

Screening strategy for anti-nuclear antibodies (ANA)

The classification of autoimmune disease (AID) distinguishes between the organ specific diseases (such as type I diabetes, autoimmune thyroiditis and autoimmune hepatitis etc.) and organ non-specific autoimmune diseases, i.e. systemic diseases including connective tissue disease (such as Systemic Lupus Erythematosus (SLE), Gougerot-Sjögren syndrome (SS), Scleroderma, dermatomyositis, polymyositis or rheumatoid polyarthritis (RP etc.) and ANCA vasculitis.

In practice, how do we detect anti-nuclear auto-antibodies (ANA)?

Their detection relies on a cascade approach: all ANA investigations start with indirect immunofluorescence screening (IIF). If the screening result is positive, the procedure is followed by an identification step in order to characterise the target antigen(s) detected on the ANAs screen.

ANA detection is performed via IIF on HEp-2 cells, human epithelial cells, which are directly cultivated on slides that come from a cancerous tracheal cell line with large activated nuclei containing multiple nuclear and cytoplasmic antigens.

A number of commercial preparations are available, including slide preparations transfected with the SSA/Ro 60 kDa gene, which over express the antigen HEp-2000®. Although new Elisa techniques have been developed over the last few years, and the future is inevitably destined to use automated, standardised and miniaturised techniques, IIF remains the reference method imposed by French NABM (nomenclature of medical pathology acts) for ANA detection.

ANA screening stage by IIF on HEp-2 cells.

The "threshold" dilution for screening is not standardised. Usually it is 1/80 or 1/100. Negative ANA screening results are not suggestive of connective tissue disease. Positive results suggest the existence of auto immune factors: connective tissue disease or organ specific auto immune disease (primary biliary cirrhosis (PBC) and autoimmune hepatitis). However, they can also be found during inflammation, infection (notably by EBV, HBV, HCV, HIV, coxsackie virus or VZV), tumours (chro-

nic lymphoid leukaemia or lymphoma), be induced by certain medications (beta blockers and anti TNF alpha treatments etc.) and can even be detected in healthy individuals.

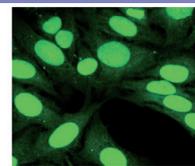
When faced with a positive ANA result, one must determine the nuclear fluorescence aspect (+/- cytoplasm and comment) as well as the successive serum dilution titre.

There are many fluorescence aspects:

- Homogeneous nuclear fluorescence, membrane fluorescence, patchy fluorescence (large grain, small grain), centromeres fluorescence, nucleoli, fluorescence, nuclear dots (multiple dots, 4-6 dots), heterogeneous fluorescence, mixed, mitotic spindle fluorescence.
- Cytoplasmic fluorescence: fine anti synthetasis grain, ribosomes, SRP, endoplasmic reticulum, lysosomes, peroxisomes, endosomes, golgi apparatus, mitochondria, actin, vimentin and tubulin.

Main fluorescent aspects:

Homogeneous fluorescence



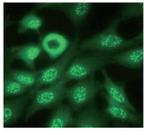
- ✓ Anti dsDNA antibodies
- ✓ Anti histone antibodies
- ✓ Anti nucleosome antibodies



- SLE
- Induced lupus
- Rheumatoid polyarthritis
- Chronic juvenile arthritis
- Systemic scleroderma

Speckled fluorescence

Large grain

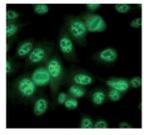


- ✓ Anti RNP abs
- ✓ Anti Sm antibodies

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- SLE
- Mixed connective tissue disease
- SS
- Scleroderma
- Raynaud syndrome

Small grain

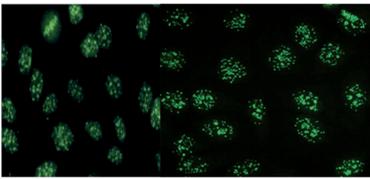


- ✓ Anti SSA/Ro 60,52 abs
- ✓ Anti AAB/La abs
- ✓ Anti Polymerase II and III RNA abs
- ✓ Anti Mi 2 antibodies

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- SLE
- Sub acute cutaneous lupus
- Neonatal lupus
- SS
- Scleroderma
- Dermatomyositis

Centromere fluorescence



- ✓ Anti centromere antibodies (A,B,C,D,E and F)

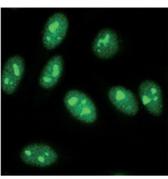
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- In 50-98% of CREST syndrome (calcinosis, Raynaud disease, esophageal dysmotility, sclerodactyly and telangiectasia)
- Reynolds disease (association CREST+PBC)
- In 20-40% of systemic scleroderma
- Raynauds disease
- SLE
- SS

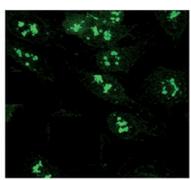
Nucleolar fluorescence

Different aspects of nucleolar fluorescence can be described but they remain difficult to distinguish in practice.

- ✓ Homogeneous aspect
- ✓ Granular aspect
- ✓ Fine speckled aspect
- ✓ Punctuated aspect



Pure nucleolar fluorescence



Nucleolar fluorescence associated with other fluorescent aspects

Identification stage: characterisation of targeted anti genes.

This is usually performed following a positive ANA result of $\geq 1/160$ (however this is open to opinion) and includes detection of anti dsDNA antibodies and anti soluble nuclear antigen antibodies (ENA).

The dsDNA antibodies are specific for lupus (according to the *American College of Rheumatology* they are part of the 11 diagnostic criteria).

Denatured anti DNA antibodies (or simple stranded DNA or ssDNA) are of no diagnostic nor prognostic interest.

For the identification of anti dsDNA antibodies, 4 method groups are available which differ by: the DNA they use (purified or recombinant), the isotypes (IgG or IgM) and the antibody avidity detected: Farr test, IIF on *Crithidia luciliae* slides, Elisa and Luminex technology. Regardless of the technology used, these characteristics must be determined.

To identify the anti-ENA antibodies, of which the main soluble and cytoplasmic antigens identified in routine testing are: Sm, RNP, SSA/Ro 60, SSA/Ro 52, SSB/La, Scl70, CENP-B, JO1 +/- PCNA, PM/Scl, Ku, Mi 2 and ribosome P, the available techniques are: Ouchterlony double immunodiffusion, Elisa, immunodot and Luminex® technology.

The anti-ENA antibodies names have several references: the first 2 letters of the name of the patient within which the antibodies were identified (Sm for Smith, Ku, Mi, La, Ro), the nature of disease where the antibody is found (SS for Sjögren Syndrome), the nature of the identified antigen (RNP for ribonucleoprotein) or the description of the gel immunodiffusion result "precipitation line". PL (PL7,PL12).

Clinical interest of anti-SSA/RO 52 kDa antibodies or anti-TRIM 21 antibodies

These antibodies are frequently associated with anti-SSA/Ro 60 kDa antibodies and sometimes with anti-JO1 antibodies. During PBC these antibodies are of a prognostic value. A number of different techniques can detect these antibodies, however they seem to be of limited clinical interest.

The frequency of the main ANAs during SLE

Specific antibodies	Non-specific antibodies
Anti ds DNA antibodies 70-95%	Anti-histones: 30-70%
Anti-Sm (Caucasians): 5-10%	Anti-U1 RNP: 20-40%
Anti-nucleosomes: 40-75%	Anti-SSA/Ro 60: 20-90%
Anti-PCNA: 10-43%	Anti-SSB/La: 10-70%
	Anti-ribosomes: 10%

Auto-antibodies and clinical symptoms during SLE.

<ul style="list-style-type: none"> ■ Anti dsDNA antibodies ■ anti-Sm antibodies ■ anti-U1 RNP antibodies ■ anti-SSA antibodies ■ anti-SSB antibodies ■ anti ribosome antibodies 	<ul style="list-style-type: none"> ■ Nephropathy ■ Nephropathy ■ Raynaud disease ■ Myositis ■ cutaneous photo sensitivity lesions, Sjogren's syndrome ■ cutaneous photo sensitivity lesions, Sjogren's syndrome ■ Nervous system and hepatic disorders
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The absence of auto antibodies does not exclude the diagnosis of an auto immune disease for several different reasons: at the beginning of an infection the antibodies may be missing and develop later on, the auto antibodies are not constant during auto immune disease, the patient may have an immunoglobulin deficiency or the antibodies may not have been detected. For therapeutic patient monitoring only the dsDNA antibodies are of interest.

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