Laboratory Testing for Antinuclear antibodies

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Background
Testing for antinuclear antibodies (ANA) is an important part of the diagnostic workup for connective tissue disease and other inflammatory autoimmune diseases. ANAs are antibodies directed against components of the cell nucleus. Examples of ANAs are antibodies to double stranded DNA and antibodies to extractable nuclear antigens (such as anti-Ro and anti-La), which are often ordered as follow on tests. These tests are used as a diagnostic adjunct to the clinical evaluation for connective tissue disease and autoimmune liver disease.

Unfortunately, inappropriate ordering of ANA is common, can be misleading, may generate anxiety and inappropriate referral wasting time and resources. ANA testing should only be carried out in the context of a clinical evaluation (full history and examination) that has led to a presumptive diagnosis of a connective tissue disease or autoimmune hepatitis.

Physicians should appreciate that ANA positivity at weak titre is common in the general healthy population (10-15%). This prevalence rises significantly in the over 65s. Hence a positive ANA in isolation does not always signify the presence of a disease process and must be interpreted within the clinical context. Positive ANAs may also be associated with certain medications or transiently during infection. Given these caveats a good understanding of why the test is being ordered is vital.

ANA testing should not be carried out as part of a general health screen.

Scope
This guideline aims to provide information on ANA testing to be used by primary care practitioners and general medicine physicians. These guidelines apply to adult and paediatric patients. The guidelines are not aimed at hospital specialists involved in the diagnosis and management of connective tissue disease or autoimmune hepatitis.

Key Recommendations
ANA testing is indicated when there are features suggestive of connective tissue diseases such as SLE, Sjogren’s syndrome, scleroderma, polymyositis/dermatomyositis or autoimmune liver disease.

If features of these conditions are not present an ANA test should not be ordered.
Testing

ANA testing in Ireland is usually carried out using an indirect immunofluorescence approach. This will generate a report that, if positive, gives a titre (or strength) of the antinuclear antibody and a pattern.

- The titre is a semi-quantitative estimate of the concentration of the antinuclear antibody. The larger the base number the stronger the antibody concentration.
- Titres of 1/160 or greater are more likely to be clinically relevant in adults in the correct clinical setting.
- Weaker titres (1/80) may still be relevant in the correct clinical context (especially in the paediatric setting), or where autoimmune hepatitis is suspected.
- Most laboratories report 4 main patterns (homogenous, speckled, nucleolar and centromere).
- Centromere ANA is the most useful pattern for the generalist and is suggestive of limited scleroderma (CREST syndrome).
- Strong positive ANA tests may require further follow on testing to give more specific diagnostic information – this may be carried out automatically by your laboratory but this practice can vary. You should clarify what your laboratory’s approach is. Possible follow on tests include
  - Antibodies to double stranded DNA (dsDNA)
  - Antibodies to extractable nuclear antigens (ENA)
    - Anti-Ro, anti-La, anti-RNP, Anti-Sm, Anti-Jo1, Anti-SCL70
    - Interpretation of positive ENAs will be available through your local laboratory handbook
    - Other panels may be available in certain circumstances (e.g. myositis).

Some laboratories test for ANA using an Enzyme Linked Immunoassay approach. Results generated by this approach may be reported numerically or semi-quantitatively. Similar principles apply, with strong positive results being more likely to be relevant in the correct clinical scenario.

Your local clinical immunology team will be able to offer advice on test selection and result interpretation.

Who to Test

Patients should have at least one of the following features that is not explained by another cause:-

- Evidence of inflammatory arthritis not consistent with rheumatoid/psoriatic arthritis (3 or more swollen joints, MCP/MTP involvement, early morning stiffness >30minutes)
- Photosensitive rash or vasculitic rash
- Skin changes of scleroderma
- Raynaud’s phenomenon
- Renal disease – laboratory evidence of renal impairment or suspected renal inflammation on urine dipstick or microscopy
- Clinical and laboratory evidence of myositis
- Haemolytic anaemia, immune thrombocytopenia, neutropaenia
- Pleurisy, pericarditis or other serositis
- Neurologic signs suggestive of autoimmune neurologic disease
- Clinical and laboratory evidence of autoimmune liver disease.
Who to Re-Test
- Repeat ANA testing is rarely required.
- Serial monitoring of ANA is not indicated even in the setting of connective tissue disease as titres do not correlate with disease activity.

Who Not to Test
- Patients in whom the clinical suspicion of connective tissue disease is low.
- Healthy patients for screening purposes.
- Patients with back pain or other musculoskeletal pain without features of connective tissue disease.
- Patients with fatigue without other features of connective tissue disease.
- Patients with joint pain likely to be degenerative (absence of early morning stiffness).

How to Test
A single serum sample is required for testing.
A completed standard laboratory or electronic test request form must be sent with all samples.

Information required on the referral form
The request form must include detailed patient and clinical information including:

- Patient demographics
  - Patient's Name
  - Patient's Date of Birth
  - Medical Record Number
  - Name of Referring Clinician
  - Name of Referring Hospital
  - Order number / external laboratory number (if applicable to external agencies only).

- Request details
  - Example - Clinical indication for testing (see list above)
  - Example - Details of any medications.

Requests received with no clinical details or with inadequate patient demographics will not be analysed.

Interpretation of tests
What does a positive Antinuclear Antibody test result mean?
- A positive result means that antinuclear antibodies have been detected.
- The interpretation of a positive result depends on the clinical scenario.
• There is no absolute cut off for a titre at which a positive ANA is clinically significant, however, high titre ANAs are more likely to be associated with connective tissue disease or autoimmune hepatitis.
• In the correct clinical scenario, a strong positive ANA should trigger further assessment for double stranded DNA and ENA antibodies. This may be ordered automatically by your laboratory but practice may vary.
• Low titre positive ANA may still be significant in the correct clinical context, especially in paediatric patients or in autoimmune hepatitis. Evaluation of results in the light of the clinical scenario is key.
• It is important to emphasise that most low titre positive ANAs are not associated with current or future connective tissue disease and neither clinical follow up or further testing is required.

What does a negative Antinuclear Antibody test mean?

• A negative ANA effectively excludes the diagnosis of SLE, scleroderma, polymyositis/dermatomyositis or autoimmune liver disease especially in the absence of a high degree of clinical suspicion.
• The predictive value of a negative result can be lower in some testing systems (solid-phase) compared with indirect immunofluorescence.
• If the index of clinical suspicion remains high you should discuss the scenario with your local clinical immunology team.
• A negative ANA does not discount rheumatoid arthritis, juvenile idiopathic arthritis, some forms of cutaneous lupus or vasculitic disorders as these are not typically associated with antinuclear antibodies.
Patients selected for ANA testing should have one of the following features not explained by another cause:
- Inflammatory arthritis
- Photosensitive or vasculitic rash
- Raynaud's or Scleroderma skin changes
- Renal disease
- Clinical or laboratory evidence of myositis
- Haemolytic anaemia, immune cytopaenias
- Pleurisy, pericarditis, serositis
- Autoimmune neurologic disease
- Suspected autoimmune liver disease

Suspected connective tissue disease or autoimmune hepatitis

- No
  - ANA testing NOT indicated

- Yes
  - Order Antinuclear antibody test
    - May not be significant - re-evaluate history
    - Weak positive 1/60 titre or less
    - Connective tissue disease very unlikely
    - Negative
    - May be significant in correct clinical context - consider further testing
    - Strong positive 1/160 titre or greater
    - Tests for antibodies to dsDNA and ENA may be considered
    - Re-evaluate clinical history with all relevant results
References and Additional Reading


