A Basic Guide to Autoimmune Testing: Part I ANA, ENA and dsDNA Antibodies

Typical scenario: A 40 year old woman presents with tiredness. She requests autoimmune tests "just to make sure", as a friend was diagnosed with Lupus some years back and has been quite unwell. She has looked it up on the internet. Blood tests reveal a normal full blood count, normal ESR, and lowish ferritin. Her ANA is 1/160, speckled pattern.

Systemic Lupus Erythematosus (SLE, or Lupus) is a complex autoimmune disease, which may present with a variety of clinical symptoms and signs. This disease is associated with various positive antibodies, some of which are specific to Lupus, some of which indicate another autoimmune disease, and some of which can occur in healthy individuals.

Antinuclear Antibodies (ANA)

An ANA is an antibody against a nuclear component of the cell. At Clinipath Pathology, the test is performed by immunofluorescence, and a titre is given, as well as the pattern of the fluorescence.

The ANA may represent many autoantibodies, so once an ANA is found, often further testing needs to be done to elucidate the type of antibody.

Titre

The titre is determined by the lowest dilution at which the fluorescence can still be seen. Hence, the higher the denominator, the stronger the intensity of the fluorescence. A 1/40 titre, therefore, is less significant than a 1/2560 titre.

In Perth some laboratories give ANA results as SI Units. With this method, the higher the SI Unit, the higher the intensity of the ANA. Due to differences in methodology, unfortunately, it is not possible to compare a result by titre with one by SI Unit.

Whether measured by titre or SI Unit, the higher the intensity, the higher the likelihood of underlying disease.

Patients with a low titre ANA are likely to be healthy.

Pattern

Many different patterns can be detected using immunofluorescence, depending upon the specificity of the underlying antibody that constitutes the ANA. An example of these patterns is illustrated in Figure 2 over the page.

A Reminder: The Clinical Manifestations of Lupus

Haematological	Anaemia, low platelets, neutropenia
Skin	Photosensitivity, rashes, alopecia, Raynauds, acrocyanosis, mouth ulcers
Joints	Synovitis, tendonitis (90% have some degree of joint involvement)
Renal	Active urinary sediment, HT
Heart, Lungs	Pleurisy most commonly
Thrombosis	Recurrent late miscarriage, IUGR, recurrent or unexpected thromboembolic disease
Cerebral	Seizures, strokes
Constitutional	Weight loss, fevers, fatigue

Further elucidation of the specificity of the antibody is done by ENA and dsDNA testing. These are useful in confirming the significance of a positive ANA and will help to lead to a diagnosis of the type of autoimmune disease.

Extractable Nuclear Antigens (ENAs)

Detecting antibodies to ENAs involves testing patient's serum for antibodies against various specific components of the cell nucleus. The nuclear antigens are extracted individually, and the patient's sera is tested against each one. Seven antibodies are routinely tested for by ELISA at Clinipath. A rough guide to the disease associations of ENAs is provided in Table 1. For some antibodies, further confirmatory testing may be required, (for example, for Jo 1 antibodies), as false positives may occur with the screening ELISA. Interpretation in the clinical context is important.

It is less likely that a patient will develop clinically significant autoimmune disease if the ENAs are all negative.

Table 1. Main conditions which may be diagnosed from ANA, ENA testing

"True Positive" ANA	"False Positive ANA"
Nuclear • Systemic Lupus Erythematosus • Sjogrens Syndrome • Scleroderma • Mixed Connective Tissue Disease • Drug Induced Lupus	*Healthy individuals, especially age >60* *Infections* • Hepatitis C • EBV • HIV • Bacterial endocarditis
Cytoplasmic • Polymyositis • Primary Biliary Cirrhosis • Autoimmune Hepatitis	*"Autoimmune diathesis"* • Rheumatoid arthritis • Juvenile Chronic Arthritis • Hashimotos • Graves Disease • Pernicious anaemia

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Double stranded DNA (dsDNA)

Antibodies against dsDNA are highly specific for SLE and are rarely found in other disorders. They are useful for confirming the diagnosis of SLE, and for monitoring disease. They predict an increased risk of Lupus nephritis. They are only positive in a proportion of patients with Lupus. (~ 70%).

Monitoring SLE

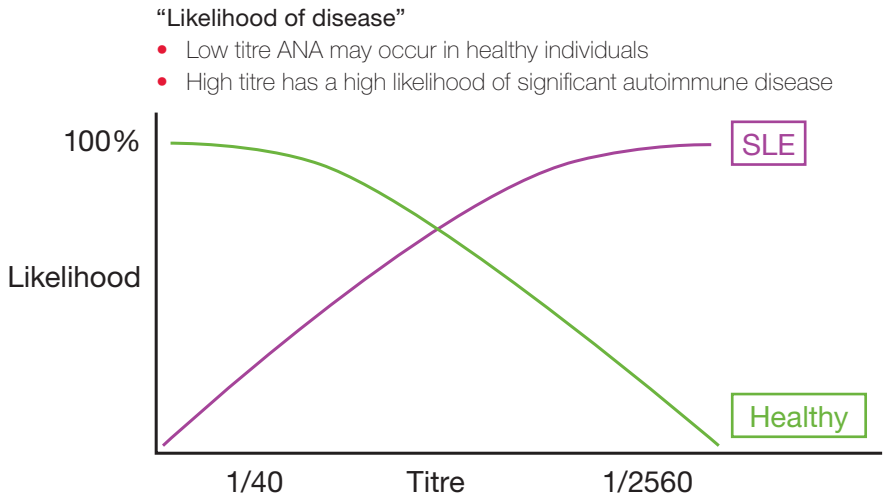
Patients with Lupus may present with a variety of clinical problems, and when monitoring these patients, their particular disease often leads to "signature" parameters to follow. This will vary depending on the patient (i.e. a patient with predominant Lupus nephritis will have different monitoring parameters to a patient with autoimmune haemolytic anaemia).

ANA and ENA antibodies are not useful for monitoring, and rarely need to be repeated after diagnosis. Tests used in monitoring are listed in Table 2.

Table 2. Which tests are useful for monitoring?

ANA	✘
dsDNA	✔
ENA	✘
C3, C4	✔
Urinary protein	✔
Creatinine	✔
ESR	✔
CRP	✘

Figure 1. How to interpret an ANA result titre



Please don't hesitate to ask the Clinical Immunologist if you are not sure how to proceed with further testing for a patient in whom you suspect autoimmune disease. It can be a complex field!

References

1. The use of laboratory tests in the diagnosis of SLE Egner, W, J Clin Pathol 2000; 53:424-432
2. Serologic Testing in Connective Tissue Diseases Habash-Bseiso et al, Clinical Medicine and Research August, 2005
3. The Management of patients with unexpected autoantibody positivity Bagnasco et al, Autoimmunity reviews 2007 347-353
4. British Columbia Guidelines for ANA testing for connective tissue disease, 2001, updated 2007 **www.BCGuidelines.ca**

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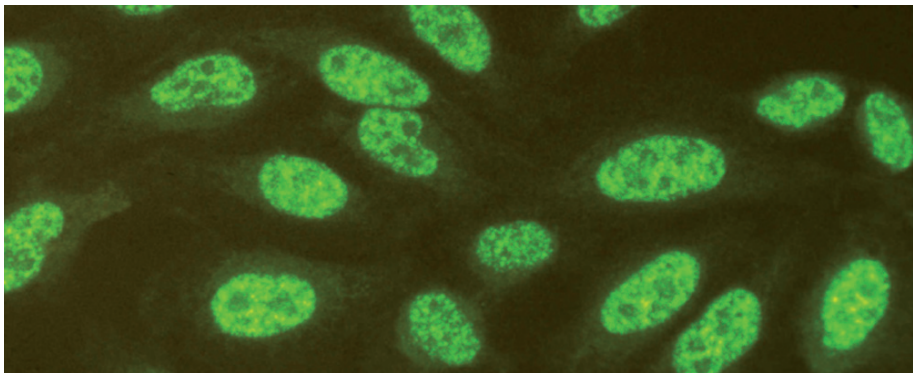

Figure 2. ANA Speckled Pattern

Table 3. Main Disease Associations with dsDNA and ENAs

Anti dsDNA	Specific for SLE
Anti SSA / Ro	SLE, Sjogrens Syndrome (The babies of pregnant women with anti SSA are at risk of neonatal heartblock)
Anti SSB / La	SLE, Sjogrens Syndrome
Anti RNP	SLE/Mixed Connective Tissue Disease
Anti Jo 1*	Polymyositis/dermatomyositis
Anti Sm	Specific for SLE
Anti Scl 70	Systemic scleroderma

** Recent assays will often show false positive Jo 1. Needs additional confirmatory testing.*

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Clinical Recommendations For ANA Testing

Recommendation 1

ANA testing should not be performed unless there is a significant clinical likelihood of autoimmune disease.

ANA should not be a first line test for the investigation of fatigue or musculoskeletal pain, unless accompanied by other clinical features to suggest autoimmune disease.

Recommendation 2

ANA testing may be indicated if patients present with one of the following:

- Arthritis/demonstrable synovitis
- Pleurisy, or pericarditis
- Photosensitive rash
- Clinical and laboratory evidence of myositis
- Skin changes to suggest scleroderma or vasculitis
- Raynauds phenomenon
- Haemolytic anaemia, thrombocytopenia or neutropenia
- Laboratory evidence of a renal disorder (eg active urinary sediment)
- Laboratory evidence of a hepatic disorder
- Evidence of a central nervous system disorder
- Recurrent thrombosis or late miscarriage

Some of the above symptoms may also occur in the setting of an intercurrent viral infection, such as CMV or EBV. These situations will lead to a false positive result.

Recommendation 3

ANA and ENA tests rarely need to be repeated. These are diagnostic, not monitoring, tests.

If an unexpected result is given, it is reasonable to repeat the test to confirm the finding. It is also useful to repeat if a person's illness has significantly changed.