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PEARLS OF LABORATORY MEDICINE

Antinuclear Antibody Testing

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CCTC Clinical Chemistry Trainee Council

Objectives

1. Define antinuclear antibodies (ANA).
2. Name common clinical disease states associated with ANA positivity.
3. Discuss ANA testing techniques, with emphasis on gold standard immunofluorescence testing.
4. Recall current clinical recommendations for ANA testing & reporting practices.

Define Antinuclear Antibodies (ANA)

Autoimmune antibodies that bind nuclear components[1,2]

- Double-stranded DNA
- Small nuclear ribonucleoproteins (eg, SS-A/Ro, SS-B/La, RNP, Smith antigen)
- Enzymes (eg, topoisomerase/Scl70)
- Histone proteins
- Centromeric proteins

>150 epitopes identified to-date[1]

Applying ANA to Clinical Diagnosis

Disease	ANA Positive in:	ANA Has Utility For:
Systemic Lupus Erythematosus/SLE	95 – 100%	Diagnosis (very useful)
Systemic sclerosis/scleroderma/SSc	60 – 80%	
Sjögren syndrome/SjS	40-70%	Diagnosis (somewhat useful)
Dermatomyositis, Polymyositis	30 – 80%	
Juvenile rheumatoid arthritis	20 – 50%	Monitoring, prognosis
Raynaud phenomenon	20 – 60%	
Drug-induced SLE	~100%	Diagnosis (part of criteria)
Autoimmune hepatitis	~100%	
Mixed Connective Tissue Disease/MCTD	~100%	

Adapted from: Kavanaugh et al. *Arch Pathol Lab Med* 2000;124:71-81 with permission.

Indirect ImmunoFluorescence Assay (IIFA)

ANA bind epitopes in the substrate → ANA bound with a secondary, fluorophore-labeled antibody → nuclear fluorescence if ANA present[2]

- Preferred substrate – Human Epithelial-2 cells
- Serial titers in1:2 increments, starting at 1:40 or 1:80
- [Check laboratory guidelines – setting a maximal dilution may be required.]

Efforts to Standardize IIFA Reporting[3-5]

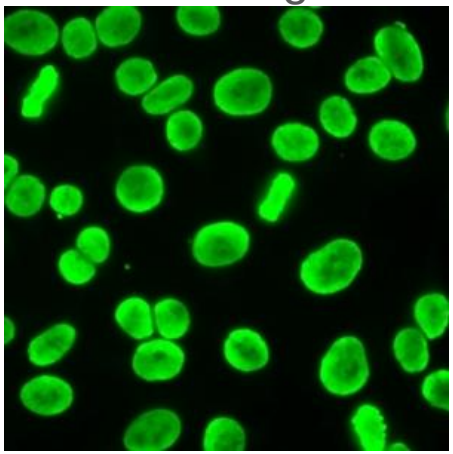
International Consensus on ANA Pattern, aka, ICAP

- Goal: standardize HEp-2 ANA reporting practice
- Consensus document, 2016[3]
  - 28 initial Anti-Cell/AC patterns
  - Nuclear, cytoplasmic, and mitotic categories
  - Organized by category, pattern, and level of training/expertise
- Regarding negative & unidentified patterns, 2018[4]; AC-0 (Negative) added
- AC-29 (DNA Topoisomerase I) added, 2018[5]

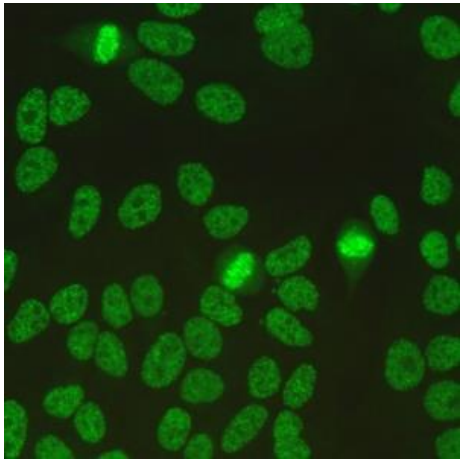
See References & www.anapatterns.org for more information.

Examples of ICAP-Classified IIFA Staining Patterns

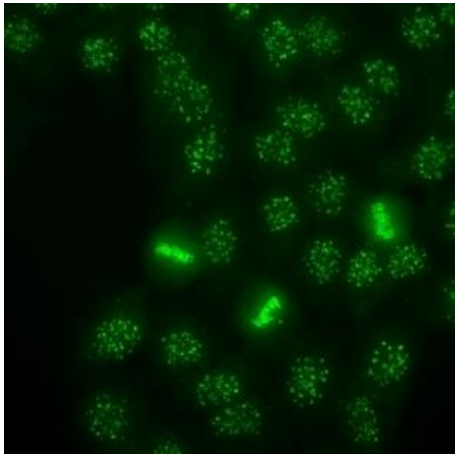
AC-1: Homogeneous



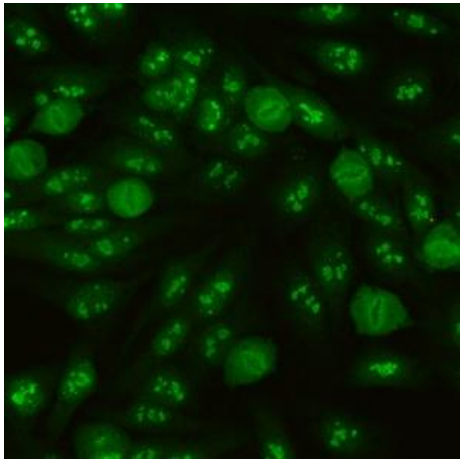
AC-2: Nuclear Dense Fine Speckled



AC-3: Centromere



AC-9: Clumpy Nucleolar



Images from www.anapatterns.org, with permission from Dr. Edward Chan.

Examples: Linking Pattern, Antigen, Disease with ICAP Classifications

Classification	Antigen Association(s)	Disease(s)
AC-1/Homogeneous	dsDNA, nucleosomes, histones	SLE, drug-induced lupus, juvenile idiopathic arthritis
AC-2/Nuclear Dense Fine Speckled	DFS70/LEDGF	(Rare) SjS, SSc, SLE
AC-3/Centromere	CENP-A/B (C)	Cutaneous SSc, PBC
AC-4/Nuclear Fine Speckled	SS-A/Ro, SS-B/La, Mi-2, TIF1γ, TIF1β, Ku	SjS, SLE, dermatomyositis
AC-5/Nuclear Large/Coarse Speckled	hnRNP, U1RNP, Sm, RNA Pol III	MCTD, SLE, SSc

PBC, primary biliary cirrhosis

Benefits & Limitations of IIFA Testing

Benefits

- Patterns correspond to disease states
- Sensitive

Limitations

- Specificity can be low, particularly at low titers
- Batched, manual preparation common
- Automation now available, but expensive
- Dark room/space & technical expertise needed

Enzyme Immunoassays (EIA) for ANA

Direct: epitopes on solid phase capture ANA in specimen → enzyme-conjugated detection antibody binds ANA → signal generated → colorimetric detection

- Semi-quantitative result vs index-based cutoff
- Qualitative interpretation – Positive, Equivocal, Negative

ANA screening by EIA

- HEp-2 cellular or nuclear homogenate coats the wells

ANA sub-serology testing by EIA

- Purified antigen in wells(eg, SS-A/Ro, SS-B/La, dsDNA…)
- Commonly used as second-level tests after initial ANA result is positive

Benefits & Limitations of EIA Testing

Benefits

- Automatable
- Relatively inexpensive
- High-capacity
- Very sensitive

Limitations

- Widely variable clinical performance
- No pattern given (if screening)
- Best when automated (expensive)

Multiplexed Immunoassay (MIA) for ANA

Direct, but in a liquid, bead-based phase

- Detection usually fluorescence-based
- Simultaneous testing for 10-12 most common ANA antigens (eg, dsDNA, SS-A, SS-B, Sm, RNP…)
- Semi-quantitative signal vs index-based cutoff
- Qualitative interpretation: Positive, Equivocal, Negative

Benefits & Limitations of MIA Testing

Benefits

- Rapid
- Automated
- Random-access
- Ability to report specific antigens/epitopes targeted
- Well-suited for basic sub-serology testing

Limitations

- Limited epitopes represented → limits clinical performance as first-level test
- Testing systems can be very expensive

Many Testing Options – (Still) One Clinical Gold Standard

American College of Rheumatology's (ACR's) Position Statement (2015)[1, 6]

- IIFA remains the gold standard for ANA testing
- Clinical performance data needed for locally-used methods
- Data available on-request
- Non-IIFA methods used for ANA detection must be demonstrably equivalent or superior to IIFA in terms of **sensitivity**

ALSO:

- Call for standardized methodology and/or reporting
- Method used stated in the result report

Conclusion/Summary

ANA testing supports Rheumatologists and other medical specialists in their efforts to diagnose, monitor, and predict outcomes for an array of disease states, most of which are connective tissue disorders.

Though there are many approaches to ANA testing, the indirect immunofluorescence assay (IIFA) remains the gold standard.

Efforts to standardize reporting for ANA testing by IIFA are driven by ICAP.

Laboratories need to generate and provide locally-sourced clinical performance data for ANA testing methods used.

References

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Thanks to Dr. Edward Chan at UFL for granting permission to use representative images of common patterns from https://www.anapatterns.org. [Accessed 21 September 2018]

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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