The Laboratory Services Reform Programme

ADVICE NOTE

Assessment of Autoantibodies against Nuclear Antigens (ANA)

Version 1 Issued 07/04/2025

CDI/0185/1.0/2025

Clinical Practice Guidance Document Cover Sheet

Document Type	Advice Note
Document Title	Assessment of Autoantibodies against Nuclear Antigens (ANA)
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Approved by:	Prof Martin Cormican
Unique Identifier Number(UID):	CDI/0185/1.0/2025
Version Number:	1
Publication Date:	07/04/2025
Recommended Revision Date: *	07/04/2027
Electronic Location:	https://www.hse.ie/eng/about/who/cspd/lsr/resources/advice.html

Version	Revision Date	List Section Numbers Changed	Author

The Laboratory Services Reform Programme offers the following advice:

1.1 Advice for Laboratory Users

1. Testing for nuclear autoantibodies is important in the diagnosis of autoimmune connective tissue diseases and some other disorders such as autoimmune liver disease.
2. A test for nuclear autoantibodies should only be requested where there is a strong clinical suspicion of autoimmune connective tissue disease. Indications are outlined in Section 1.2.18. Please state specific indications using these terms, or other similar terms based on local laboratory advice on every request for assessment of nuclear autoantibodies
3. Tests for nuclear autoantibodies should not be ordered in asymptomatic individuals or individuals with non-specific symptoms. These tests should never form part of a health screening approach.
4. Users should be aware of the terminology used in testing for nuclear autoantibodies. Terms used can include Antinuclear antibody (ANA) and Connective Tissue Disease (CTD) test or “CTD screen”.
5. Strong positive results (either a higher quantitative value, or where titres are reported, a large base numerical value) are more likely to be clinically significant and to provide a sound basis for diagnostic decisions than weak positive results.
6. Weak positive results (either a lower quantitative value, or where titres are reported, a small base numeric value) are less likely to be clinically significant and should be interpreted with caution, in particular when the pre-test probability of a connective tissue disease is low
7. A clinical diagnosis of an autoimmune disease should never be based on nuclear autoantibody test results alone. Users should interpret results alongside clinical findings and other laboratory results. A positive result does not in itself confirm a diagnosis and a negative or not detected result does not exclude all autoimmune diseases
8. Users should have a basic understanding of the test methodology used in their local laboratory as this can have a significant impact on interpretation of results.
9. The two main methods used in the assessment of nuclear autoantibodies are indirect immunofluorescence and solid phase assays such as ELISA or EliA. Table 1, Section 2 provides a comparison of these methods.
10. Users should be aware that laboratories will often progress to the performance of additional tests if a sample tests positive on the initial test (this is known as reflex testing). Users should be aware of the reflex testing cascade implemented in their local laboratory for further characterisation of positive results. This may involve assessment for specific autoantibodies such as dsDNA or extractable nuclear antigen (ENA) panels on samples that test positive at an initial stage. Available panels may differ between laboratories.
11. Users of laboratories that primarily test for nuclear autoantibodies using solid phase assay methodologies need to be aware that a negative, or not detected, test result in a clinical setting where the clinical suspicion (pre-test) probability of disease is high, should prompt a request for assessment by a highly sensitive method (*eg* indirect immunofluorescence) to exclude the presence of less well characterised nuclear autoantibodies.

12. Users should be aware that certain clinical circumstances require nuclear autoantibody assessment by a specific test methodology (for example by indirect immunofluorescence in autoimmune liver disease or certain paediatric settings) and should specifically request this, with relevant clinical details, from their local laboratory.
13. Users should be aware that assessment for nuclear autoantibodies does not test for all autoimmune diseases, or all connective tissue diseases. For example, when vasculitis or autoimmune myositis is suspected, different tests will be required.
14. Unnecessary testing for nuclear autoantibodies results in substantial costs, avoidable risks of needle exposure and generates unnecessary clinical and laboratory waste.
15. Weak positive nuclear autoantibody results in settings where the suspicion of a related disease being present, before testing, is low are generally clinically irrelevant. Users should avoid this situation by appropriate test requesting. If users request nuclear autoantibodies in settings where the clinical suspicion of a related disease is low, they should ensure that they have the requisite skills to manage the issues that arise without recourse to onward referral.
16. Misinterpretation of nuclear autoantibody results, particularly in settings of low pre-test probability, can result in the initiation of an avoidable, inappropriate referral cascade that leads to anxiety and impacts on waiting times for patients with clinically significant findings.
17. Where there is uncertainty about test selection or interpretation of results, users should consider discussion with the laboratory team or with appropriate clinical specialty.

1.2 Advice for Laboratories and Users

1. Indications for the assessment of nuclear autoantibodies include the following features not explained by another cause. Requests for testing for nuclear autoantibodies should include one or more of the following or other relevant clinical details.
    (a) Evidence of inflammatory arthritis not consistent with rheumatoid/psoriatic arthritis (3 or more clinically inflamed swollen joints, small joint involvement, early morning stiffness)
    (b) Sicca syndrome
    (c) Photosensitive rash, Vasculitic rash, Discoid rash
    (d) Skin changes suggestive of scleroderma
    (e) Raynaud's phenomenon
    (f) Renal disease with evidence of renal inflammation on urine dipstick or microscopy
    (g) Clinical, laboratory or radiological evidence of myositis
    (h) Haemolytic anaemia, immune thrombocytopaenia, autoimmune neutropaenia
    (i) Pleurisy, pericarditis or other serositis
    (j) Symptoms or signs suggestive of autoimmune neurological disease
    (k) Clinical and laboratory evidence of autoimmune liver disease
2. Assessment for nuclear autoantibodies is not indicated in settings such as "fatigue" and "arthralgia", not accompanied by any of the features outlined in Section 1.2.1. Laboratories should consider that these do not represent relevant clinical details. In such settings of very low pre-test probability laboratories and users should be aware that positive results may be misleading.

3. Repeat testing for nuclear autoantibodies is rarely required unless there is a significant change in clinical presentation. In some circumstances monitoring of levels of individual nuclear autoantibodies, eg dsDNA, may be considered. Laboratories and Users should be satisfied that such monitoring approaches are evidence based and that the methodology employed in the local laboratory is compatible with this use and should take particular care when comparing results that may use different methodologies.
4. Users should be aware that laboratories may implement minimum retesting intervals. These intervals do not represent endorsement of repeat testing. Exceptions occur, for example in a patient who develops new symptoms suggestive of a new connective tissue disease. In such cases clinicians should discuss the requirements for a repeat test with their laboratory.

1.3 Advice for Laboratories

1. Laboratories should communicate to laboratory users the indications for assessment of nuclear autoantibodies.
2. Measurement of nuclear autoantibodies should be performed when relevant and legible clinical details and requestor information are provided on the request (electronic or paper) accompanying the sample and where the sample received is suitable for analysis.
3. To the greatest extent that is practical, requests for nuclear autoantibodies that do not meet these requirements or where the information received is inadequate should not be processed.
4. If samples are not processed, a report should issue to the effect that testing for nuclear autoantibodies was not performed because testing criteria were not met. Individual laboratories may wish to develop a process to store samples for a defined period of time, to allow users to update a request with appropriate information.
5. Laboratories may wish to consider implementing a minimum retesting interval. A 12 month general minimum retesting interval is recommended. Laboratories may wish to consider the requirements of their specific clinical users and the capabilities of the laboratory information system when implementing minimum retesting rules. Exceptions to such rules do occur and laboratories should have a framework for engaging with clinicians to address this.
6. Laboratories should communicate to laboratory users the main testing modality employed for the assessment of nuclear autoantibodies. Laboratories should consider including this information on results.
7. Where laboratories use a primary testing modality such as ELISA or EliA (solid phase assays), they should also offer pathways for access to nuclear antibody assessment by indirect immunofluorescence for defined groups that require access to a specific testing modality in specific clinical circumstances *e.g.* autoimmune liver disease or certain paediatric connective tissue diseases. Testing by indirect immunofluorescence can be used in these groups in other circumstances where there is a high degree of clinical suspicion (high pre-test probability) despite negative or not detected test results using solid phase assays.
8. Laboratories should have a clear operating procedure for reflex testing and this should be included in the laboratory user manual

9. Laboratories should consider engaging with key user groups, such as rheumatologists, to refine testing algorithms and to identify and engage with inappropriate practice.
10. Laboratories should consider developing educational strategies to help users optimise test selection and interpretation within their own clinical practice.
11. Laboratories should endeavour to provide comments to guide result interpretation, in so far as is possible within their Laboratory Information System framework.

1.4 Background

Nuclear autoantibodies, also known as antinuclear antibodies (ANAs) are autoantibodies that target components within the cell nucleus. They are commonly associated with systemic autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Sjogren syndrome or other members of a family of conditions known as connective tissue diseases. ANAs are also important in the diagnosis and classification of specific other disorders such as autoimmune hepatitis. In addition, they can be observed frequently in other conditions such as certain malignancies, thyroid disease and multiple sclerosis, where the presence of nuclear autoantibodies does not impact on the diagnosis. Nuclear autoantibodies may also be present, often at low titre in healthy individuals. Therefore, the interpretation of results must be informed by the context in which the test is ordered.

Knowledge of the method used to test for nuclear autoantibodies is important to aid the clinical interpretation of results. Indirect immunofluorescence is considered by some to be the gold standard method for nuclear autoantibody detection. This involves incubating patient serum with a substrate of slide mounted cultured cells, typically HEp-2 cells. A fluorescently labelled secondary antibody allows scientists to indirectly visualise bound nuclear autoantibodies by fluorescence microscopy. End point titration allows the strength of autoantibody to be assessed and fluorescence patterns can be reported. Fluorescence patterns can offer useful information that can support the diagnosis of specific connective tissue diseases, for example, centromere patterns are frequently associated with limited scleroderma. Indirect immunofluorescence is highly sensitive for the detection of nuclear autoantibodies. The cellular substrate used means that hundreds of antibody specificities can potentially be detected, but only a handful of these are known to be clinically significant. This means that this approach is very highly sensitive for the detection of autoantibodies associated with connective tissue disease. Unfortunately, this high sensitivity is coupled with a poor specificity.

More recently, laboratories have adopted solid phase assays to measure nuclear autoantibodies. These approaches use well characterised, clinically relevant, recombinant nuclear antigens mounted on plates or caps (the solid phase). Solid phase assay approaches are highly scalable, often automated and suitable for high throughput work. These assays typically have moderate to high sensitivities and specificities. However, solid phase assays may not identify less well characterised nuclear autoantibodies that are very occasionally important. This means that in settings where there is a high clinical suspicion of a connective tissue disease and a solid phase assay has yielded an initial negative or not detected result, clinicians should request confirmation by indirect immunofluorescence (as a method with a very high sensitivity). Table 1 briefly compares indirect immunofluorescence with solid phase assays for nuclear autoantibodies. Communication between laboratory and clinical teams on assay selection is vital given the sizable differences in sensitivity and specificity between familiar indirect immunofluorescence methods and newer solid phase assay approaches.

A positive indirect immunofluorescence or solid phase assay result typically results in further 'reflex testing' by the laboratory. This can involve measurement of levels of specific well characterised autoantibodies such as anti-double stranded DNA, and those directed against extractable nuclear antigens (ENAs), classically Anti Ro, Anti La, Anti RNP, Anti-SCL70, Anti Sm and Anti Jo1. These second line tests are usually solid phase assays and yield quantitative results. Laboratories may differ in the repertoire of tests ordered as reflex tests.

	Indirect Immunofluorescence	Solid Phase Assays
Detection method	Visual pattern recognition by fluorescence microscopy on substrate cells	Automated measurement of a binding signal (eg colorimetric signal)
Result output	Qualitative / Semi-quantitative Pattern and titre	Quantitative/ Qualitative
Pattern recognition	Report of fluorescence patterns may assist diagnosis	Does not provide pattern information
Sensitivity	Very high sensitivity	Moderate-high sensitivity, may not identify less well characterized nuclear autoantibodies
Specificity	Low specificity due to detection of clinically insignificant nuclear autoantibodies	Moderate-high specificity for defined nuclear autoantigens
Throughput	Lower throughput and often longer turnaround time due to multiple manual steps	High throughput and automated

Table 1: *Comparison of indirect immunofluorescence and solid phase approaches for nuclear autoantibody detection*

In common with almost all laboratory tests, the interpretation of results is significantly influenced by the pre-test probability – the likelihood of a disease being present before testing. Clinicians should use their clinical skills to improve pre-test probability by selecting patients in which there is a reasonable basis for requesting nuclear autoantibody tests based on clinical history, signs and other laboratory parameters. Tests for nuclear autoantibodies should not be used in settings where the pre-test probability is very low, as positive (and particularly weak positive) results in such settings are likely to be clinically irrelevant. Tests for nuclear autoantibodies should never be used in health screening, in evaluation of asymptomatic patients or in the investigation of individuals with non-specific symptoms such as fatigue, when not accompanied by features that might reasonably suggest a connective tissue disorder. Increasingly, clinicians use pre-defined batteries of tests or 'care-sets' when assessing certain patient cohorts.

Caution should be used in considering if tests for nuclear autoantibodies are included in such 'care-set' panels and the advice of Consultant Immunologists should be taken. Results should be interpreted within the clinical context of the individual patient, reflecting on the pre-test probability of disease. Users who have to date deployed tests for nuclear autoantibodies in low pre-test probability scenarios, should understand that clinically irrelevant, false positive results will be generated and are advised to change practice. If users continue to request nuclear autoantibodies in settings where the clinical suspicion of a related disease is low, they should ensure they have a plan and requisite skills to manage the issues that arise without recourse to onward referral.

In summary, tests for nuclear autoantibodies are a valuable diagnostic tool when used appropriately. An understanding of the methodologies involved, and a consideration of the pre test probability are essential to interpret results correctly and to avoid pitfalls associated with false positive or false negative results.

References

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