



Resident's Page

Anti-nuclear antibodies: A practical approach to testing and interpretation

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ABSTRACT

Anti-nuclear antibodies (ANAs) are a group of antibodies that are characteristically associated with connective tissue diseases (CTDs). Indirect immunofluorescence antibody technique, having a high sensitivity, is the most common technique used for detection, results of which are expressed in terms of the pattern of fluorescence, substrate used, and the titer of a positive test. Other methods include solid-phase assays. ANA test must be performed only when there is a clinical suspicion of an autoimmune CTD. ANA should not be used as a screening tool for asymptomatic individuals. It is essential in clinical practice to be aware of when to order ANA testing, and how to correctly interpret the test results.

Keywords: Antinuclear antibodies, Connective tissue diseases, Systemic lupus Erythematosus, Indirect immunofluorescence

INTRODUCTION

Anti-nuclear antibodies (ANAs) are a group of antibodies that target macromolecules which bind DNA, RNA, proteins, and their complexes.^[1,2] Although they bind cytoplasmic components too, the term ANA was retained for historical reasons.^[3] Characteristically associated with connective tissue diseases (CTD), ANA may also be detected in other conditions and even in general population.^[4]

HISTORY

LE cell phenomenon was described by Hargraves, Richmond, and Morton in 1948.^[5] Kidney or liver sections from rats or mice were used as substrates initially to detect ANAs with indirect immunofluorescence antibody (IFA) technique, which are now replaced with human epithelial type-2 (HEp-2) cells.^[6-8]

Techniques to detect ANA

The methods to detect ANA can be broadly classified as indirect immunofluorescence technique and solid-phase assays.

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Indirect IFA technique/fluorescent ANA (FANA)

The method of choice for the detection of ANA is the indirect IFA technique, otherwise known as FANA technique.^[2,9-11] It involves incubation of serum or plasma with either a tissue section or a cell line fixed to a glass slide. Large nuclei of HEp-2 cells make the detection of fluorescence staining patterns easier. Serial dilutions of positive samples are tested to obtain an endpoint titer, and dilution prior to this endpoint is reported as ANA titer.^[1,2,4,6] Three parameters are evaluated – the pattern of fluorescence, substrate used, and the titer of a positive test [Figure 1].^[4]

Interpretation of ANA titer by immunofluorescence

ANA titer is the quantitative expression of ANA concentration, in dilution. There is a lack of consensus regarding recommended initial dilution for screening purpose, although a titer of 1:160 is taken as significant.^[4] Lower titers are also suggested by some experts.^[5,6,10-13]

STAINING PATTERNS

The staining patterns of ANA on HEp-2 cells can be broadly divided into nuclear, cytoplasmic, and mitotic patterns [Tables 1 and 2].^[14] The intensity of staining expressed in qualitative scale + to ++++ should also be reported, as fluorescence intensity is considered proportional to antibody concentration, which may predict the severity of the CTD. Although staining patterns may provide a clue to the underlying CTD, they cannot be considered specific.^[4,14]

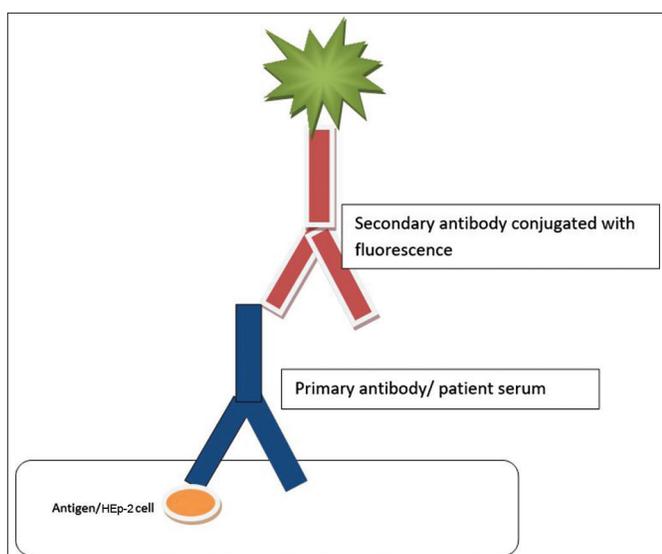


Figure 1: Diagrammatic representation of the detection of antinuclear antibody using immunofluorescence technique.

Advantages of IFA technique for ANA

1. A large number of autoantibodies can be detected using the HEp-2 cells
2. It has high sensitivity (95%–100% for diagnosis of SLE)
3. The pattern of staining can provide a clue to the diagnosis.^[10,11]

Disadvantages

1. The specificity is low – approximately 43% at 1:40 dilution and 63% at 1:80 dilution^[15]
2. The test can be affected by many variables, such as the quality of ingredients, the specificity of substrate, the conjugate, microscope bulb, and reader^[11,16]
3. HEp-2 cells may lack some antigens, such as the Ro-60 antigen and the ribosomal-P^[17]
4. It is labor and skill intensive.

SOLID-PHASE ASSAYS

A panel of purified autoantigens is prepared, and each antigen is immobilized on a solid surface (microtiter plate, fluorescent microsphere, or membrane). Diluted human serum is incubated with the immobilized antigen and a secondary antibody is used to detect bound autoantibodies. These include:

1. Enzyme-linked immunosorbent assay [Figure 2]
2. Immunoblot assay
3. Line immunoassay

Table 1: Patterns of immunofluorescence on IFA test.

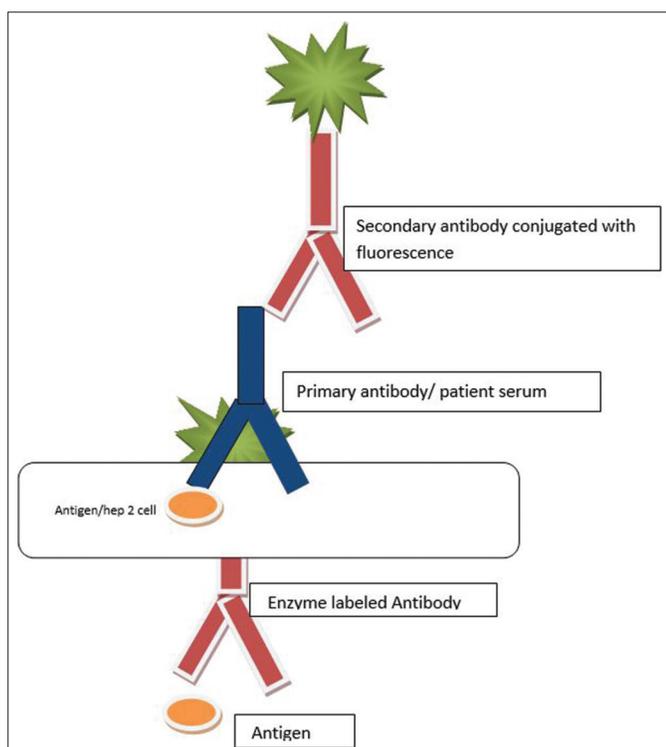
Patterns	Subtypes
Nuclear patterns	Homogeneous
	Speckled (fine and coarse)
	Peripheral/rim
	Nucleolar
	Centromeric
	PCNA
	Nuclear dots
	Nuclear membrane
	Diffuse grainy
	Cytoplasmic patterns
Mitochondrial-like	
Ribosomal-like	
Golgi apparatus	
Lysosomal-like	
Cytoskeletal filaments (actin, vimentin, and cytokeratin)	
Mitotic patterns	Mitotic spindle
	Centrosomes
	NuMA mid-body
	CENP-F

IFA: Indirect immunofluorescence antibody test, PCNA: Proliferating cell nuclear antigen, NuMA: Nuclear mitotic apparatus, CENP-F: Centromere protein F precursor

Table 2: Common IF-ANA patterns and associated diseases.

ANA pattern	Antigen	Associated diseases
Homogenous/diffuse	dsDNA, nucleosomes, histones	SLE, juvenile idiopathic arthritis, drug-induced LE
Speckled/granular	ENA, RNP, Sm, SSA/Ro, SSB/La, Scl-70, Jo-1, ribosomal-P	SLE, mixed CTD, systemic sclerosis, primary Sjogren's syndrome, polymyositis
Peripheral	RNP, Sm, Ro/SSA	SLE, systemic sclerosis
Nucleolar	Anti-PM-Scl, anti-RNA polymerase I-III, anti-U3-RNP	Systemic sclerosis, polymyositis
Centromere	CENP A-E	Limited cutaneous systemic sclerosis

IF-ANA: Immunofluorescence antinuclear antibody, SLE: Systemic lupus erythematosus, LE: Lupus erythematosus, CTD: Connective tissue disease, ENA: Extractable nuclear antigen, DNA: Deoxyribonucleic acid, RNP: Ribonucleoprotein, RNA: Ribonucleic acid, PM: Polymyositis, CTD: Connective tissue disease, CENP: Centromere protein

**Figure 2:** Diagrammatic representation of the detection of antinuclear antibody using enzyme-linked immunosorbent assay technique.

4. Chemiluminescence immunoassays
5. Multiplex bead-based assays
6. Chip-based assays.^[2,4]

Advantages of solid-phase assays

1. They are suitable for high throughput testing
2. Semi-quantification of results is possible
3. Automation can increase efficiency and decrease labor cost
4. Simultaneous identification of the responsible autoantibody.^[11]

Disadvantages of solid-phase assays

1. They have low sensitivity, hence less suitable for screening. Depending on the test kit, sensitivity can vary from 69% to 98% with an average of 87%^[4]
2. Many antigens present in HEp-2 cells are absent in solid-phase substrate, hence, false-negative results may be high.^[4]

ANA profile

Many laboratories provide a bouquet of antibody tests in addition to FANA. The components of this panel can be different in different laboratories, but usually include antibodies to single-stranded and double-stranded DNA, ribonucleoprotein antigens, Ro, La, Sm, and topoisomerase I (Scl-70) antigens. ANA profile has higher sensitivity than FANA. ANA profile can also provide additional diagnostic and prognostic information depending on the type of antibody present. Different laboratories use different techniques for the performance of ANA profile, and unfortunately, the performance of many of these tests is not consistent always.^[18]

Fallacies of ANA testing

Although rheumatic diseases are quite uncommon in general population, the frequency of ANA positivity determined by IFA in an otherwise healthy population can be high. Most people who are ANA positive will never develop a rheumatic disease.^[1]

Just like any other test, the higher the pre-test probability that a patient has a rheumatological disease, the more likely that an ANA test will be of use in establishing the diagnosis. Hence, ANA should not be used as a screening tool for asymptomatic individuals. Indiscriminate testing will cause positive results in 5% at the predetermined screening dilution (usually 1:160).^[1] A positive ANA test is important only in conjunction with clinical evaluation. A positive

result of ANA or diagnosis of SLE should not lead to all clinical features being interpreted as manifestations of SLE (Greenwald's law of lupus).^[4]

However, screening of patients could be valuable for identifying individuals prone to develop SLE who have a pre-autoimmunity state, where abnormalities of the immune system cause autoreactivity, but clinical disease is yet to manifest.^[1,19,20] The titer and binding pattern of ANAs through IFA and the identification of specific ANAs such as anti-dsDNA, anti-Ro60, or anti-La antibodies may be helpful in such situations.^[1,17,21]

Conditions other than CTDs which cause positive ANA

A number of non-rheumatological conditions can yield a positive ANA test [Table 3].^[22]

ANA testing: Which test to order first

The American College of Rheumatology ANA task force in 2009 recommended that the immunofluorescence test should remain the gold standard for ANA testing, due to high sensitivity (>95%).^[23,24]

When to test for ANA

ANA test must be performed only when there is a clinical suspicion of an autoimmune CTD. If screening test for ANA is positive, further tests for specific autoantibodies must be ordered, based on the clinical features and possible diagnosis. If ANA screening is negative, patient must be reevaluated and kept under follow-up, and test must be repeated only if clinically indicated.^[4] If ANA is negative in a patient with

a high degree of clinical suspicion for SLE, ANA may be repeated. If negative again, anti-SSA antibody may assist in diagnosis of ANA-negative SLE.^[4,25]

When not to test for ANA

1. For confirming rheumatoid arthritis or osteoarthritis, as it is not considered helpful
2. To evaluate fatigue, back pain, or other musculoskeletal pain, unless they are accompanied by clinical features suggestive of a CTD
3. For screening of asymptomatic individuals
4. ANA tests do not need to be repeated, as changes in ANA titer are not of value in monitoring disease activity
5. Negative tests need to be repeated only if there is a strong suspicion of an evolving CTD, or a change in the patient's illness that suggests a revision in diagnosis.^[4]

ANA-negative lupus

Several cases of ANA negative lupus have been published, and its prevalence may be as high as 5%–10%.^[26] The causes of ANA-negative lupus include antigen-deficient substrate and leaching of antigens, concurrent immunosuppressive treatment, persistent renal loss of proteins, and a false diagnosis of lupus.^[27]

CONCLUSION

ANA testing is an important prong in the diagnosis of rheumatological diseases, but in itself has no value in the absence of clinical correlation. A standalone positive or negative ANA test offers nothing to the patient or clinician, and hence, an awareness regarding the judicious use of the same is imperative in clinical practice.

Declaration of patient consent

Not required as there are no patients in this article.

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Conflicts of interest

Dr Kidangazhiathmana Ajithkumar is on the editorial board of the Journal.

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Table 3: Conditions other than autoimmune connective tissue diseases causing antinuclear antibody positivity.

Normal population	
Liver disease	Chronic hepatitis Primary cirrhosis Autoimmune hepatitis
Infections	Tuberculosis Viral hepatitis Parasitic diseases Syphilis
Skin diseases	Psoriasis Lichen planus
Malignancies	Breast cancer Prostate cancer Hodgkin's lymphoma Leukemia
Other autoimmune diseases	Type 1 diabetes Addison's disease Autoimmune anemia Hashimoto's thyroiditis

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