling Patient:** ANA negative: anti-dsDNA positive
  - DNA the antigen
  - Anti-DNA Antibodies and Assays
- **Summary and Future: different approaches and technologies**

For PDF version of this presentation email: fritzler@ucalgary.ca

Mentors Make a Difference: Dr. Eng M. Tan

August 26, 1926 - March 9, 2024

The Kodachrome Legacy
The Dark & Bright Side:

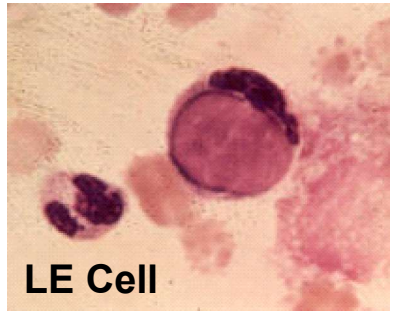
"If I look back on all the crap I learned in high school, it's a wonder I can think at all."
(Paul Simon)

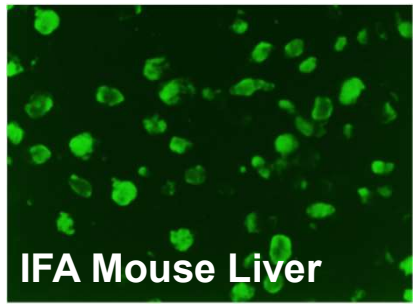
The bright side:
"Kodachrome, gives those nice bright colors,
They give **us the greens** of summers,
Makes you think all the world's a sunny day, oh yah!"

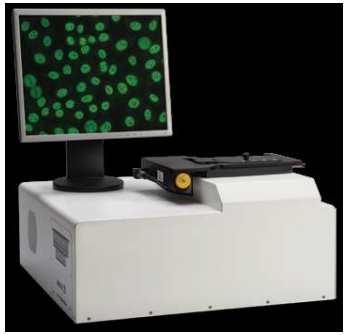
The DARK SIDE
"Everything looks worse in black and white"

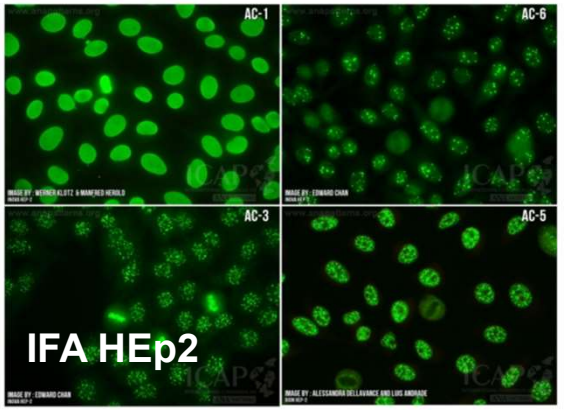
Brief History: ANA Testing Assays

- 1948 Hargraves discovers LE cell at Mayo Clinic
- 1950s – 1980s Rodent cryopreserved organ sections
- 1980 – present. Fixed HEp-2 substrate
- 1990s – present. ELISA using cell extracts spiked with 'missing' antigens. Higher throughput than HEp-2 IFA.
- 2000s Addressable Laser Bead Assays (ALBIA), Chemiluminescence (CIA). Even higher throughput.
- 2000s Automated digital ANA IFA testing

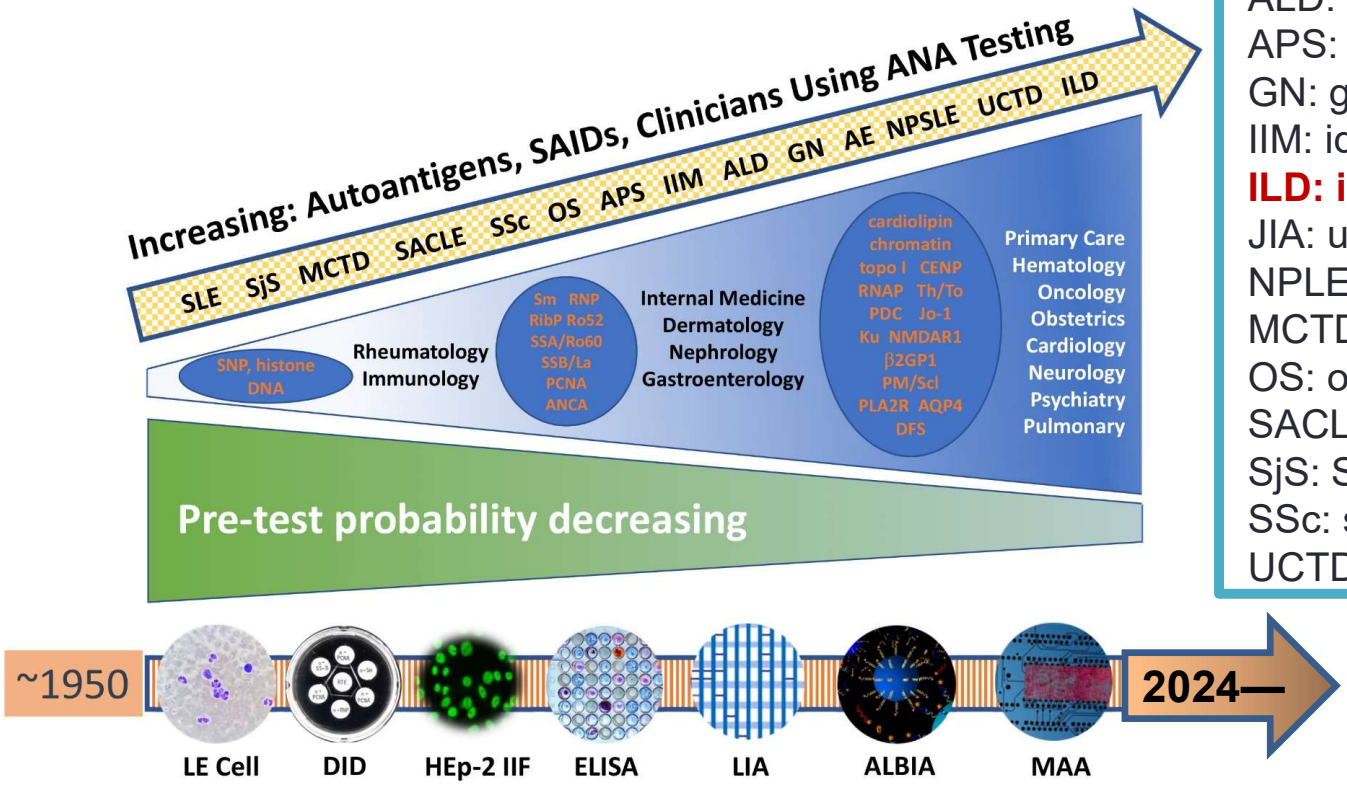








Changing Bandwidth of ANA Testing



AE: autoimmune encephalitis
ALD: autoimmune liver disease
APS: anti-phospholipid syndrome
GN: glomerulonephritis
IIM: idiopathic myopathies
ILD: interstitial lung disease
JIA: uveitis
NPLE: neuropsychiatric LE
MCTD: mixed conn. tissue dis.
OS: overlap syndromes
SACLE: sub-acute cutaneous LE
SjS: Sjögren syndrome
SSc: systemic sclerosis
UCTD: undifferentiated CTD

Clinical scenarios where ANA testing is sued

- **Intent to REFER: Primary Care Screening > CASE FINDING**
  - **The most value in Mid-Range Pre-Test Probability (Aches and pains, rashes, and other symptoms with no explanation)**
- **Intent to TREAT: Specialist Establish/Confirm DIAGNOSIS**
  - **High Pre-Test Probability**
    - Guide to further investigations (biopsy, imaging, etc.), tertiary care referrals and TREATMENT
  - **Follow clinical course:** Guide to treatment effectiveness
    - **Confirm remission and/or flares (controversial)**

- **Intent to PREVENT: Earlier and Accurate Diagnosis**
  - **Annual Check-ups**
  - **Low Pre-Test Probability**
  - **Not widely used**
  - **Cost–Value Equation important: VALUE = COST/OUTCOMES**

Other clinical uses of anti-nuclear antibodies (ANA)

- **Fulfill Disease Classification Criteria (Research)**
  - **SLE:** https://www.ncbi.nlm.nih.gov/pubmed/31385462
  - **Autoimmune Liver Disease:** https://www.ncbi.nlm.nih.gov/pubmed/24418295
- **Predict clinical risk**
  - **JIA: risk of uveitis** https://www.ncbi.nlm.nih.gov/pubmed/35398272
  - **Raynaud's: risk of systemic sclerosis (especially anti-centromere)**
  - **Undifferentiated Connective Tissue Disease (UCTD) progression to SARD**

ANA Testing/Screening

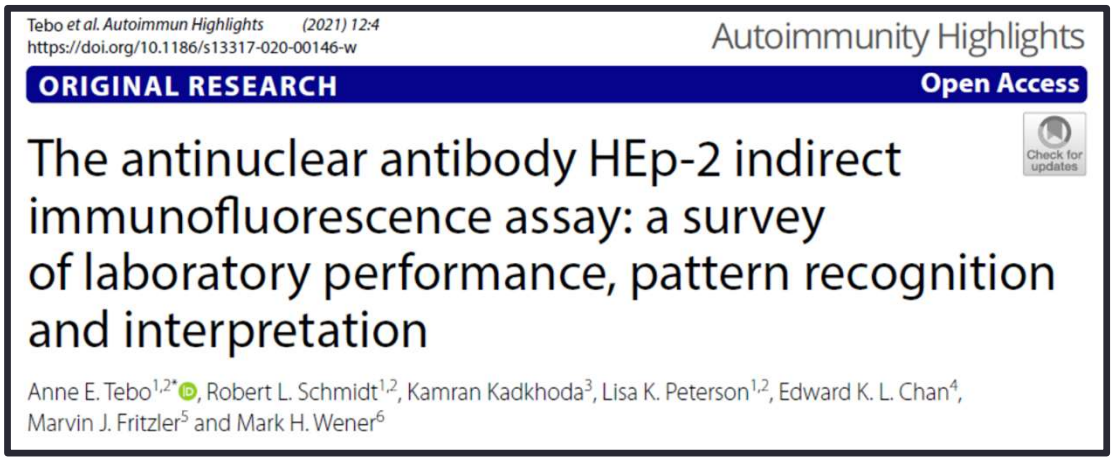
- **HEp-2 IFA patterns and titers matter**
  - See publications below and references therein

DE GRUYTER

Clin Chem Lab Med 2020; aop

Lisa K. Peterson[a], Anne E. Tebo[a,*], Mark H. Wener, Susan S. Copple and Marvin J. Fritzler

Assessment of antinuclear antibodies by indirect immunofluorescence assay: report from a survey by the American Association of Medical Laboratory Immunologists



Tebo *et al. Autoimmun Highlights* (2021) 12:4
https://doi.org/10.1186/s13317-020-00146-w

Autoimmunity Highlights

ORIGINAL RESEARCH

Open Access

Check for updates

The antinuclear antibody HEp-2 indirect immunofluorescence assay: a survey of laboratory performance, pattern recognition and interpretation

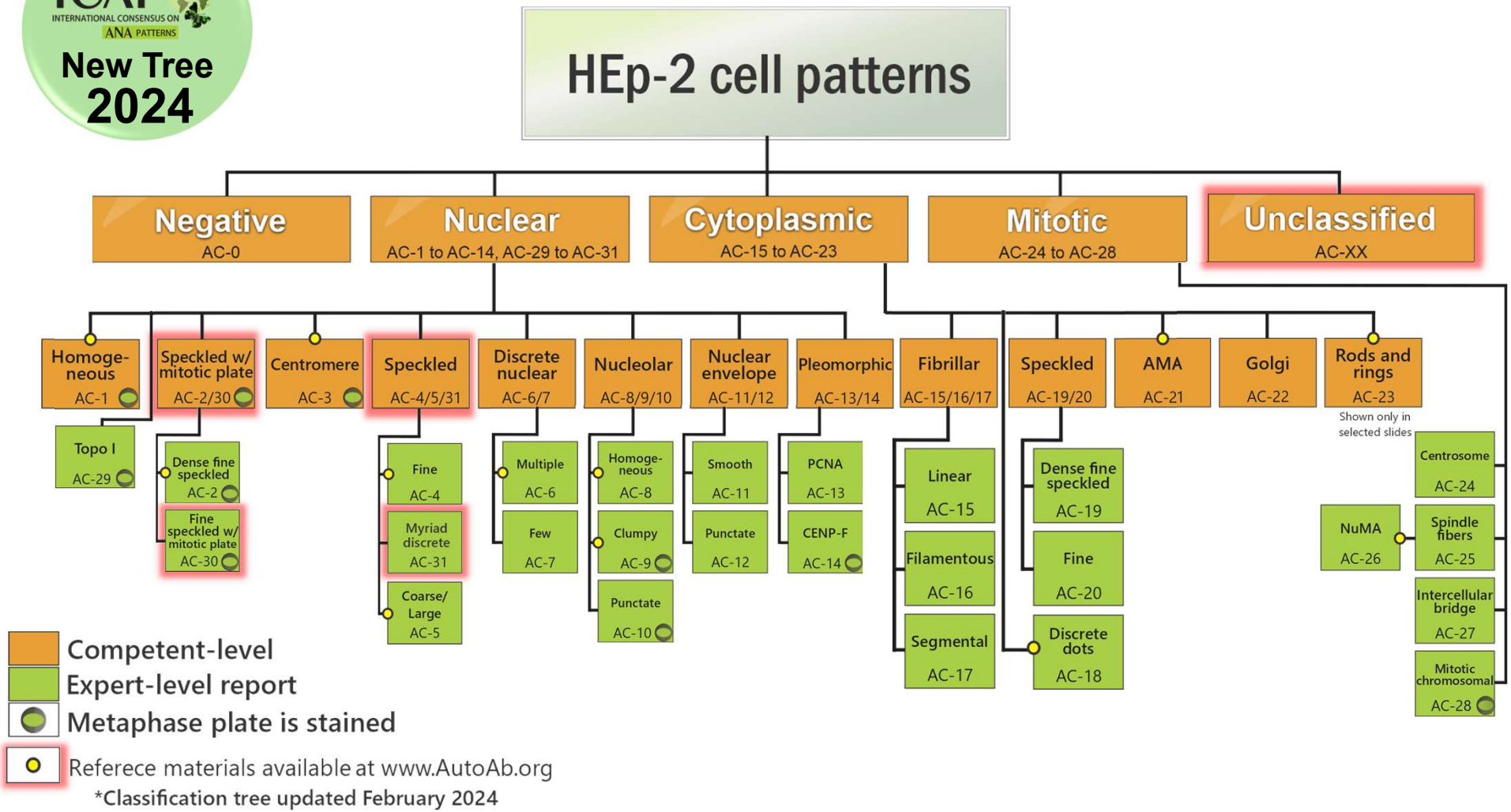
Anne E. Tebo[1,2*], Robert L. Schmidt[1,2], Kamran Kadkhoda[3], Lisa K. Peterson[1,2], Edward K. L. Chan[4], Marvin J. Fritzler[5] and Mark H. Wener[6]

ANA Testing/Screening

- **HEp-2 IFA Limitations**
  - In Practice: even though >1000 autoantibody targets present in HEp-2, not all are detected by IFA using human sera
  - Inter-manufacturer variation
    - Growth conditions and processing
    - HEp-2 cells stabilized and permeabilized by a variety of fixatives: altered native epitopes
  - Interlaboratory variations in reporting, nomenclature and interpretation being addressed **International Consensus on Autoantibody Anti-Cell (AC) Patterns**

ICAP
INTERNATIONAL CONSENSUS ON ANA PATTERNS

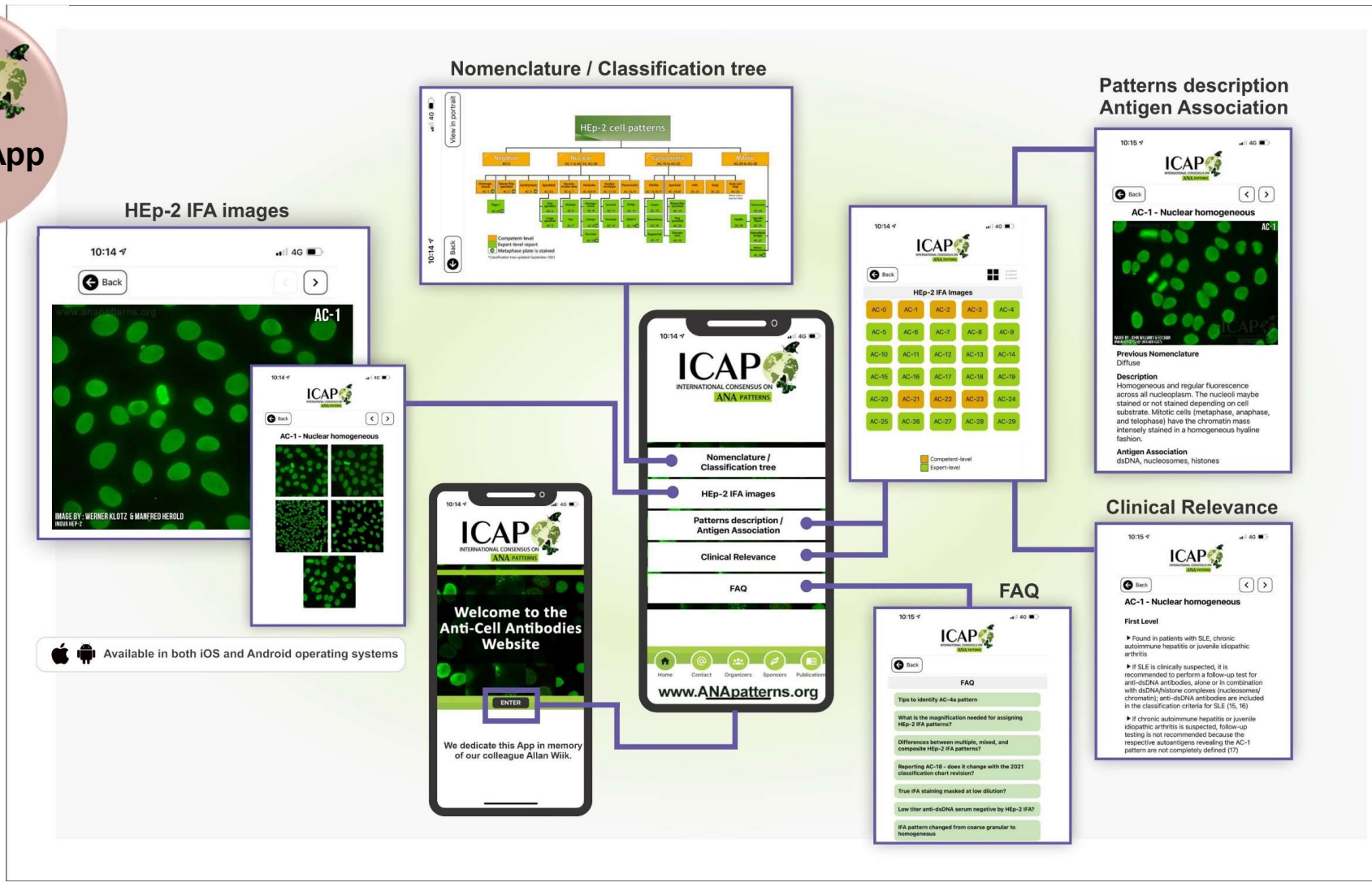
New Tree 2024



*Classification tree updated February 2024

ICAP
INTERNATIONAL CONSENSUS ON ANA PATTERNS

iPHONE App



ANA IFA: Key Population Studies

Country	Study	Fqy %	Comments	References
Brazil (2011)	N=918 Healthy Individuals 1:80	13	53.4% had titer <=1:160 vs. 10.8% AARD cohort DFS in 33% HI vs 0% in SARD	Arthritis Rheum 64: 2319-27, 2012
USA (2012)	N=4754 ≥4 years 1:80	14	Details later	Arthritis Rheum 64: 2319-27, 2012
China (2014)	N=20970 2-88 years ($\bar{x}$=32) 1:100	14	Females>Males Elderly>Young	Curr Ther Res Clin Exp 76: 116-119, 2014
Germany (2017)	N=1199 ≥20 years 1:80	33	29% had titer 1:80 or 1:160 Females > Males	Arthritis Res Ther 19:127, 2017
Belgium (2018)	N=279 18-69 ($\bar{x}$=46) years 1:80 CAD	34		Autoimmun Rev 17: 533-540, 2018

NaHaNES: National Health and Nutrition Examination Survey

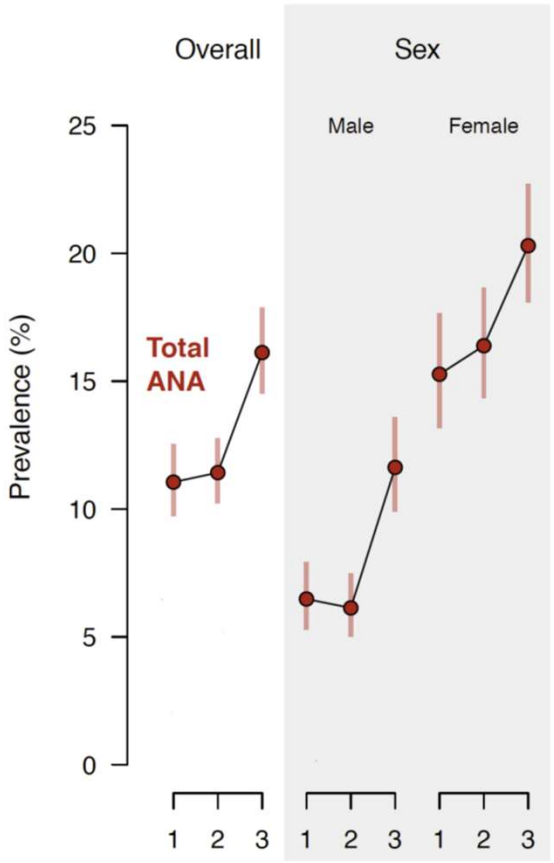
Dinse GE, Parks CG, Weinberg CR, et al. Increasing Prevalence of Antinuclear Antibodies in the United States.
Arthritis Rheumatology 74: 2032-41, 2022

AIM: Determine if prevalence of ANA changed over a 25-year span in USA.

- 13,519 participants age >/=12 years
- 3 time periods studied: 1988-1991, 1999-2004, and 2011-2012.
- Included demographic, environmental variables
- **The same assay/diagnostic platform used for all studies**

- **The prevalence of ANA:**

Prevalence ANA Over Three Periods 1988 – 2012

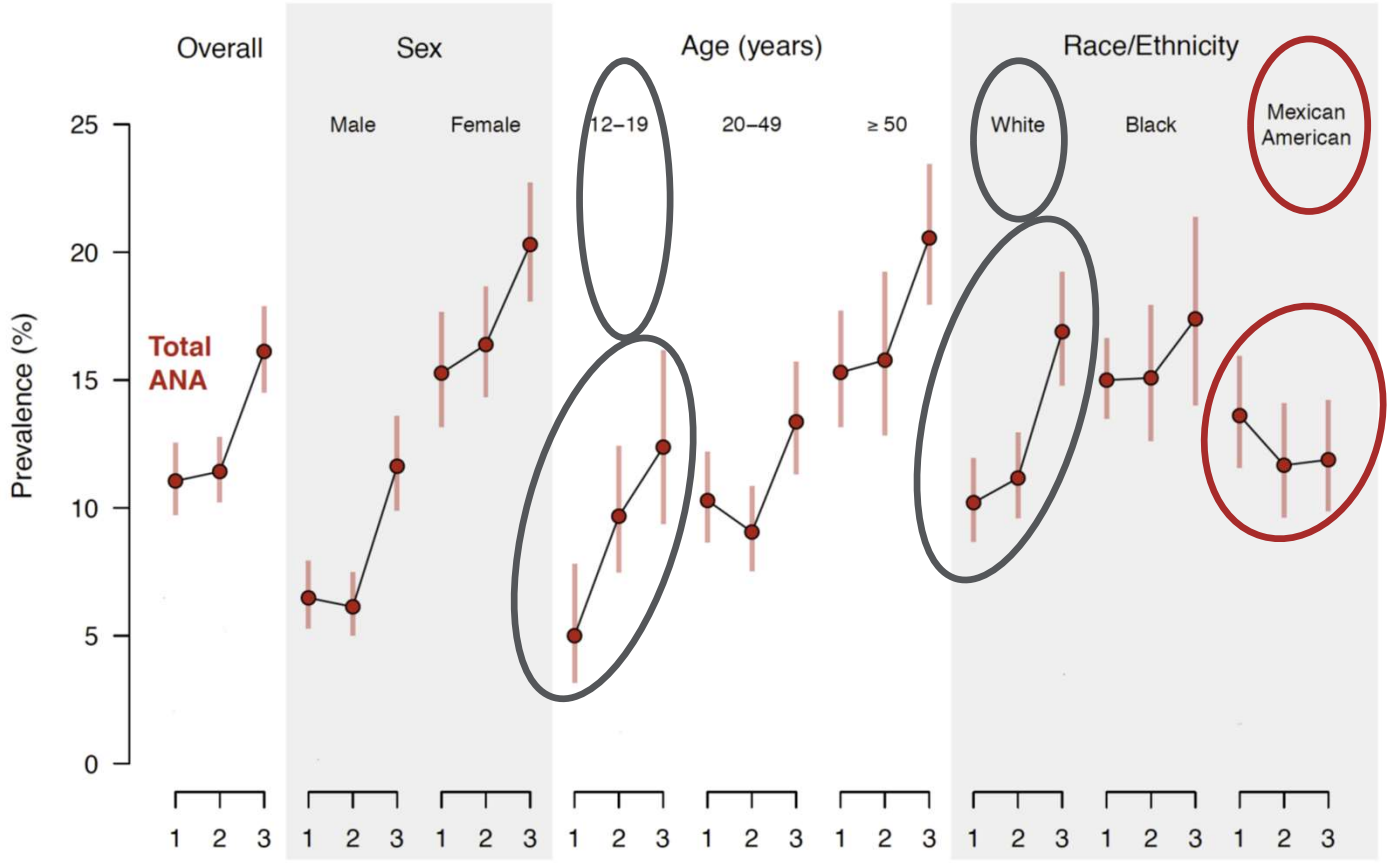


OVERALL Increase

1 = 1988-92	**11.0%**
2 = 1999-04	**11.7%**
3 = **2011-12**	**14.0%**

Source: Dinse et al. DOI: 10.1002/art.42330

Prevalence ANA Over Three Periods 1988 – 2012



Source: Dinse et al. DOI: 10.1002/art.42330

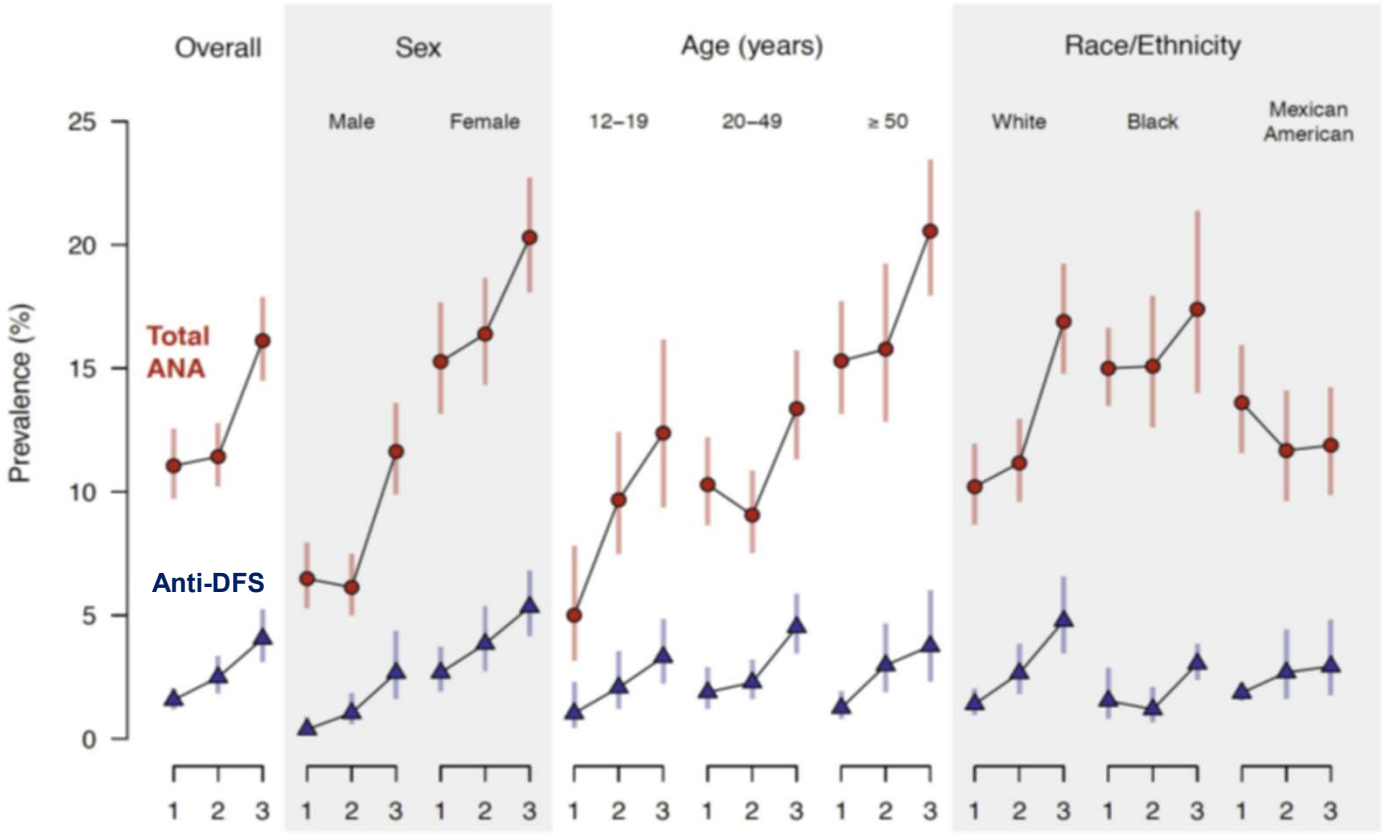
National **H**ealth and **N**utrition **E**xamination **S**urvey: **NaHaNES**

Dinse GE, Parks CG, Weinberg CR, et al. Increasing Prevalence of Antinuclear Antibodies in the United States. *Arthritis Rheumatol.* 74(12):2032-41, 2022.

- **The prevalence of +ANA increased**
  - 11.0% 1988-92 (95% confidence interval [95% CI] 9.7-12.6%) ~22.3 million
  - 11.4% 1999-2004 (95% CI 10.2-12.8%) , ~26.6 million
  - 16.1% 2011-2012 (95% CI 14.4-18.0%) ~41.5 million
- **Greatest change was among adolescents 12-19 years** (OR 2.07 (95% CI 1.18-3.64) and 2.77 (95% CI 1.56-4.91)(P for trend = 0.0004).
- **ANA prevalence increased** in both sexes (especially in **men**), **older adults** (age >/=50 years), and **non-Hispanic white** individuals.
- Increases in ANA prevalence were not explained by concurrent trends in weight (obesity/overweight), smoking exposure, or alcohol consumption.
- **Unfortunately, geographic clustering not done**

What about changes in specific autoantibodies?

Prevalence of DFS AC-2 antibody 1988 – 2012



Anti-DFS
1. 88-92 **1.6%**
2. 99-04 **2.5%**
3. 11-12 **4.0%**

Source:G. E. Dinse, B. Zheng, C. A. Co, C. G. Parks, C. R. Weinberg, F. W. Miller, et al. Front Immunol 14: 1186439, 2023 Accession Number: 37426660 PMCI[PMC10326272 DOI: 10.3389/fimmu.2023.1186439 https://www.ncbi.nlm.nih.gov/pubmed/37426660

ARTHRITIS & RHEUMATISM
Vol. 63, No. 1, January 2011, pp 19–22
DOI 10.1002/art.30078

EDITORIAL

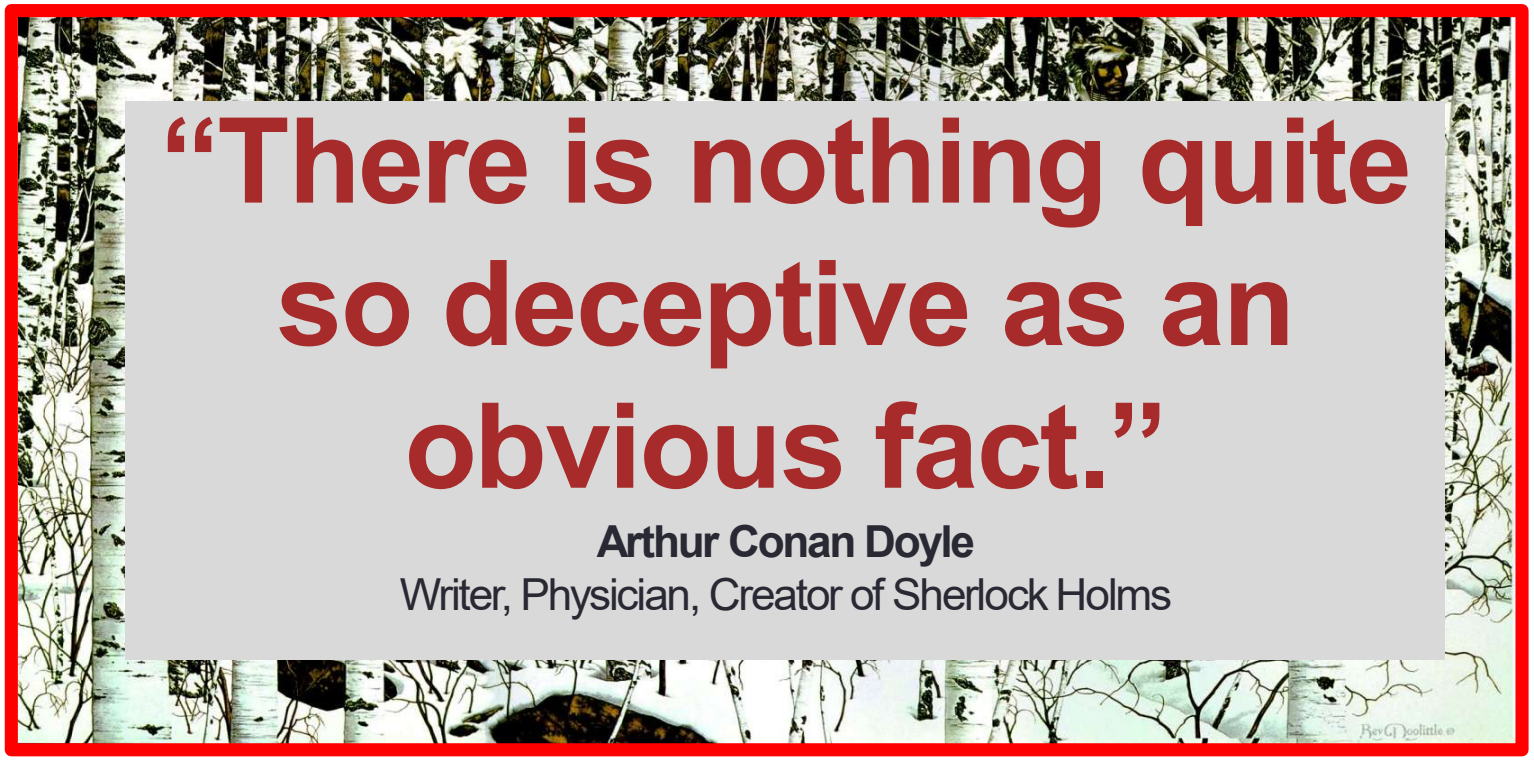
The Antinuclear Antibody Test: Last or Lasting Gasp?

Marvin J. Fritzler

If submitted to FDA or Health Canada today, would HEp-2 IFA be approved?

Anti-dsDNA antibodies

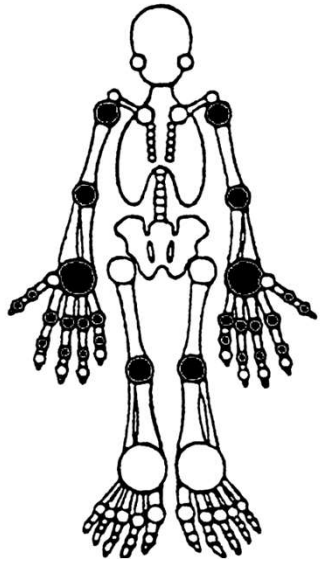
What is anti-DNA?



“Red Fox” by Bev Doolittle

ANA Negative — anti-dsDNA Positive = "false positive"?

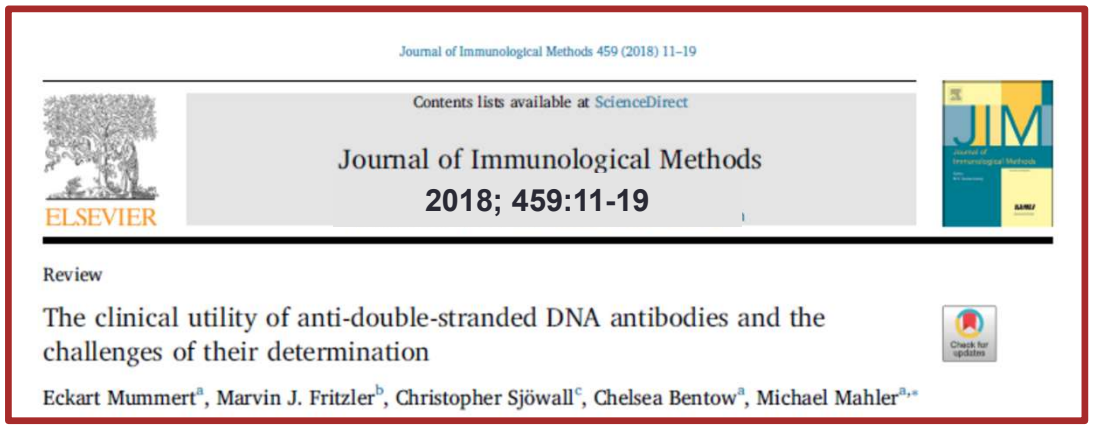
- A 69-year-old female presented with a 1-month history of symmetric polyarthritis, pleuritis and 10 kg weight loss.
- Initial laboratory analysis:
  - Lymphopenia 0.3 x 10^9/L
  - Elevated ESR 48mm/hr, CRP 93.0mg/mL
  - IFA ANA was **NEGATIVE** on HEp-2 cells
  - **But…high titer anti-dsDNA on Crithidia (CLIFT) and Chemiluminescence Assay (CIA)**



- **Is this a False +ve anti-dsDNA or a False –ve ANA?**
- **ANA negative: does not meet 2019 EULAR/ACR SLE Criteria**

Case Courtesy Drs. M.Y. Choi & H. Arbillaga, University of Calgary

Key References



Journal of Immunological Methods 459 (2018) 11–19

Contents lists available at ScienceDirect

Journal of Immunological Methods

2018; 459:11-19

Review

The clinical utility of anti-double-stranded DNA antibodies and the challenges of their determination

Eckart Mummert[a], Marvin J. Fritzler[b], Christopher Sjöwall[c], Chelsea Bentow[a], Michael Mahler[a,*]

frontiers in Immunology

2019 10: 1104

HYPOTHESIS AND THEORY

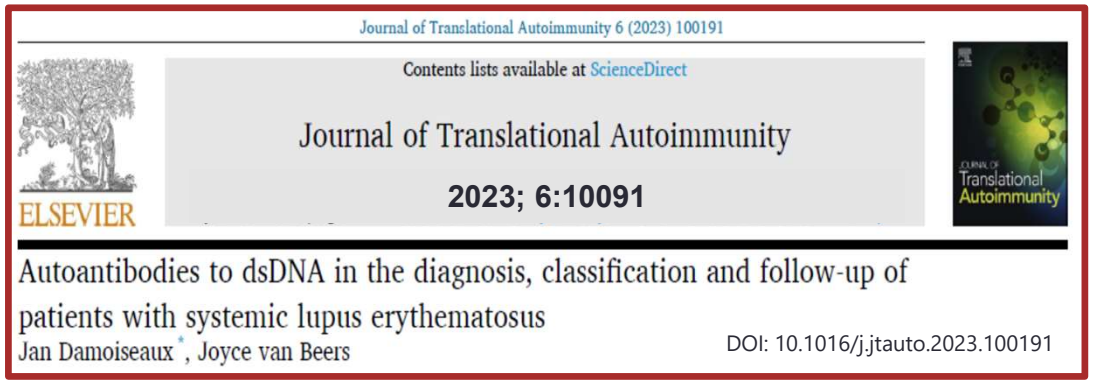
published: 15 May 2019

doi: 10.3389/fimmu.2019.01104

The dsDNA, Anti-dsDNA Antibody, and Lupus Nephritis: What We Agree on, What Must Be Done, and What the Best Strategy Forward Could Be

*Ole Petter Rekvig**

Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway



Journal of Translational Autoimmunity 6 (2023) 100191

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

2023; 6:10091

Autoantibodies to dsDNA in the diagnosis, classification and follow-up of patients with systemic lupus erythematosus

Jan Damoiseaux*, Joyce van Beers

DOI: 10.1016/j.jtauto.2023.100191

Review

The role of DNA in the pathogenesis of SLE: DNA as a molecular chameleon

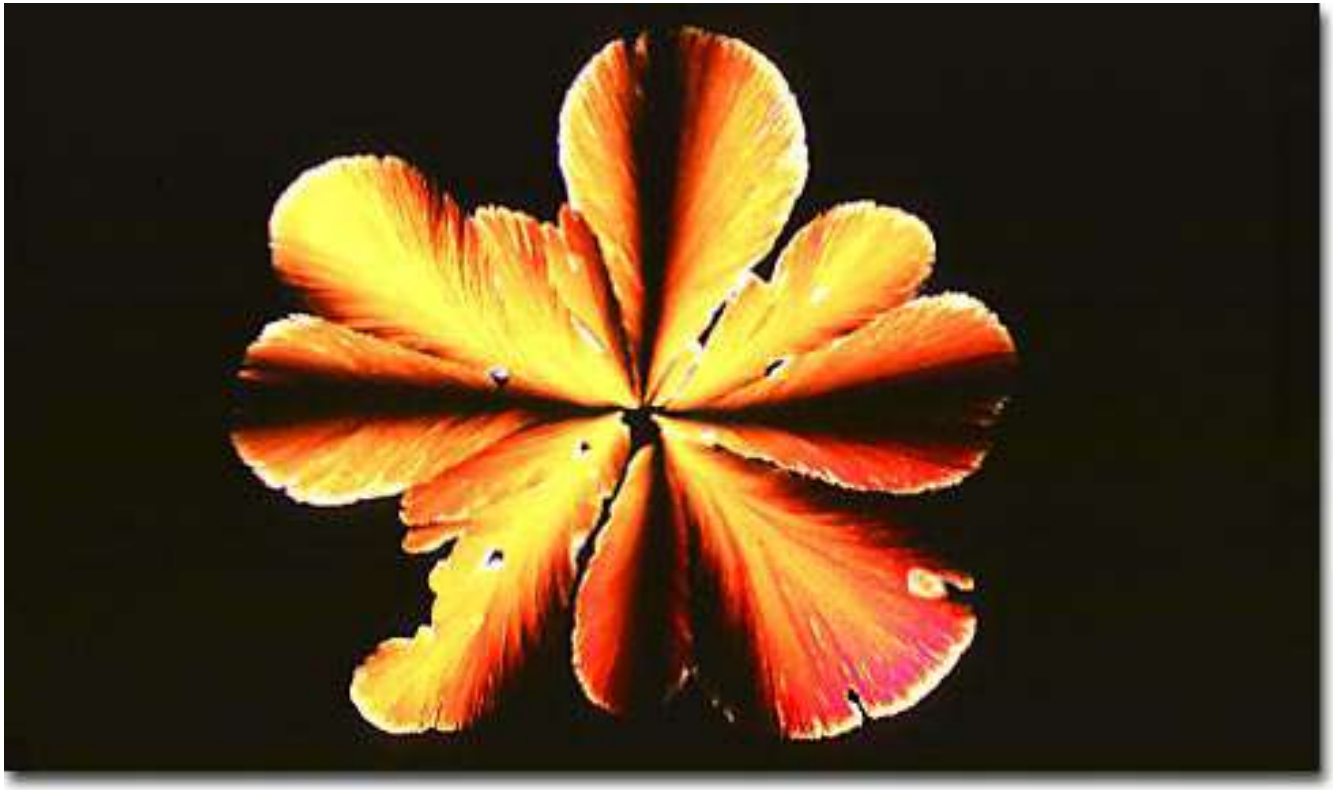
David S Pisetsky [1,2] Alan Herbert [3]

Ann Rheum Dis 2024; **0**:1–8.
doi:10.1136/ard-2023-225266

A Short History of DNA

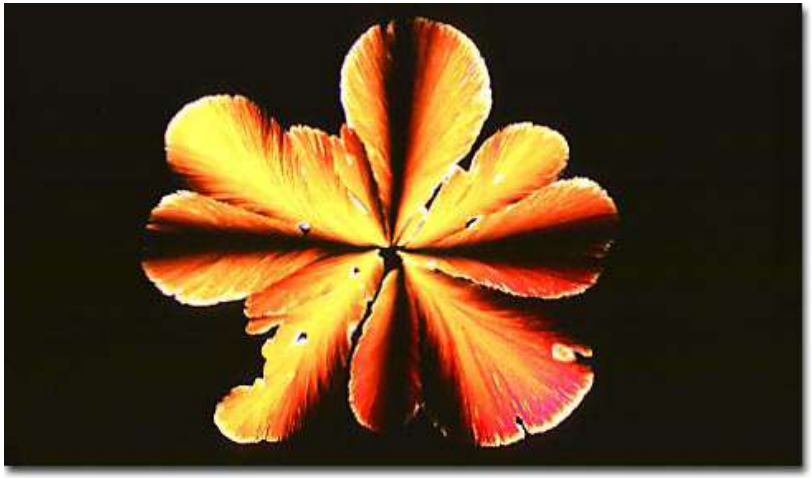
- **1860s-1920s Johann Friedrich Miescher**: identified **"nuclein“** as a substance in cell nuclei.
- **1944 Avery, MacLeod, and McCarty:** Identified DNA as substance responsible for bacterial heredity.
- **1952 Hershey & Chase** experiments confirmed that **DNA is not protein**, but the ‘genetic’ material.
- **1953 Watson and Crick:** proposed the double-helix model.
- **1957-58 Kornberg A et al:** DNA replication elucidated .
- **1960s Genetic Code and RNA:** The genetic code – DNA sequences translated into proteins deciphered. The role of RNA (mRNA, tRNA, rRNA) in protein synthesis became clearer.
- **1970s Recombinant DNA Technology** (Paul Berg) **:** Development of recombinant DNA technology allowed the manipulation and cloning of DNA leading to genetic engineering.
- **1990 – 2003 Human Genome Project:** Mapped and sequenced the human genome.
- **2000s – present:** Technological advancements: next-generation sequencing, accelerated DNA research.

Human DNA



- A single cell contains **~3 billion** DNA base pairs
- Human body **~30 trillion cells**
- Human Genome Project identified genes/exons expressing **~20,000 proteins**

Human DNA



- **Junk DNA:** short and long interspersed nuclear elements (LINEs and SINEs)
- February 2024 University of Toronto : **"Dark DNA" = 1 million "new" exons identified** "Researchers discover one million new components of the human genome" (medicalxpress.com)
  - Nicholas Stepankiw et al, The human genome contains over a million autonomous exons, *Genome Research* (2023). DOI: 10.1101/gr.277792.123

A Short History of Anti-DNA

- **1938-39**: Identification of anti-DNA induced by bacterial infections

  Rekvig O: Front Immunol 10: 1104, 2019

- **1957: Holman & Kunkel DNase of DNP eliminated the LE cell.**

  Science 126: 162-3, 1957

- **1966**: Tan, Schur, Carr et al. DNA and anti-DNA in SLE

  J Clin Invest 45: 1732-40, 1966

- **1967**: Koffler, Schur & Kunkel elute **anti-DNA** from SLE kidney.

  J Exp Med 126: 608-24, 1967

- **1982**: Anti-dsDNA included in ARA (ACR) Revised SLE Criteria

  Arthritis Rheum 25: 1271-7, 1982

- **1995**: Moens, et al. anti-dsDNA induced by DNA virus protein

  ***PNAS** 92:12393, 1995*

- **2000's**: Link to viruses and oncogenes: Numerous reports of anti-dsDNA in cancer

  Rekvig: Front Immunol 10: 1104, 2019

- **2006:** Link to increased Type I Interferon and eventually the IFN "signature"

  Arthritis Rheum 54:1906, 2006

- **2012**: anti-dsDNA included in SLICC criteria

  Arthritis Rheum 64: 2677, 2012

Clinical Applications Anti-dsDNA

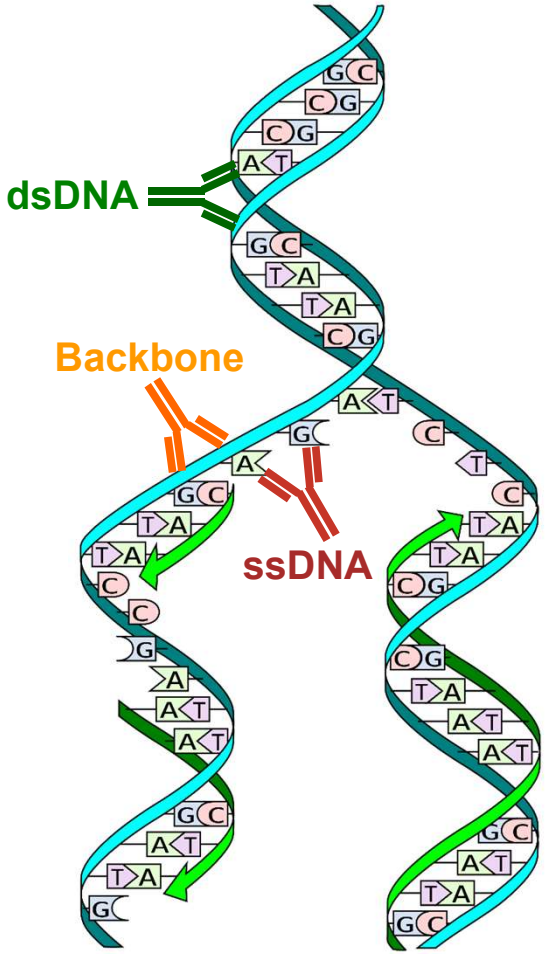
- **"Marker" autoantibody for Systemic Lupus Erythematosus (SLE)**
  - For most immunoassays: specificity >80%; sensitivity 40-75% (depends on cohort makeup)
- **A central and historic criterion for classification of SLE**
  - ACR (1982) – 11 criteria; must have 4 to be classified as having SLE
  - SLICC (2012) – 17 criteria; must have 4 and at least one clinical and one immunological
  - EULAR/ACR (2019) – **Anti-dsDNA has weighted score of 6** – 10 required for "definite" SLE
- **Linked to pathogenesis of SLE*: controversial**
- **Anti-dsDNA levels fluctuate with disease activity**
  - Anti-dsDNA levels used to indicate/predict SLE flare
  - **Limitations discussed later**
- **Anti-dsDNA/DNA immune complexes activate classical pathway complement**
  - Deposited or formed *in situ* in the glomerulus leading to inflammation and lupus nephritis
  - Decreased C3, C4, increased sC5b-9 (MAC).
- **BUT…** transient anti-dsDNA can occur in the context of an infection.
  A single positive test in time might not be "diagnostic.

* See Damoiseaux & van Beers. J. Translation. Immunol. 6:10091, 2023

The spectrum of anti-DNA targets in SLE

- Bases (purines, pyrimidines: AG CT)
  - **ssDNA**
- **Sugar-Phosphate backbone**
- Double helix
  - **dsDNA**
- Numerous other DNA conformations*
  - **"Bent" or "Kinked"**
  - **Elongated/linear**
  - ***Z* (left-handed)**
  - **"Triplex" – "I" motif**
  - Cruciform
  - DNA/RNA hybrids

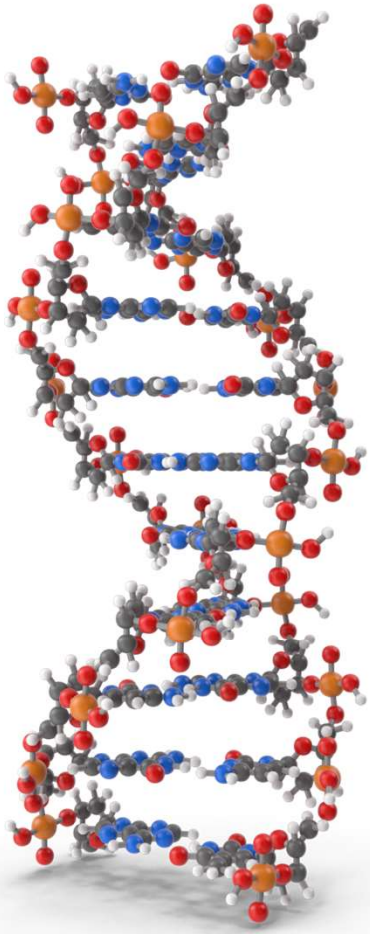
***THE** Reference: Stollar BD. Molecular analysis of anti-DNA antibodies. FASEB J 8:337, 1994



dsDNA: two main structural variants

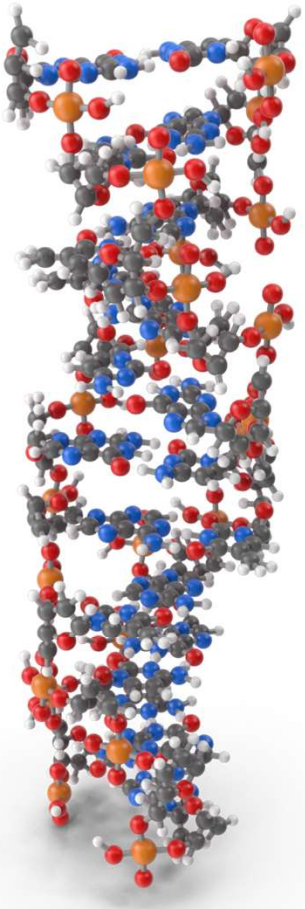
β DNA: right-handed

Immunogenic only under special circumstances



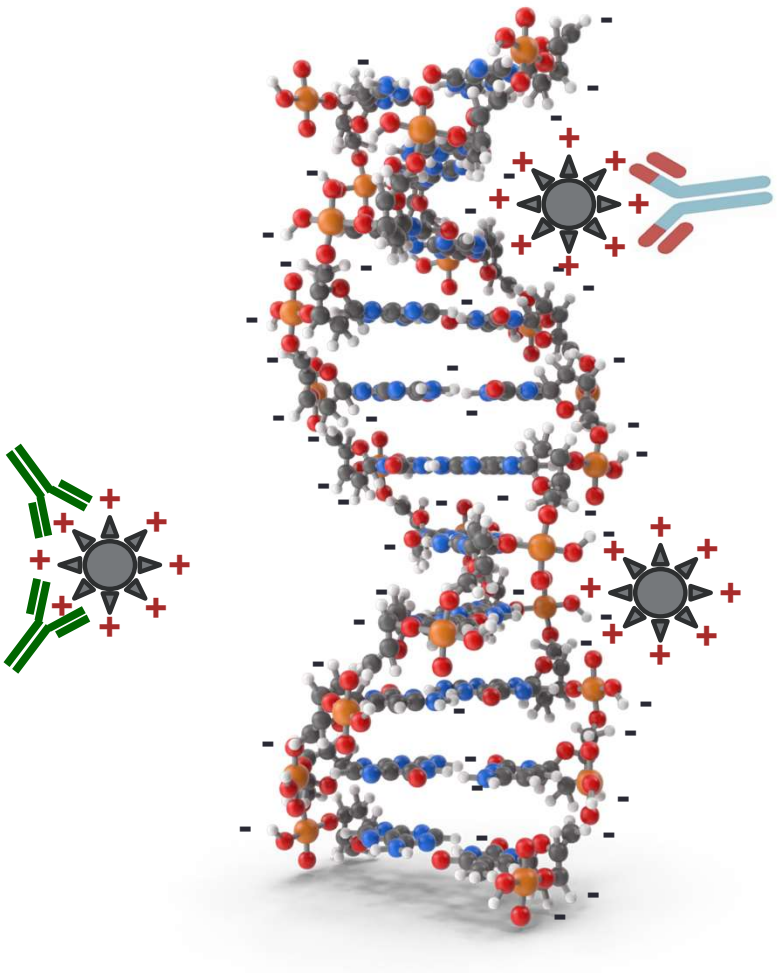
Z DNA: left-handed

Potent immunogen but tends to be unstable



Stollar, B.D. Why the difference between B-DNA and Z-DNA? Lupus **1997**, 6, 327–328.

dsDNA is highly anionic due to deoxyribose phosphate groups

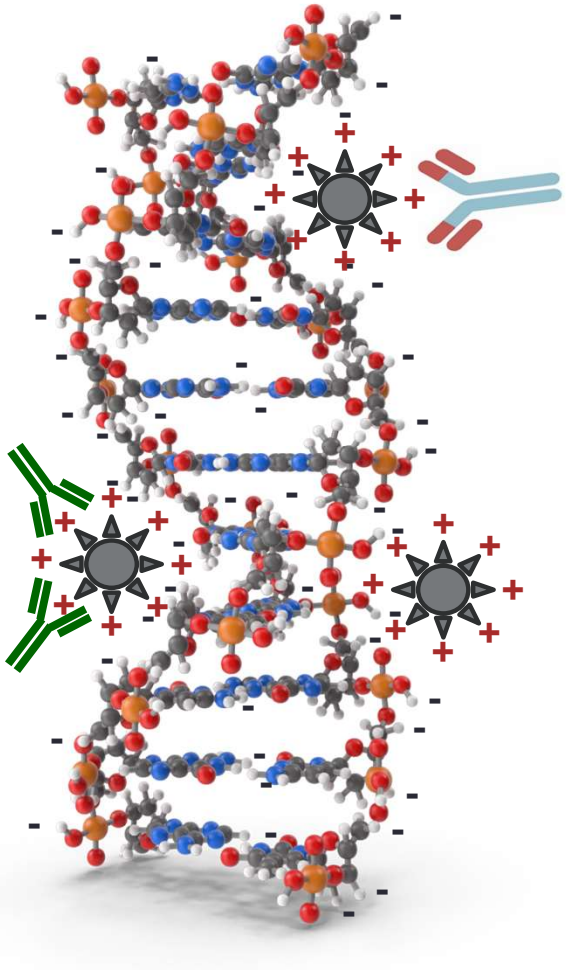


Numerous (cationic) proteins bind to DNA

- **Histones forming chromatin/nucleosomes**
- **C1q**
- **b2 glycoprotein 1**
- **Myeloperoxidase (MPO)**
- **Topoisomerase I (Scl-70)**
- Eosinophil cationic protein (ECP)
- Eosinophil peroxidase (EPX)
- Major basic proteins 1 & 2
- The Fab antibody binding site contains arginine residues
- MANY Others

- **Cationic Immune complexes bind DNA**

dsDNA is highly anionic due to deoxyribose phosphate groups



Numerous (cationic) protein ligands

- **Histones forming chromatin/nucleosomes**
- **C1q**
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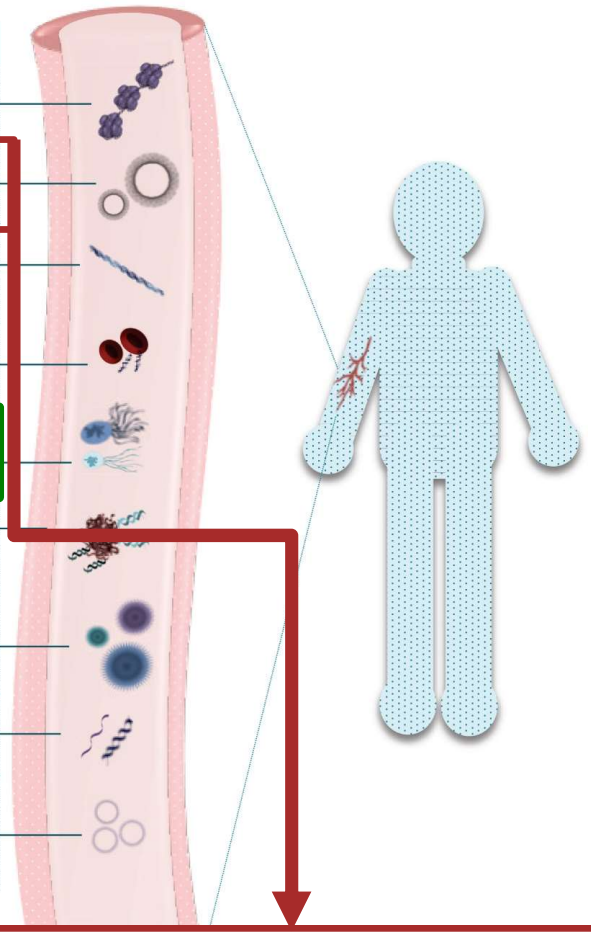
- **MPO (myeloperoxidase)**
- **Topoisomerase I (Scl-70)**
- Eosinophil cationic protein (ECP)
- Eosinophil peroxidase (EPX)
- Major basic proteins 1 & 2
- The Fab antibody binding site contains arginine residues
- MANY Others

Origins of dsDNA antigens driving B cell responses in SLE?

- DNA released from PMNs to extracellular biofilms.
- Complement opsonizes bacterial DNA to promote clearance.
- HMG box containing proteins like HU and IHF **from bacteria** and HMGB1 **from the host** promote the formation of B-DNA/Z-DNA junctions (BZj) that stabilize Z-DNA formation by NETs.

Pisetsky & Herbert. Ann Rheum Dis 2024

Cell-free DNA release mechanisms and types	
Apoptosis	• Nucleosomes
Necrosis Pyroptosis Autophagy Phagocytosis Mitotic catastrophe	• Mitochondrial DNA • Long cfDNA fragments
Erythroblast enucleation	• Erythroblast DNA
NETosis	• Neutrophil extracellular traps (NETs)
Macromolecular structures	• DNA-protein complexes • Virtosomes
Extracellular vesicles (EVs)	• Apoptotic bodies • Microvesicles • Exosomes
Various	• Single- and double stranded DNA
Micronucleation by chromosomal instability	• Extrachromosomal circular DNA • Other



Oxidized mitochondrial DNA released from stimulated PMN = DAMP
Frontiers Biosci.(Landmark.Ed). 22:1011, 2017.

Interest in Mitochondria in SLE

Nat Rev Rheumatol 2022 18 (11): 621-640, 2022

REVIEWS

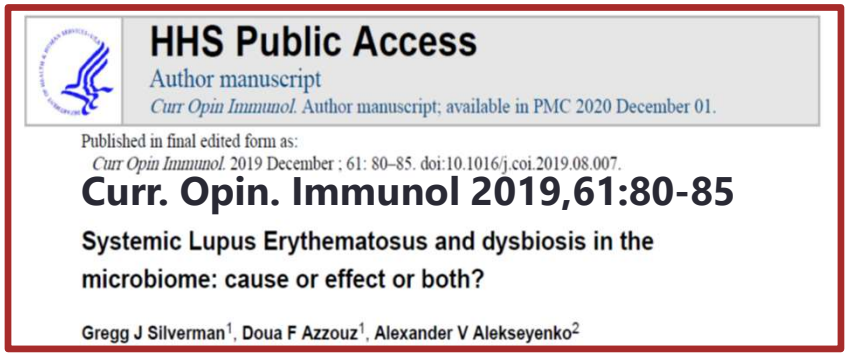
The role of mitochondria in rheumatic diseases

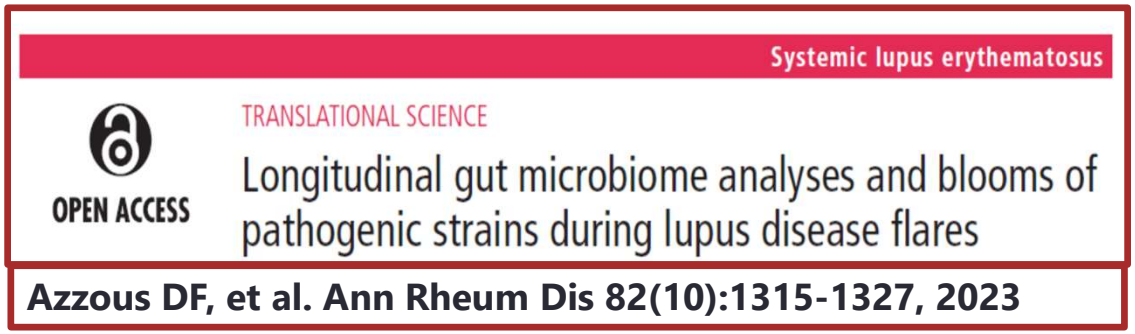
Yann L. C. Becker[1,2,3,6], *Bhargavi Duvvuri*[4,6], *Paul R. Fortin*[1,2,5], *Christian Lood*[4] ✉ *and Eric Boilard*[1,2,3] ✉

"Mitochondria are immunogenic, and anti- mitochondrial antibodies (for example, antibodies that target cardiolipin, mitofusin 1 (MFN1), **mitochondrial DNA or mitochondrial RNA) are commonly seen in…" (SLE)**.

Is the Gut Microbiome a Factor?

- Dysbiosis of the gut microbiome: segmented filamentous bacteria – ***Ruminococcus gnavus*** (an anaerobe associated with Crohn disease) predominated
- Transplantation of fecal samples from "lupus" mice into germ-free (GF) mice **induced significant levels of anti-dsDNA antibodies**. Ma Y, et al. Mol Med 2019 [PubMed: 31370803]
- Fecal transfer from SLE patients to germ-free mice caused lupus-like features, **including increased serum autoantibodies and 'imbalanced' immune system**.
  Ma Y, et al. Clin Immunol 2021 [DOI 10.1016/j.intimp.2020.106948]



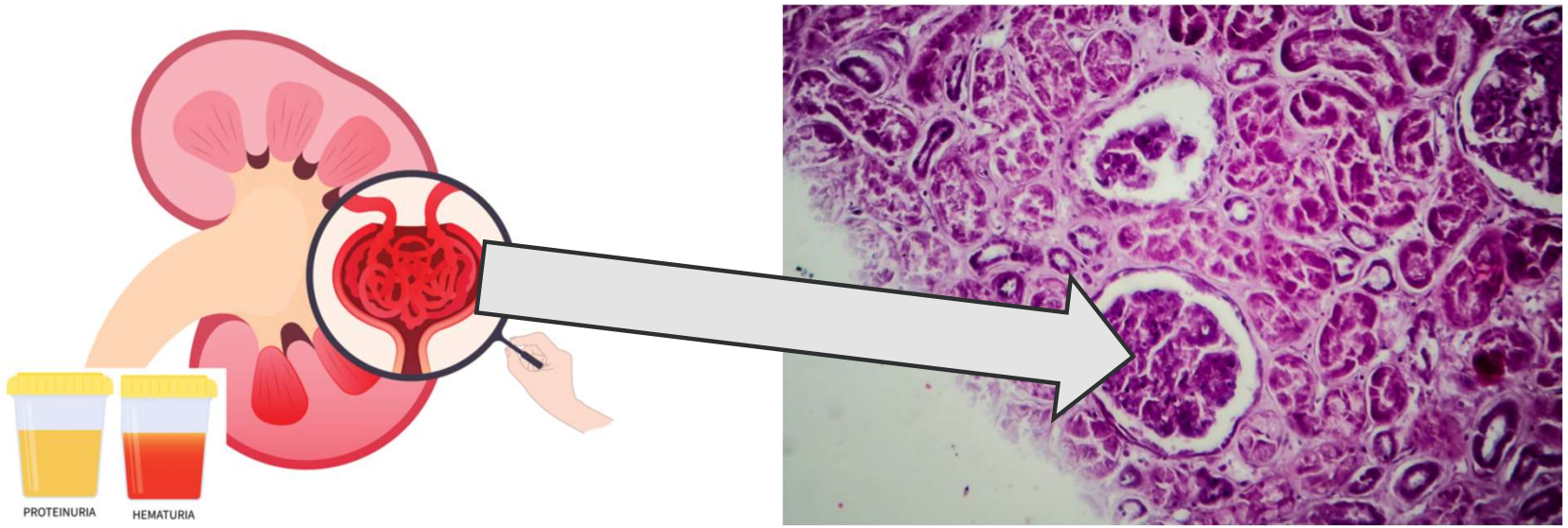


Azzous DF, et al. Ann Rheum Dis 82(10):1315-1327, 2023

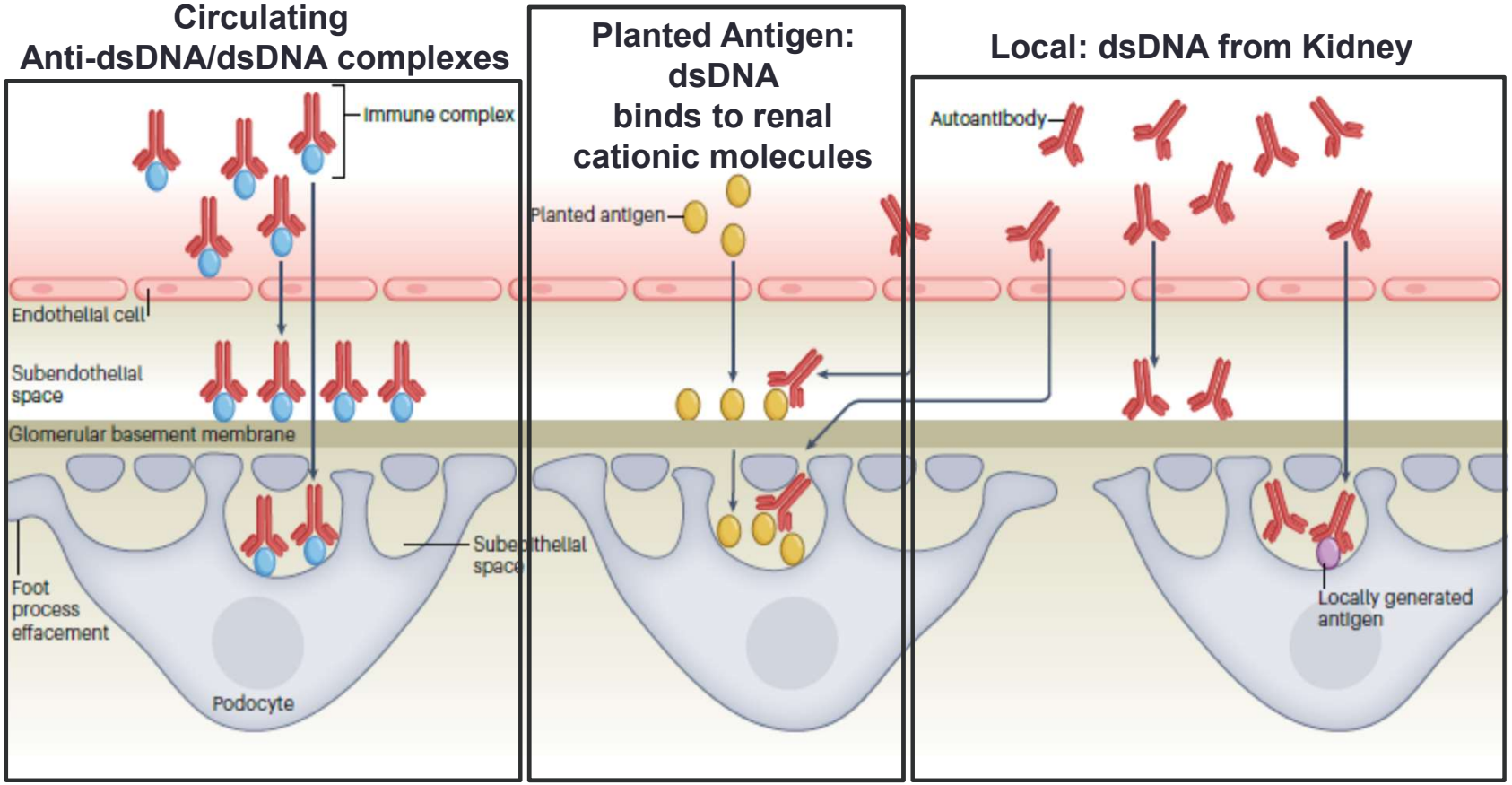
Key factors in induction and persistence of anti-dsDNA

- **Antigen (dsDNA) 'persistence'**
  - **Deficiency of DNase degradation**
    - Genetic deficiency of DNase(s)
    - Caspase-activated DNase deficiency: Arthritis Rheum 64(4): 1247-56, 2012
  - **Autoantibodies to DNase that block its activity:** recent focus on **DNaseIL3**
    - Autoimmunity 50(2): 125-132, 2017
    - J Exp Med 218(5): e20201138, 2021
    - Nat Commun 14(1): 1388, 2023
- In pediatric SLE (CARRA): 24% +ve associated with anti-dsDNA, -RibP, -Sm, –U1RNP
- **Limitations**
  - Does not explain appearance of autoantibodies that accompany anti-dsDNA
  - DNase does not degrade all chromatin (core histones need to be removed)
  - In our studies so far, there is no direct correlation of antibodies to DNaseIL3 with anti-dsDNA

Anti-dsDNA and Pathogenesis of Glomerulonephritis/'Lupus Nephritis'



Main Considerations: Pathogenesis of Glomerulonephritis



From: Pisetsky D. Pathogenesis of Autoimmune Disease DOI: 10.1038/s41581-023-00720-1

But: are anti-dsDNA actually pathogenic?

- Despite over 50 years of research and study, controversial and debatable.

frontiers in Immunology

HYPOTHESIS AND THEORY
published: 15 May 2019
doi: 10.3389/fimmu.2019.01104

The dsDNA, Anti-dsDNA Antibody, and Lupus Nephritis: What We Agree on, What Must Be Done, and What the Best Strategy Forward Could Be

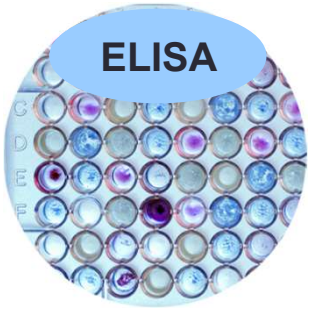
*Ole Petter Rekvig**

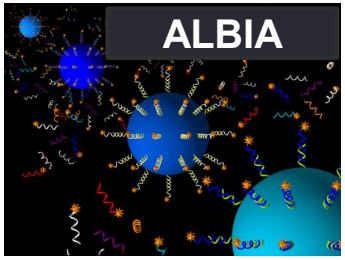
Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway

https://www.ncbi.nlm.nih.gov/pubmed/36469011
https://www.ncbi.nlm.nih.gov/pubmed/36896834
https://www.ncbi.nlm.nih.gov/pubmed/37543287
https://www.ncbi.nlm.nih.gov/pubmed/35797522

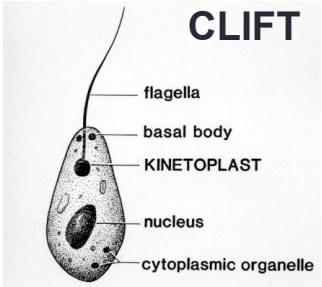
Contemporary Anti-dsDNA Immunoassays

- **ELISA** note: typically, dsDNA is bound to ***poly L-lysine*** coating the plate
- **Line assays:** Dot Blot + Lateral Flow
- **Fluorometric Enzyme Immunoassay (FEIA)**
- **Bead-based assays**
  - Addressable Laser Bead Immunoassay (ALBIA)
  - Chemiluminescence immunoassays (CIA)
  - Particle Based Multi-Analyte Technology (PMAT)
- **Indirect Immunofluorescence assays: Crithidia luciliae (CLIFT)**





CIA/PMAT

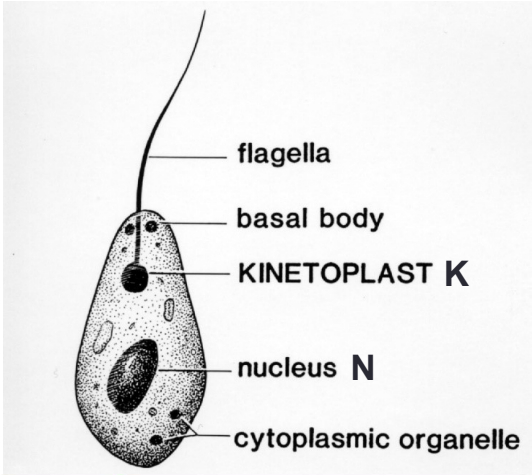


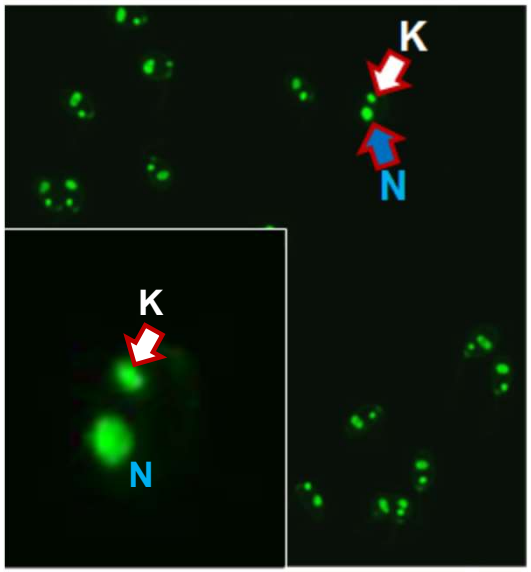
CLIFT : Comments

- SLE: High specificity (>90%);
  Low sensitivity <30%)
- ~1/150 +ve Kinetoplast staining — ANA negative
- **Kinetoplast is a modified mitochondrion**
  - Unique epitope of 'kinked' DNA
  - Concatenated DNA maxi- and mini-microcircles
    - Maxi encode Oxid Phosphor genes
    - Mini encode "guide" RNA: editosomes

Note:
60% PBC/AIH Overlap syndrome have +CLIFT*
Muratori, A. et al. *Am.J.Gastro. 104:1420, 2009.*

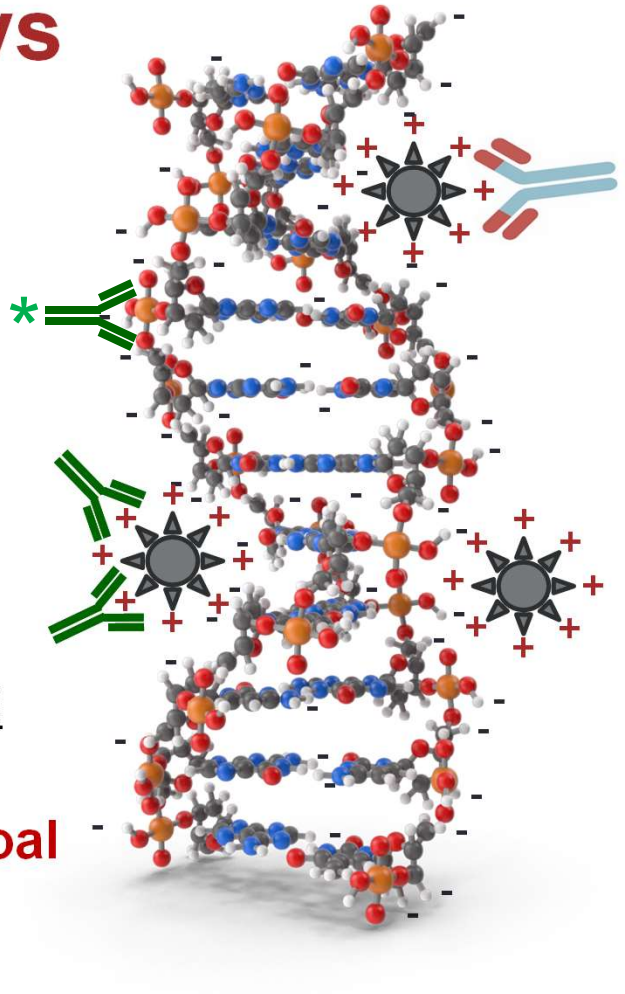
Nguyen, Swain, Norman, Fritzler replicated findings
***BUT not** anti-dsDNA +ve in other immunoassays.*
PLoS One 2018 Vol. 13 Issue 3 Pages e0193960





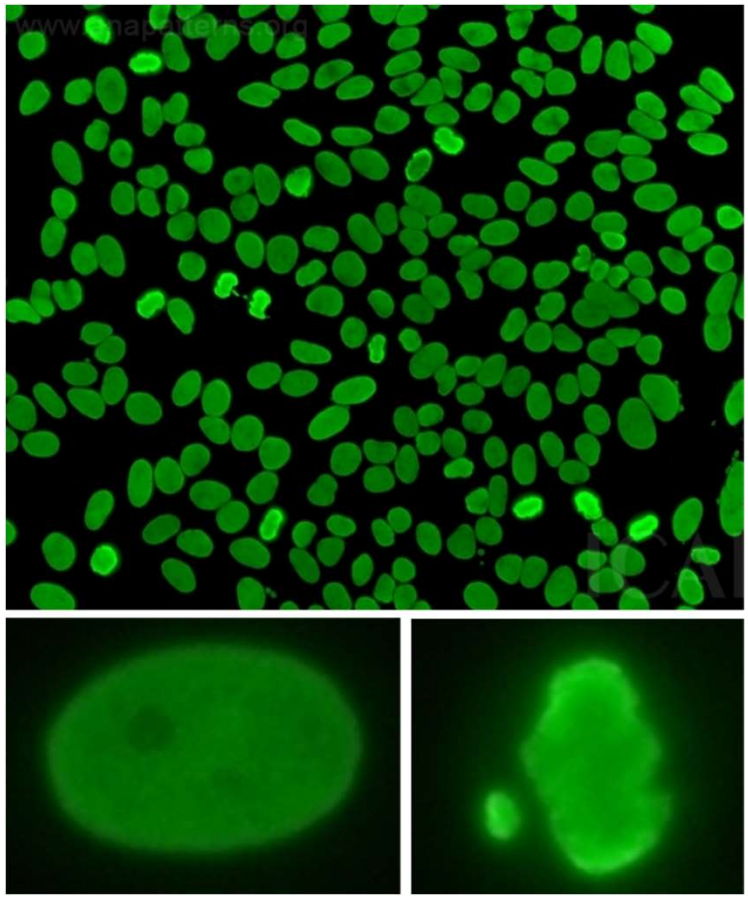
Considerations: anti-dsDNA Assays

- **Patient: unique** epitope, isotype, affinity, glycosylation
- **Source**: human/mammalian
  - Purified calf thymus or human cellular = “native”
  - Purified Synthetic: ~22-30 nucleotides; closed circular; “trade secrets”
- **Purity**
  - “Contaminating” ssDNA
  - **Secondary binding of cationic serum/plasma molecules.**
- Preferably detect **high avidity** antibodies
  - correlated with diagnosis and probability of renal involvement in SLE
- **Reference standard sera are available:**
  WHO and ASC/PSG harmonization of assays still a goal



Anti-dsDNA in an Inception Cohort of SLE

- **1,137 SLICC patients enrolled within 6 months of diagnosis**
- 66.4% anti-dsDNA positive by chemiluminescence (conventional cut-off)
- ~50% anti-dsDNA+ using SLICC-12 cut-off
- Anti-dsDNA correlated with SLEDAI or renal disease?
  - 31.6% had evidence of renal disease at first visit
- Follow-up longitudinal data – DOI: 10.1136/annrheumdis-2022-222168
- Anti-dsDNA **not always associated** with homogenous (AC-1) HEp-2 IFA pattern.



Choi MY, et al. Lupus 2017 DOI: 10.1177/0961203317692437

Anti-dsDNA in 717 SLICC patients at Enrollment*

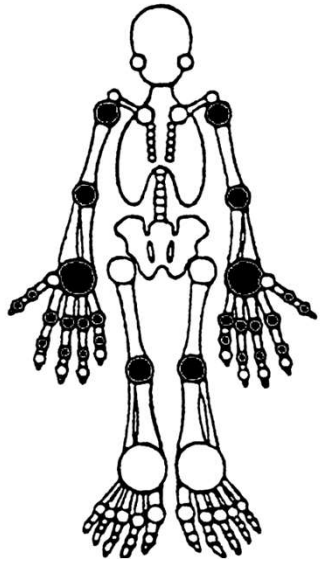
- **524/717 (73.1%) anti-dsDNA +ve**
- 73 (10.2%) monospecific dsDNA+ (ENA negative)
- 37 (5.2%) monospecific dsDNA+ if **ENA and APLA** negative
- Of the 524 anti-dsDNA +ve
  - **46.8% (245) dsDNA+ had anti- SSA/Ro60+**
  - **41.2% (216) dsDNA + had anti-histone**
  - **40.6% (213) dsDNA + had anti-Ro52/TRIM21**
  - **33.8% (177) dsDNA+ had anti-Ribo P**
  - **30.9% (162) dsDNA+ had anti-Sm/RNP**

  - **Data does not remarkably change at 1 year after enrollment**

* Extracted from SLICC Dataset:
Choi MY, et al. Ann Rheum Dis 81(8): 1143-1150, 2022

ANA Negative — anti-dsDNA Positive = "false positive"?

- A 69-year-old female presented with a 1-month history of symmetric polyarthritis, pleuritis and 10 kg weight loss
- Initial laboratory analysis:
  - Lymphopenia 0.3 x 10^9/L
  - Elevated ESR 48mm/hr, CRP 93.0mg/mL
  - HEp-2 IFA ANA was **NEGATIVE**
  - **But…High titer anti-dsDNA on Crithidia (CLIFT) and BioFlash Chemiluminescence Assay (CIA)**



- **ANA negative: does not meet EULAR/ACR Criteria for SLE**
- **Is this a False +ve anti-dsDNA or False –ve ANA?**

Case Courtesy Drs. M.Y. Choi & H. Arbillaga, University of Calgary

Positive dsDNA but “negative” ANA?

Antibody Profile	ANA HEp-2 IFA		dsDNA			ENA		Chromatin	C1q	CCP	RF	SSc Panel
Assay (Units)	Inova (1/80)	EUROIMM (1/80)	CIA (IU/mL)	Quanta LITE (AU)	Crithidia luciliae (dil 1:20)	MagPix (CU)	Euroline (DU)	ELISA (Inova)	ELISA (Inova)	ELISA (Inova)	(kU/L)	Euroline LIA
Result	Cytoplasmic speckled 1/160	Negative	Positive (667)	Positive (399.2)	Positive (1/320)	dsDNA (419)	dsDNA (28)	Negative	Negative	Negative	Negative	Negative

“Negative” ANA on 3 different HEp-2 slides: but positive speckled cytoplasmic ACA (AC-19) on one.

Highly positive dsDNA on multiple assays

How common is ANA –ve / dsDNA +ve?

- **<1%:** MDx lab audit of 500 anti-dsDNA results

- 1137 SLICC Adult SLE Enrollment Baseline
  - 6.7% ANA negative
  - **0** of ANA negative were anti-dsDNA +ve
    BUT
- 258 CARRA Pediatric SLE Study (Dr. Peter Nigrovic: Harvard)
  - **2.3%:** 6 anti-dsDNA + ANA negative (AC-0)

Anti-dsDNA on Metaphase Chromosomes

Remove histones and other chromatin proteins with 0.1N HCl

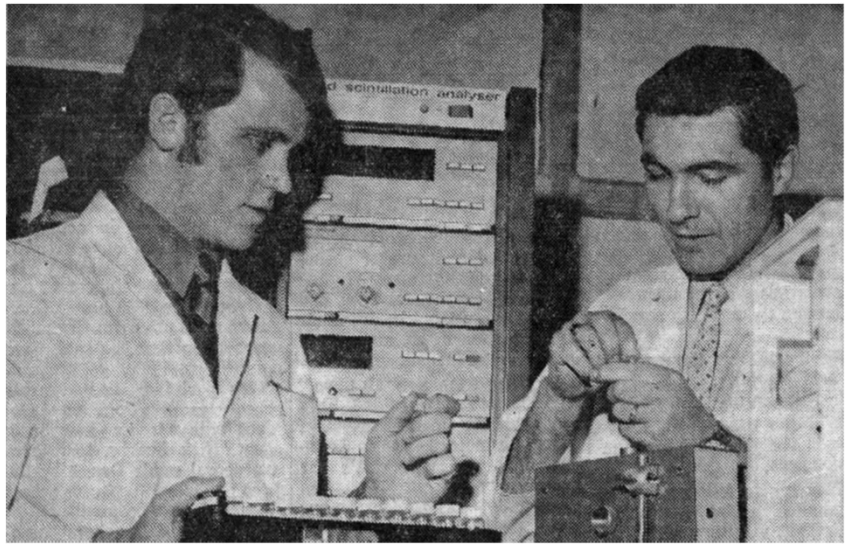
LOCALIZATION OF ANTI-NUCLEAR ANTIBODIES ON HUMAN METAPHASE CHROMOSOMES

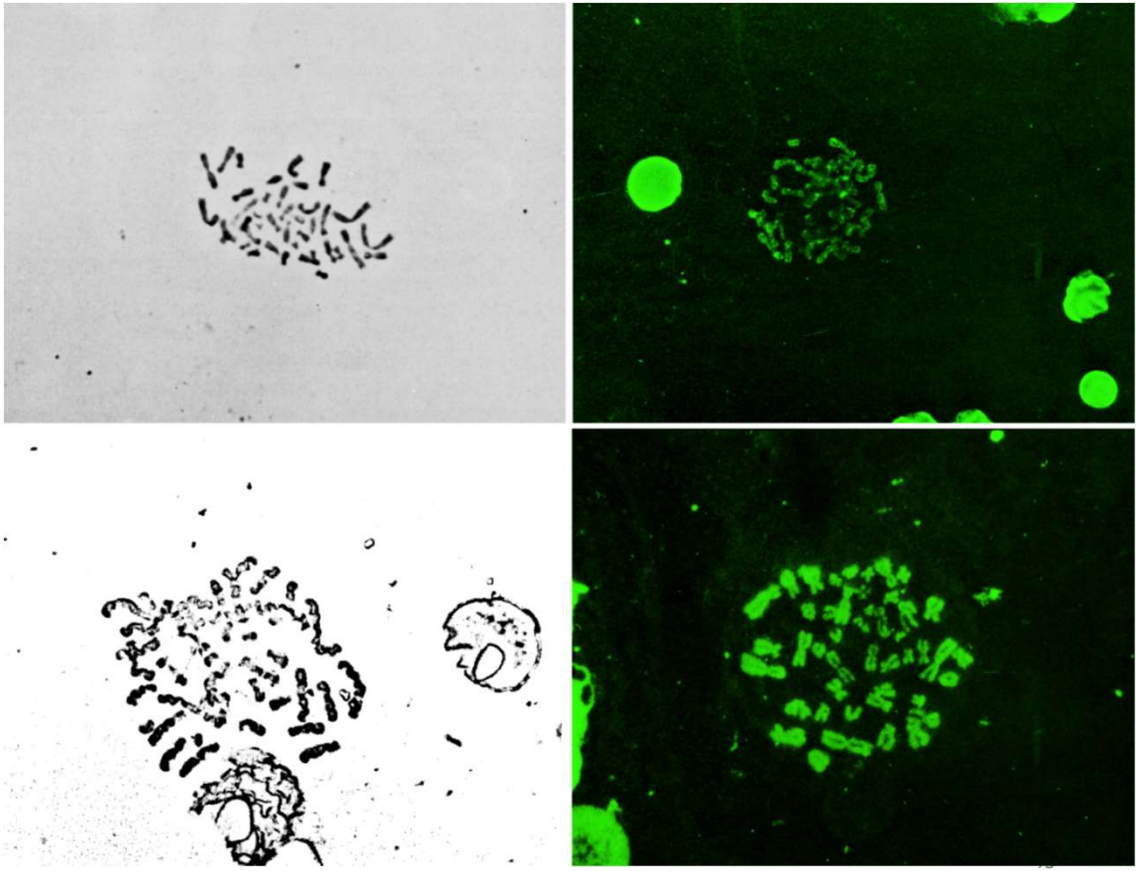
M.J. FRITZLER*, J.I. WATSON and R.B. CHURCH*

Divisions of Medical Biochemistry and Medicine, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4, Canada*

Journal of Immunological Methods 5 (1974) 21–31 © North-Holland Publishing Company

Received 2 October 1973 Accepted 28 December 1973



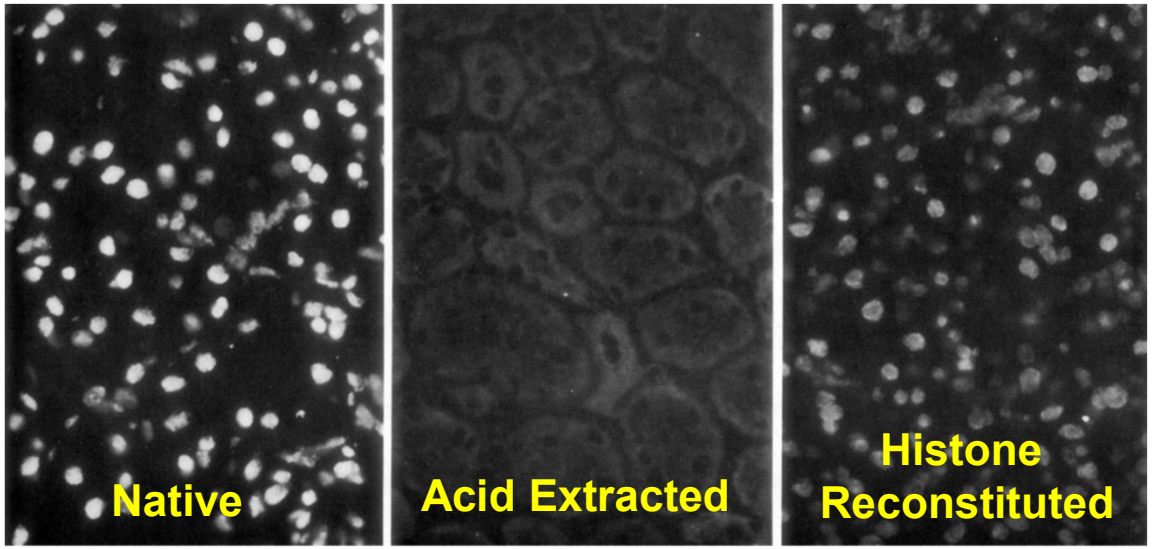


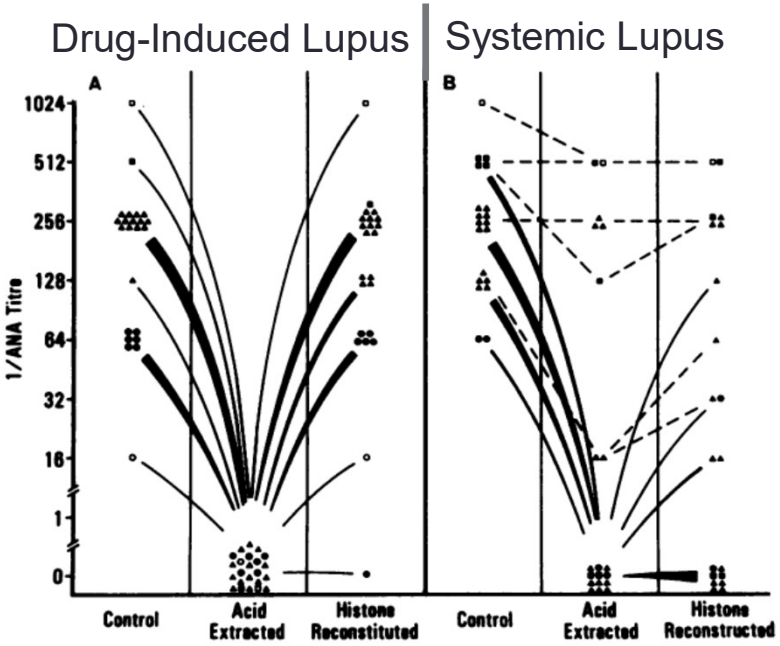
Drug-Induced Lupus (DIL)

Antibodies to Histones in Drug-Induced and Idiopathic Lupus Erythematosus

M. J. FRITZLER and E. M. TAN, *Division of Rheumatic Diseases, University of Colorado Medical Center, Denver, Colorado 80262*

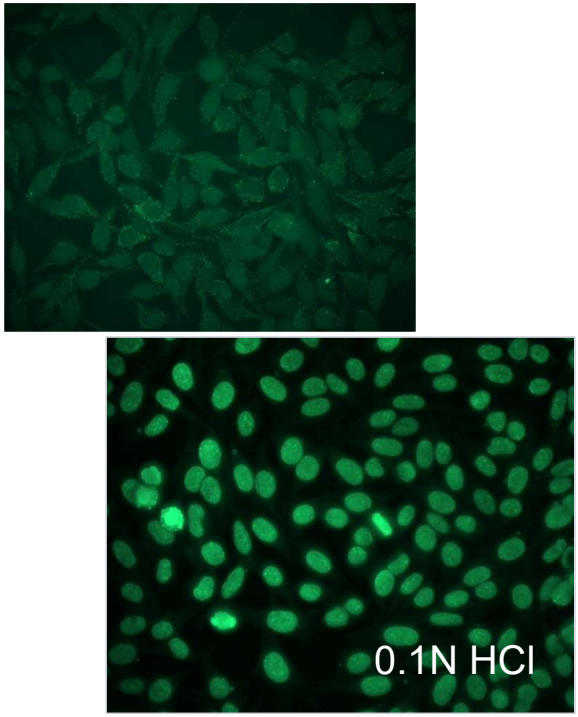
J Clin Invest 62 (3): 560-7, 1978



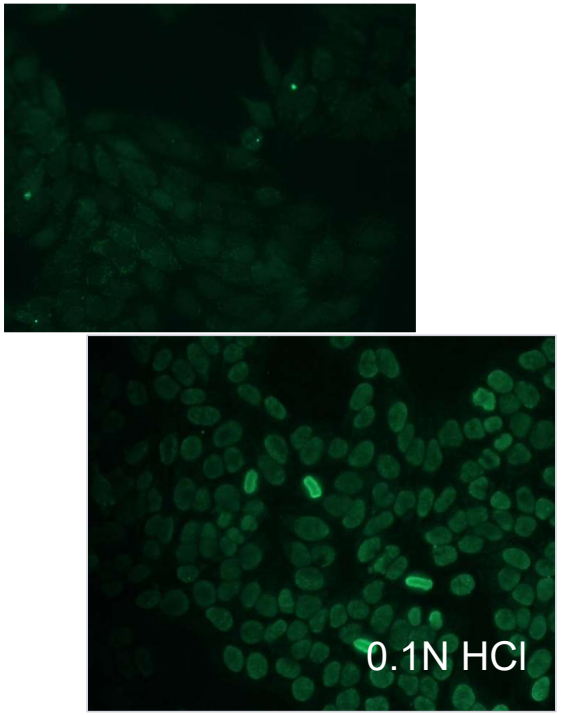


Nuclear homogenous IFA appears after 0.1N HCl protein extraction

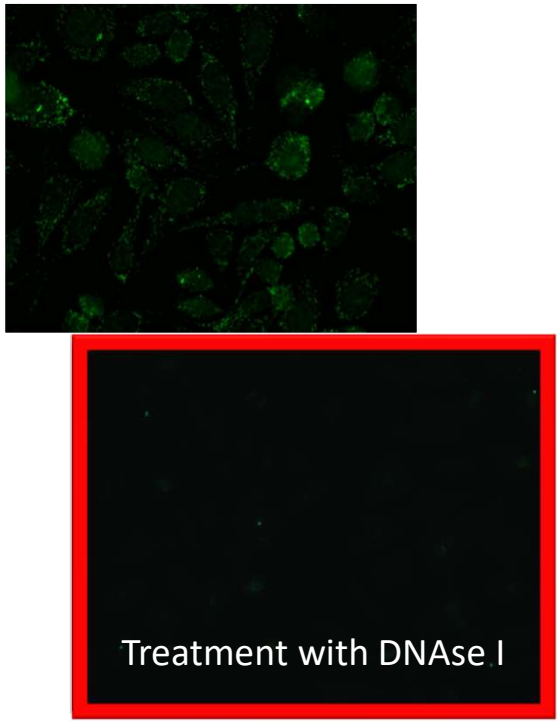
HEp-2 #1



HEp-2 #2



HEp-2

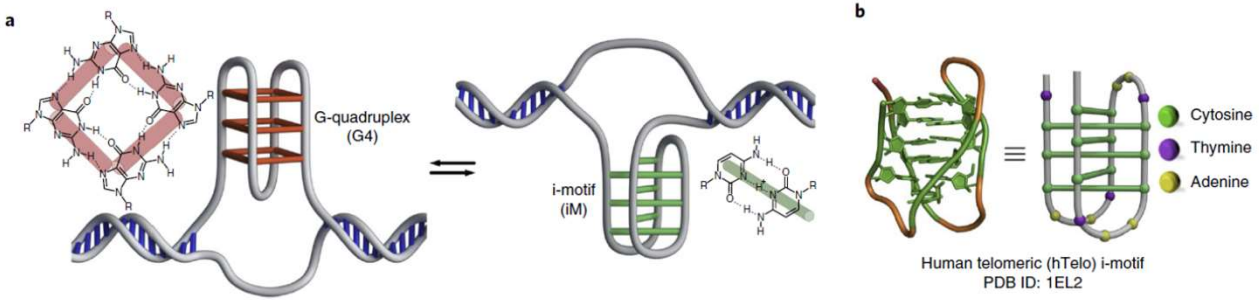


nature chemistry
ARTICLES
https://doi.org/10.1038/s41557-018-0046-3

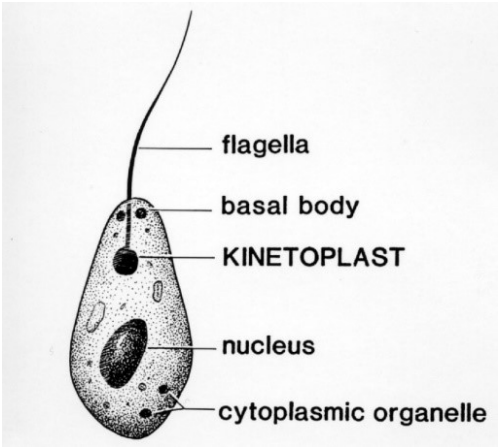
I-motif DNA structures are formed in the nuclei of human cells

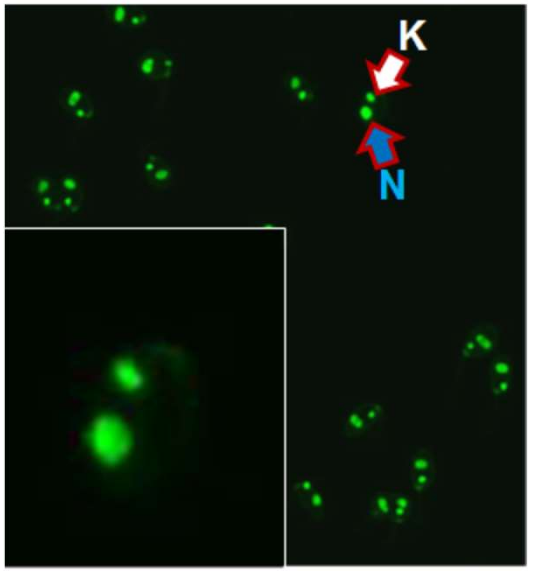
Mahdi Zeraati[1,2], David B. Langley[1], Peter Schofield[1], Aaron L. Moye[3], Romain Rouet[1], William E. Hughes[1,2], Tracy M. Bryan[3], Marcel E. Dinger[1,2*] and Daniel Christ[1,2*]

- **"4-stranded" DNA**



- Found in:
  - Telomeres (NOR-90/hUBF and other telomere autoantigens TERF2) see: https://www.ncbi.nlm.nih.gov/pubmed/36634459.
  - Centromeres
  - Promotor regions of oncogenes
  - **Areas of "Molecular Crowding" (think Crithidia kinetoplast)**





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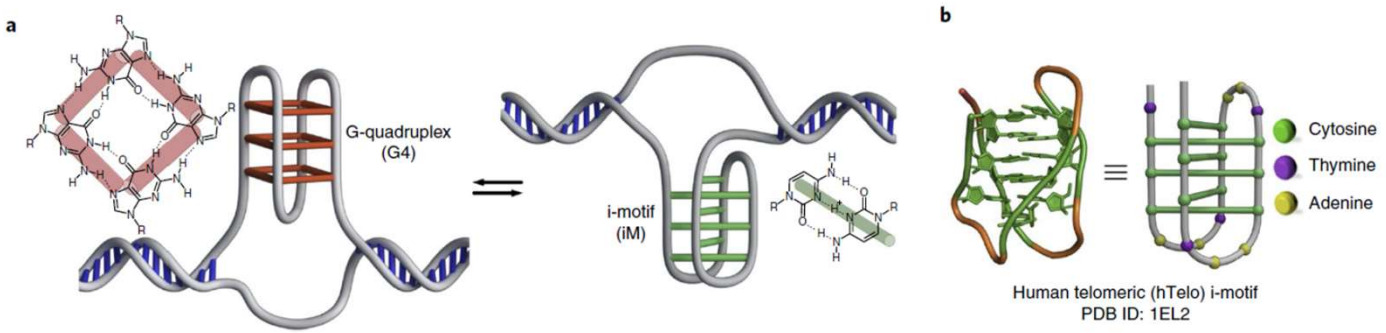
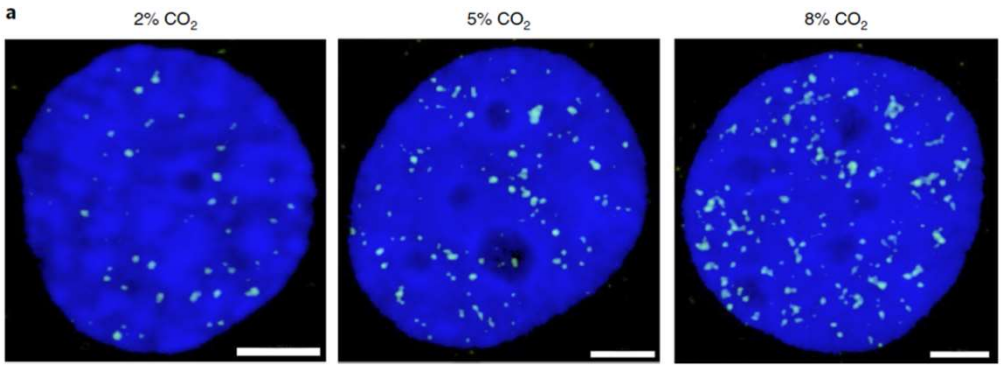
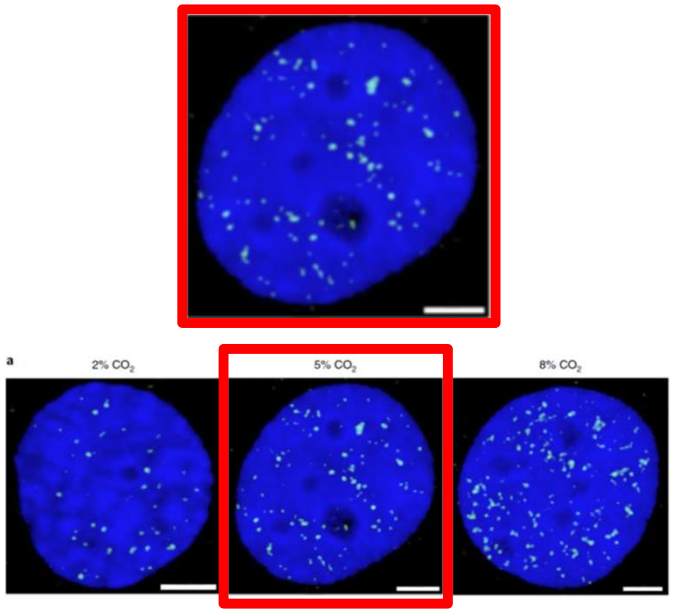
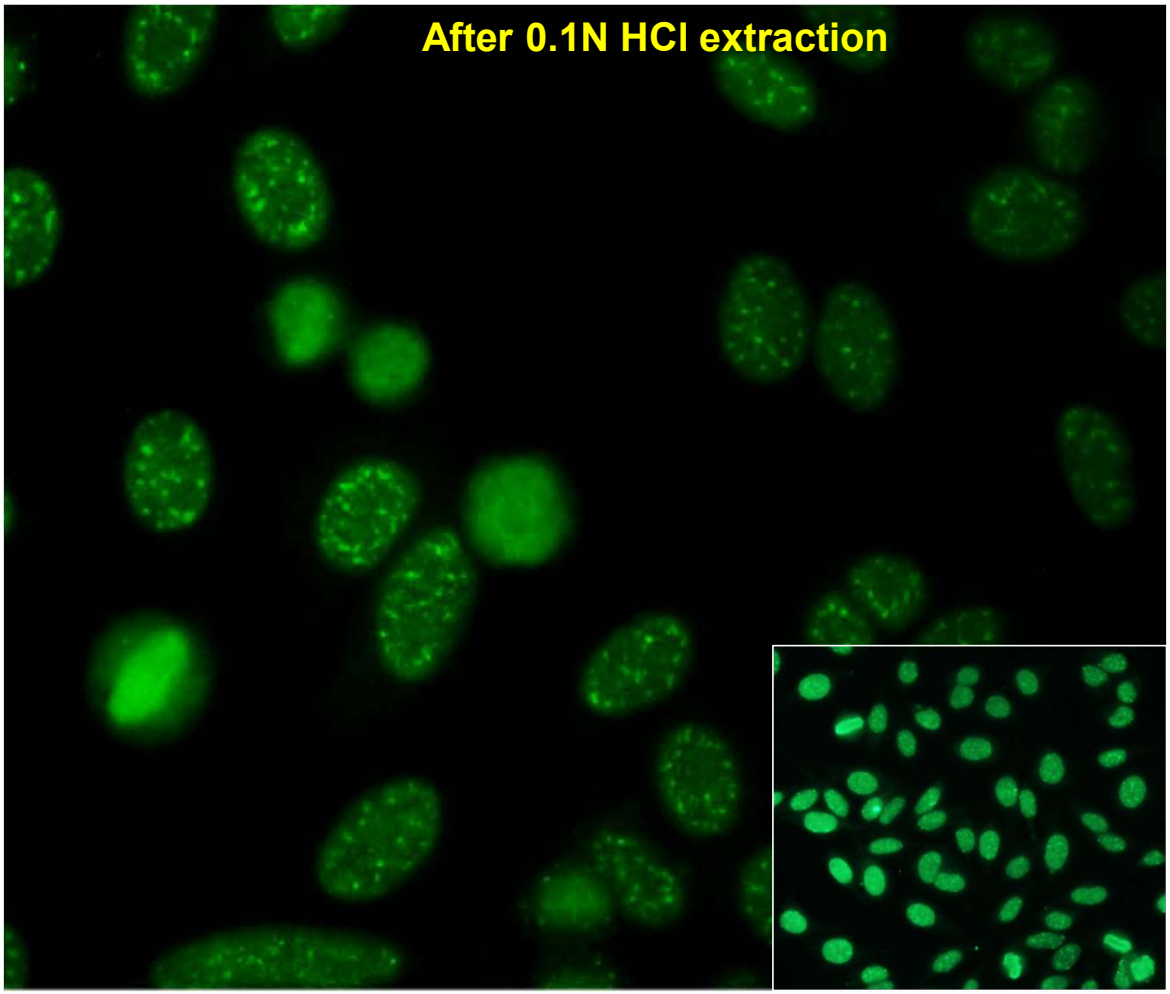




ANA –ve / dsDNA+ve





Anti-DNA: Summary and Future Considerations

Summary: Anti-dsDNA Antibodies

- Anti-dsDNA antibodies part of SLE criteria for over 45 years!!
  - Are current SLE classification criteria appropriate for a clinically heterogeneous disease?
- Despite over half a century of anti-DNA research no “gold standard” or harmonized anti-dsDNA immunoassays
- Monitoring SLE disease activity or prediction of clinical relapses/remissions using the level of anti-dsDNA antibodies has challenges.
  - Current approved assays are not marketed for this purpose.
  - No universal measure of flares: SLEDAI-2K or BILAG not particularly useful
- Anti-dsDNA antibodies are best interpreted in conjunction with anti-nucleosome and anti-C1q antibodies and components of the complement pathway (sC5b-9).

The Bigger anti-DNA Picture

“There is nothing quite so deceptive as an obvious fact”. Arthur Conan Doyle

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  - Dr. Jenny Walker — University of Adelaide
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- Dr. Yves Troyanov — University of Montréal
- Dr. Jean-Luc Senécal — University of Montréal
- Dr. Michael Mahler — Werfen, San Diego
- Dr. Hani El Gabalawy — University of Manitoba
- >50 graduate students, fellows and post-docs
- Dr. Marinka Twilt — Alberta Children's Hospital
- Dr. Heinrike Schmeling — Alberta Children's Hospital
- Dr. Rae Yeung — Hospital for Sick Children, Toronto
- Dr. Alan Rosenberg — University of Saskatchewan
- Dr. Anne Stevens — Johnson & Johnson/CARRA
- Dr. Kathryn Torok — University of Pittsburgh/CARRA
- Dr. Peter Nigrovic — Harvard University/CARRA

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