

November 2022

UPDATE: AUTOIMMUNE DIAGNOSTIC WORKUP

Connective tissue disease (CTD) is a heterogeneous group of diseases characterised by antinuclear antibodies (ANA), as well as other distinctive autoantibodies. In the first version of this LabUpdate, we announced a simplified approach to the diagnosis of CTD, with updated antibody profiles and terminology. In this drastically changing milieu, with ever-increasing cost-to-patient considerations, the latest developments (with updated terminology on available autoimmune profiles) are described below.

UPDATED TERMINOLOGY

- **ENA/CTD screen**

The previously offered CTD Profile 1 and 2 are now offered as the ENA/CTD **profile**. The ENA/CTD profile, a line immunoassay, is the most comprehensive and cost-effective test for detecting a wider array of auto-antibodies.

The ENA/CTD **screen** (consisting of RNP, Sm, SSA (Ro-60), SSB (La), Scl-70 (Topoisomerase I), Jo-1, Centromere-B, PM-Scl, Mi-2, Fibrillarin, PCNA, Ribosomal P-protein, dsDNA antibodies, RNA Pol III and Ro-52) will be reported as **positive** or **negative**.

If the ENA/CTD **screen** is **positive**, it is imperative to request an ENA/CTD **profile**, which reports antibodies qualitatively, so as to identify the specific autoantibody.

The ENA/CTD **profile** includes 16 main auto-antibodies: Sm/RNP, Sm, SSA (Ro-60), Ro-52, SSB (La), Scl-70 (Topoisomerase I), PM-Scl, Jo-1, Centromere B, PCNA, Nucleosome, Histone, Ribosomal P-protein, AMA-M2 and DFS-70.

Accordingly, this profile presents a cost-effective option and, additionally, is free from the interfering streptavidin biotin system, which is incorporated in many immunological assays. Streptavidin antibodies can lead to false positive results.

If the ENA/CTD **screen** is positive, but the ENA/CTD **profile** is negative, a myositis line immunoassay or systemic sclerosis line immunoassay should be considered, depending on your clinical findings. Individual ENA/CTD antigens can be requested.

- **Double-stranded DNA**

A dsDNA ELISA (dsDNA complexed with nucleosome, thus excluding non-specific binding) is available to get quantitative data for monitoring. This test must be specifically requested.

- **Cytoplasmic antibodies**

If cytoplasmic antibodies are identified on the ANA screen, this will be reported with an option to do a cytoplasmic autoimmune profile.

TABLE 1: UPDATED AUTOIMMUNE PROFILES

Profile	Components
Cytoplasmic profile	AMA-M2, 3E (BPO), Ribosomal P, Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52
Myositis profile (previously Myositis Western Blot)	Mi -2 α (Mi - 2alpha); Mi -2 β (Mi - 2beta), TIF1 γ , MDA5, NXP2, SAE1, Ku, PM-Scl 100, PM-Scl 75, Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52
Systemic sclerosis profile (previously Systemic Sclerosis Western Blot)	ScI-70 (Topoisomerase I), Centromere A, Centromere B, RNA Pol III (RP 11), RNA Pol III (RP 155), Fibrillarin, NOR-90, Th/To, PM-Scl 100, PM-Scl 75, Ku, PDGFR and Ro-52
Autoimmune liver profile (previously Liver Western Blot)	AMA-M2, 3E (BPO), Sp 100, PML, gp210, LKM-1, LC-1, SLA/LP and Ro-52
ENA profile (previously ENA Western Blot)	Sm/RNP, Sm, SSA (Ro-60), Ro-52, SSB (La), ScI-70 (Topoisomerase I), PM-Scl, Jo-1, Centromere B, PCNA, Nucleosome, Histone, Ribosomal P-protein, AMA-M2 and DFS70
Neuronal profile	IFA: Yo, Hu and Ri Amphiphysin, CV2, PNMA2(M α -2/T α), Ri, Yo, Hu, Recoverin, SOX1, Titin, Zic4, GAD65, Tr (DNER)
NMDA profile	NMDA, AMPA 1, AMPA 2, GABA-B-receptor, CASPR2, LGI-1
Ganglioside profile	GM1 IgM, GM1 IgG, GM2 IgM, GM2 IgG, GD1a IgM, GD1a IgG, GM3 IgM, GM3 IgG, GD1b IgM, GD1b IgG, GT1b IgM, GT1b IgG, GQ1b IgM, GQ1b IgG
Other organ-specific profiles and antibodies are available as indicated on the autoimmune test request form.	

RECOMMENDATIONS

If a connective tissue disease is suspected, the following **autoimmune profiles** are recommended:

- ANA + ENA/CTD screen with breakdown if positive
- Why not only ANA?
 - Even with a negative ANA, an ENA/CTD screen is always a good idea. Some autoantigens (including Ro-52, SSA (Ro-60), ribosomal P-protein and inflammatory myositis-associated antigens) may not be detected with the gold standard immunofluorescent ANA test.
 - Combining the ANA with an ENA/CTD screen can lead to early diagnosis and treatment of autoimmune diseases.

IN CONCLUSION

Ampath remains committed to implementing novel and relevant autoimmune testing, as well as to simplifying the process of requesting diagnostic tests for autoimmunity, while containing costs to the patient. It is in the best interest of patients to have both an ANA and ENA/CTD screening test, as this will increase the detection of connective tissue diseases. An updated comprehensive autoimmune request form is available. Tests can be ordered electronically, while personalised test request forms can be ordered from your Ampath representative. In this rapidly developing field, rare and new autoantibodies can be requested as a send-away test to international centres.