THE PATHCARE NEWS

AUTOIMMUNE DISEASE:
USE OF ANTINUCLEAR AND SPECIFIC ANTIBODIES FOR DIAGNOSIS

Autoimmune conditions pose a diagnostic challenge as the presenting clinical symptoms may be non-specific, vary from patient to patient and often overlap. **Moreover, there is no single diagnostic test for any one autoimmune disease**. Diagnosis is most often based on a combination of clinical information, patient history, laboratory testing and in some cases imaging studies. **Laboratory testing includes relatively non-specific antinuclear antibody (ANA) testing and/or tests for individual antibodies that are more disease specific.**

ANAs refer to a collection of autoantibodies that target a variety of nuclear and cytoplasmic antigens. First described more than 50 years ago, ANAs still remain the most sensitive serologic marker for evaluating patients with suspected connective tissue diseases, also referred to as ANA Rheumatic Diseases (AARDs). Several test methods for ANA testing are available in clinical laboratories including the immunofluorescence assay (IFA), enzyme linked immunoassays (ELISA) and multiplex immunobead assays. **The American College of Rheumatology (ACR) recommend using the IFA with Hep-2 cells, mainly because of the high sensitivity of this assay.**[1] This high sensitivity stems from the high number of autoantigens (up to 150) present in the HEP-2 cells.

With the IFA test method ANA is observed and reported as a fluorescence pattern. These patterns are not specific for a specific disease type, but the information may guide towards follow-up disease-specific antibody testing, which may aid clinical diagnosis.

A positive ANA result does not necessarily indicate the presence of an autoimmune condition. Healthy individuals, especially in older age, and those with infectious diseases or malignancies, may present with positive ANA test results that may be unrelated to an autoimmune condition. Therefore, ANA must be reviewed in the proper clinical context. In contrast with the ANA IFA test method, the immunoassay specific test methods may have a higher specificity for a specific autoimmune disease but lack the sensitivity of the ANA IFA.

There is no single best way to approach laboratory testing for autoimmune diseases – the approach depends on the clinical picture of the patient. PathCare offer the following testing strategies:

1. ANA IFA (Hep-2 cells) + reflex specific autoantibody testing as indicated by the ANA IFA pattern
2. Clinically driven test panels as recommended by rheumatology associations such as the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR)

ANA on the IFA on Hep-2 cells method lack the sensitivity to detect low levels of SSA and dsDNA antibodies, therefor the ANA IFA are offered in combination with SSA and dsDNA antibody-specific immunoassays in the ANA Screen test, in order to increase sensitivity and specificity of antinuclear antibody testing.*

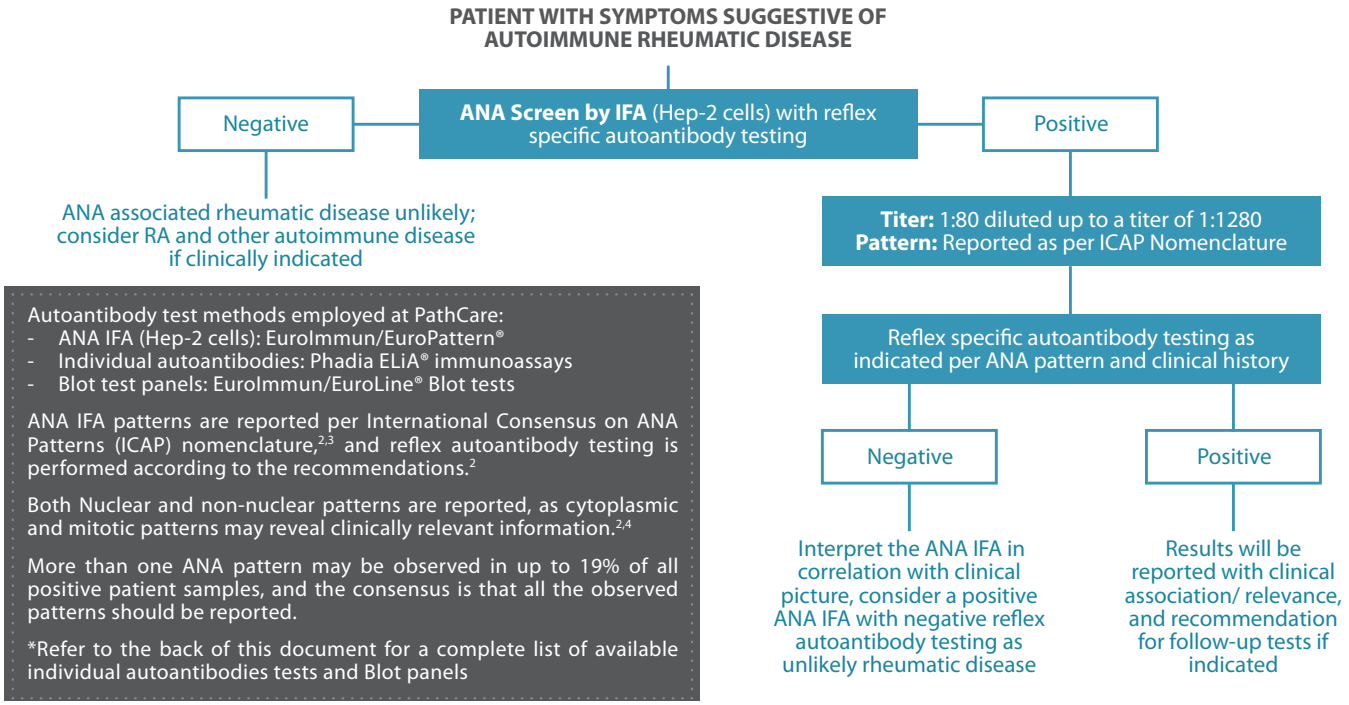

Figure 1. PathCare protocol for ANA screening in patients with suspected autoimmune rheumatic disease

Clinical driven test panels:

The recommended panels below are in accordance with pushed criteria from clinical associations such as the American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) per autoimmune condition.

Clinical Condition	Recommended tests
SLE	ANA Screen*, FBC+ Diff + platelets, Antiphospholipid antibodies, Lupus anticoagulant, C3+C4, dsDNA & Sm antibodies, urine protein: creatinine.
Sjögren syndrome	ANA Screen*+ SSA + SSB antibodies.
Systemic Sclerosis	ANA Screen*+ Systemic Sclerosis" Blot test 13 antibodies.
Rheumatoid Arthritis	RF-IgM, anti-CCP, ESR and CRP.

Clinical Condition	Recommended tests
Autoimmune Liver Conditions	ANA Screen*+ IgG + IgM + Liver autoantibody and related Blot test autoantibodies (14 Antibodies including anti-mitochondrial, Liver-Kidney microsomal-1 antibodies).
Drug Induced Lupus	ANA Screen*+ dsDNA + Histone antibodies.
Autoimmune Myositis	ANA Screen*+ Myositis and related autoantibody Blot test (16 Antibodies).

Other Autoimmune tests and test panels:

Test/Condition	Test Description
ANA Screen*	ANA IIF (HEp-2 cells) + dsDNA + SSA antibodies. Relevant ENA antibodies as indicated by the observed ANA pattern will be reflexed from titres 1:160.
CTD Screen	The ELiA® CTD screen is a mixture of 14 autoantibodies and are resulted qualitatively as positive/negative. Antibodies included in the mixture: dsDNA, Scl-70, Centromere B, U1-RNP, fibrillarin, Sm, SSA52/60, SSB, Jo-1, Ribosomal P, PM-Scl, Mi-2, RNA-polymerase III, PCNA (These antibodies can be requested separately).
ENA Antibodies	ENA antibodies that are available as individual analytes: Scl-70, Centromere B, U1-RNP, fibrillarin, Sm, SSA52/60, SSB, Jo-1, Ribosomal P, PM-Scl, Mi-2, RNA-polymerase III, PCNA, Histone antibodies.
dsDNA	dsDNA is performed on the Phadia ELiA® immunoassay with a quantitative result. If the dsDNA result is discrepant with the ANA IIF pattern (nuclear homogeneous) a nucleosomal dsDNA ELISA assay will be reflexed.
ANA Antigen Blot test	Antibodies included in this panel (23): DFS-70, PCNA, gp210, RP155, RP11, PM-Scl100, Scl-70, PML, Sp100, CENP B, CENP A, Ku, Mi-2α, Mi-2β, Sm, RNP/Sm, SSB, Ro52, SSA, Histones, Nucleosomes, dsDNA.
Systemic Sclerosis Blot test	Antibodies included in this panel (13): R0-52, PDGFR, Ku, PM-Scl75, PM-Scl100, Th/To, NOR90, Fibrillarin, Rp155, RP11, CENP B, CENP A, Scl-70.
Myositis Blot test	Antibodies included in this panel (16): Ro-52, OJ, EJ, PL-12, PL-7, SRP, Jo-1, PM-Scl75, PM-Scl100, Ku, SAE1, NXP2, MDA5, TIFγ, Mi-2α, Mi-2β.
Autoimmune Liver Condition Blot test	Antibodies included in this panel (14): PDGH, CENP B, CENP A, Scl70, Ro-52, SSA, SLA/LP, LC-1, LKM-1, gp210, PML, Sp100, M2-3E, AMA-M2+ total IgG and IGM.

Test/Condition	Test Description
Autoimmune Encephalitis /Paraneoplastic panel(including anti-Cerebellum antibodies)	Antibodies included in this test panel: NMDA, AMPA1&2, VGKC (CASPR2, LGI1, DPPX. Ri, Yo, Hu, Ma/Ta, CV2, Purkinje, Neurone, Amphiphysin, PNMA2, SOX1, Titin, Zic4, Tr (DNER) and GAD65 (Blot)
Ganglioside antibodies Blot	Antibodies included in this test panel: GM1, GM2, GM3, GD1a, GD1b, GQ1b, GT1b IgM & IgG.
Stiff man Syndrome	GAD65.
Neuromyelitis Optica (NMO)	AQP-4 Antibodies and MOG.
Myasthenia Gravis	ACH-R antibodies. Muscle Specific Kinase antibodies (send overseas).
ANCA	Test includes p- and c-ANCA on IIF, quantitative PR3 and MPO antibodies on ELiA immunoassay.
Rheumatoid Arthritis markers	RF-IgM and anti-CCP (ELiA® immunoassay).
Thyroid Antibodies	TG, TPO and TSH-Receptor antibodies are all available as individual markers.
Coeliac Disease Markers	TTG-IgA, Gliadin IgG, TTG-IgG, Gliadin-IgA, Endomesial antibodies, Total IgA as well as HLA DQ2 and DQ8 are available as individual requests or as part of Coeliac screen (TTG-IgA + Gliadin IgG).
Inflammatory Bowel Disease	ANCA, Saccharomyces cerevisiae antibodies, faecal Calprotectin.
Autoimmune Gastritis	Parietal cells and Intrinsic factor antibodies.
Diabetes Mellitus Type1	GAD65 + IA2 antibodies.
Antiphospholipid antibodies	Cardiolipin and B2-glycoprotein IgG & IgM.
Glomerulonephritis	ANCA and GBM-Antibodies (as per immunoassay) available as separate requests.
Primary Membranous Nephropathy	PLA-R2 receptor antibodies.
Autoimmune Pancreatitis	IgG4.
Ankylosing spondylitis	HLA B27.
Other	Other autoantibodies not listed may be available. Please enquire at PathCare laboratory for the availability of the tests.

A PathCare Autoimmune test request form is available, and may be requested from a local PathCare laboratory or at clients@pathcare.co.za

Compiled by: Drs Pierre Schoeman and Mariana Lloyd, PathCare Pathologists, 021 596 3400

References:

1. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis (2010) doi:10.1136/ard.2009.127100.
2. Damoiseaux J Et al. Clinical relevance of HEP-2 indirect immunofluorescence patterns: The International Consensus on ANA patterns (ICAP) perspective. Ann Rheum Dis 2019; 78:879-889.
3. International Consensus on ANA Patterns (ICAP): anapatterns.org