# Connective Tissue Disease

## Tools to Aid in the Accurate Diagnosis of Connective Tissue Disease

Inova Diagnostics

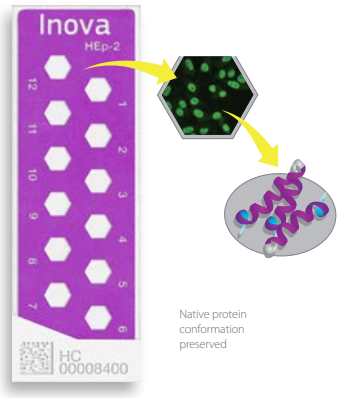
A Werfen Company

# High quality assays and novel tests

Inova offers a complete array of assay methods, including immunofluorescence (IIF), enzyme linked immunosorbant assay (ELISA), and chemiluminescence immunoassay (CIA).

The **QUANTA Lite®** line of ELISAs covers the spectrum of CTD testing from screening assays with 4 or more antigens to specific extractable nuclear antigens (ENA). The **QUANTA Flash® CIA** assays offer broad dynamic range, high sensitivity and specificity. The QUANTA Flash assays for use on the BIO-FLASH® system offer a robust menu including critical assays like QUANTA Flash dsDNA® to maximize the utility of this fully automated random access system.

One of the most important first steps in CTD diagnosis is performing a screening test. Screening ANA by IIF is the gold-standard, as recently recommended by the American College of Rheumatology Task Force. To that end, Inova has revolutionized HEp-2 ANA screening by utilizing optimized antigen expression to allow identification of the most complicated cell structures with the **NOVA Lite®** assays. A completely unique assay, NOVA Lite® HEp-2 Select®, reduces the rate of false positivity in routine ANA screening samples. This culminates into overall healthcare cost savings and efficiency.



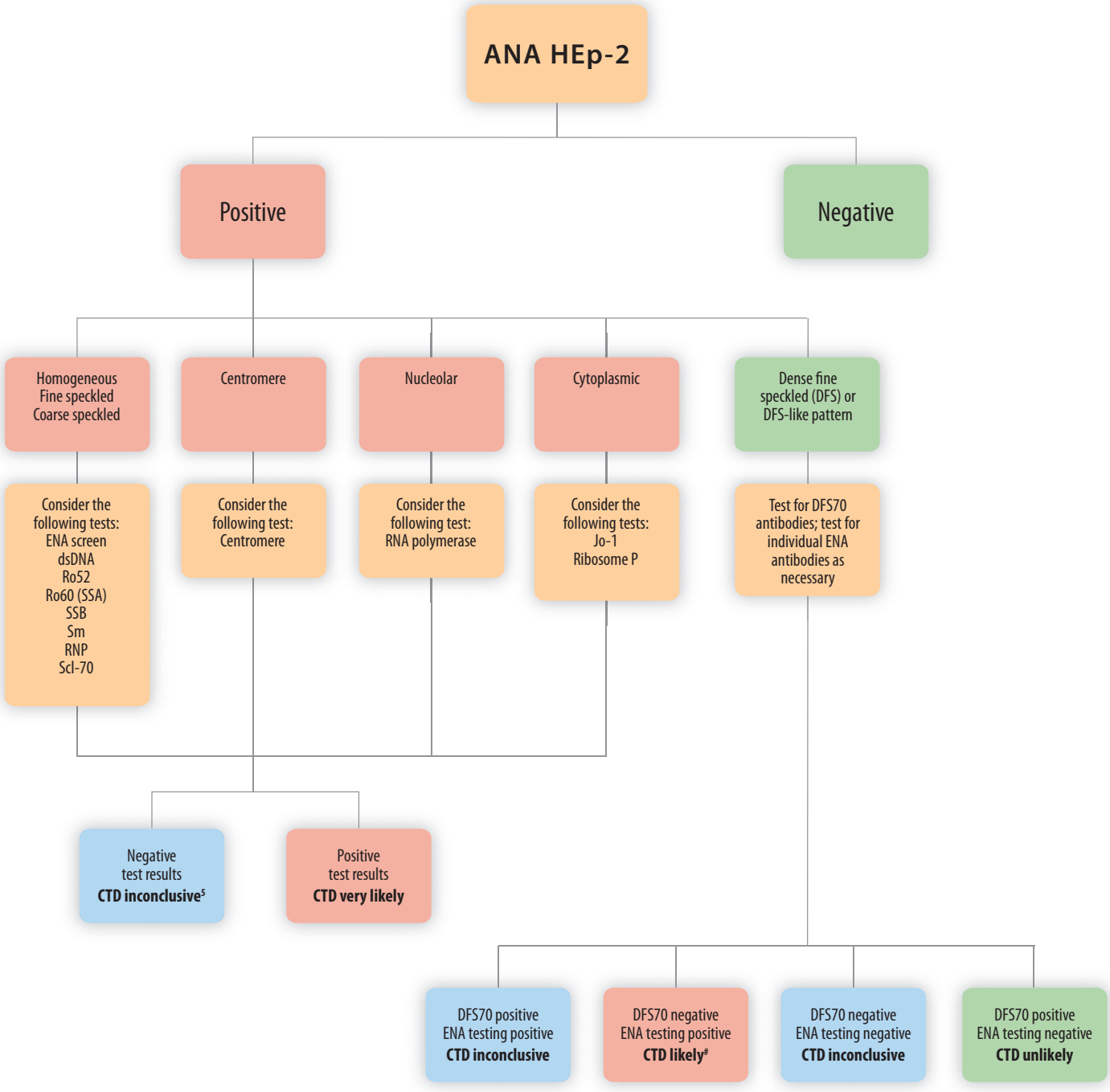

## Disease overview

The complex diagnosis of CTD involves a multitude of sometimes ambiguous clinical symptoms. The guide below highlights the most important clinical features and disease statistics.

| Disease | Epidemiology |
|---|---|
| **Systemic Lupus Erythematosus**<br>Systemic lupus erythematosus (SLE) is a disease which affects multiple organs. The classic triad of fever, joint pain, and rash in a woman of childbearing age should prompt investigation into the diagnosis of SLE. | - Worldwide, the prevalence varies by race; typically 50 cases per 100,000 persons<br>- African American women have a higher rate of SLE than women of any other race, followed by Asian women and then white women<br>- Female to male ratio is 9:1; onset is usually after puberty |
| **Systemic sclerosis**<br>Systemic sclerosis (SSc) is a chronic, multisystem disorder characterized by thickening of the skin and accumulation of connective tissue in various organs. | - Prevalence is estimated at 240 cases per 1,000,000 persons<br>- Female to male ratio ranges from 4-9:1; onset typically occurs at 30-50 years of age |
| **Sjögren's syndrome**<br>Sjögren's (SjS) syndrome is characterized by dry eyes and dry mouth. | - Affects 0.1-4% of the population; this wide range reflects the lack of uniform diagnostic criteria<br>- Homogeneous disease occurring worldwide with similar prevalence<br>- Female to male ratio is 9:1; onset typically occurs 40-50 years of age |
| **Idiopathic inflammatory myopathies**<br>Idiopathic inflammatory myopathies are a group of chronic conditions characterized by inflammation and degeneration of skeletal muscles.<br><br>These disorders include dermatomyositis (DM), polymyositis (PM), and inclusion body myositis (IBM). | - Very low prevalence disease; incidence is 4-10 per 1,000,000 persons<br>- Age of onset varies<br>  - DM is bimodal with disease occurring in childhood and 50-70 years of age<br>  - PM typical onset is >20 years of age<br>  - IBM typical onset is >50 years of age<br>- Females to male ratio is 2:1 in DM and PM<br>- Males to female ratio is 2:1 in IBM |
| **Mixed Connective Tissue Disease**<br>Mixed Connective Tissue Disease (MCTD) patients exhibit varied combinations of features common to other autoimmune diseases such as SLE, polymyositis, rheumatoid arthritis, or scleroderma. | - Prevalence of 1-2 per 100,000 persons<br>- Typically occurs 15-25 years of age<br>- Female to male ratio is 3:1 |

**References**
- SLE - Danchenko, N et al. Lupus 2006; 15(5):308-318. Blank, M et al. Lupus 2009; 18(13): 1136-1143. Klein-Gitelman, M et al. Rheum Dis Clin North Am. 2002; 28(3): 561-577.
- Scleroderma - Walker, J et al. Curr Opin Rheumatol. 2007; 19(6):580-591. Hsu, V et al. J Rheumatol. 2008; 35(3): 458-465.
- Sjögren's syndrome - Helmick, C et al. Arthritis Rheum. 2008; 58(1):15-25.
- Inflammatory myopathies - Nasr, R et al. Curr Opin Rheum. 2012; 24(6): 609-615. Ernste, F et al. Mayo Clin Proc. 2013; 88(1): 83-105.
- MCTD - Aringer, M et al. Best Pract Res Clin Rheumatol. 2007; 21(6): 1037-1049. Swanton, J et al. Rheum Dis Clin North Am. 2005; 31(3): 421-436.

# Potential Testing Algorithm for Connective Tissue Disease




[5] Likelihood depends on IIF pattern obtained
[#] CTD is likely if results can be confirmed (e.g., ENA sub-differentiation). Further studies are needed to determine the likelihood.

Testing
CTD likely
CTD unlikely
CTD inconclusive

Testing strategies provided for informational purposes only.
Testing and diagnosis should be determined by a licensed clinician.

# Important assays you should know about

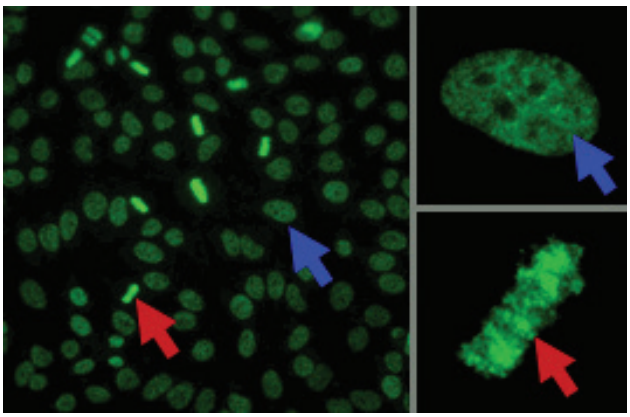
## Critical answers to complicated cases

### High prevalence of anti-DFS70 antibodies in routine screening cohorts

Screening patients suspected of having systemic autoimmune rheumatic disease by HEp-2 is the gold-standard method. However, based on the population being tested, the frequency of false positive ANA results may be quite high. It has been recently discovered that a majority of such positives are the result of anti-DFS70 antibodies. Anti-DFS70 antibodies are responsible for as much as 15% of positive HEp-2 results in routine testing even though these antibodies are not associated with systemic autoimmune rheumatic disease.

- ANA positive results often contribute to high anxiety both among patients and clinicians.
- Confirming isolated anti-DFS70 positivity reduces unnecessary reflex and follow-up testing, while lessening patient concerns.
- Adsorption of anti-DFS70 antibodies by NOVA Lite HEp-2 Select, reduces HEp-2 positivity rates thereby increasing overall healthcare efficiency
- The presence of anti-DFS70 antibodies can be positively confirmed by using the QUANTA Flash DFS70 assay

**Characteristic dense fine speckled pattern is a challenge to interpret by IFA**



Dense fine speckled pattern of resting cell (blue arrow)
Chromatin staining of mitotic cell (red arrow)

### Anti-C1q antibodies may be prognostic for lupus flares

Over 50% of SLE patients will display nephritis. Predicting flare up activity is essential to preventing further relapses and subsequent renal damage. According to Akhter, E. et al. 2010:

- Anti-C1q may play a role in the propagation of SLE by enhancing the development of anti-dsDNA and other glomerular-targeting autoantibodies, by reducing the amount of C1q available for effective clearance of these antigens or by enhancing the pathogenicity of C1q-containing immune complexes.
- Compared to dsDNA, chromatin, and ribosomal P autoantibodies, anti-C1q is the only marker associated with both global and renal activity as well as the SLICC renal activity score.

### Anti-HMGCR antibodies and their association with statin associated myopathies

Statins are among the most commonly prescribed medications that significantly reduce cardiovascular risk. However, these drugs can also be associated with muscle symptoms ranging from mild myalgias to severe rhabdomyolysis.

- Although statin myotoxicity is usually self-limited, in some instances statin-exposed subjects can develop an autoimmune myopathy typically characterized by progressive weakness, muscle enzyme elevations, a necrotizing myopathy on muscle biopsy, and autoantibodies that recognize 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the pharmacologic target of statins.
- These antibodies are also found in some autoimmune myopathy patients without statin exposure.
- Testing for anti-HMGCR antibodies may help differentiate those with self-limited statin myopathy who recover after statin discontinuation from those with a progressive statin-associated autoimmune myopathy who require immunosuppressive therapy.

**References**
- DFS70 – Mahler, M et al. J Rheum 2012; 39 (11); 2104-2110.
- C1q – Akhter, E et al. Lupus 2011; (20) 12: 1267-1274.
- HMGCR – Mohassel, P. et al. Muscle & Nerve 2013; DOI 10.1002/mus. 23854

*QUANTA Flash DFS70 is available outside the USA only

# Connective Tissue Disease

| | Prevalence of autoantibodies in various conditions and clinical associations[#] | | |
|---|---|---|---|
| | **Autoantibody** | **Estimated autoantibody frequency** | **Clinical association(s)** |
| SLE specific | dsDNA | 30-90% | Classification marker for SLE ; lupus nephritis |
| | Sm | 10-30% | Classification marker for SLE ; frequent in African Americans |
| | Ribosomal P | 10-20% | Frequent in Asians; can be present in isolation to other SLE autoantibodies |
| | Chromatin | 30-90% | Helpful in cases of anti-dsDNA negative SLE patients; lupus nephritis |
| SLE associated | Histone | 50-70% | Helpful for differentiating between SLE and drug induced lupus |
| | C1q | 30-60% | SLE and lupus nephritis |
| | RNP | 25-40% | Not specific for SLE |
| | Ro52 | 40-60% | Ro52 autoantibodies are usually detected in conjunction with Ro60 (SSA); congenital heart block in babies born to women with SLE |
| | Ro60 (SSA) | 20-60% | Secondary SjS; discoid rash and photosensitivity |
| | SSB | 10-30% | Secondary SjS; discoid rash and photosensitivity |
| SSc | Centromere | 20-60% | Classification marker for SSc; CREST syndrome, limited SSc, Raynaud's phenomenon, low occurance of pulmonary fibrosis |
| | Scl-70 | 15-40% | Classification marker for SSc; diffuse skin involvement, pulmonary interstitial fibrosis, increased mortality rate |
| | RNA Polymerase III | <20% | Classification marker for SSc; highly specific for diffuse cutaneous disease; heart and kidney involvement |
| | Th/To | <10% | Limited cutaneous SSC and interstitial lung disease |
| SjS | Ro52 | 37-75% | Ro52 autoantibodies are usually detected in conjunction with Ro60 (SSA) autoantibodies |
| | Ro60 (SSA) | 50-80% | Classification marker for SjS |
| | SSB | 40-60% | Classification marker for SjS |
| Idiopathic inflammatory myopathies | Jo-1 | 10-40% | Interstitial lung disease, Raynaud phenomenon, and antisynthetase syndrome |
| | PM/Scl | 10-25% | SSc polymyositis overlap |
| | Ro52 | 20-35% | Polymyositis with antisynthetase syndrome; often detected in conjunction with Jo-1 autoantibodies |
| | HMGCR | <10% | Statin associated myopathy; useful to differentiate patients with self-limited statin myopathy vs patients with a progressive statin-associated autoimmune myopathy who require immunosuppressive therapy |
| | Mi-2 | <25% | Very specific marker; often seen in dermatomyositis |
| | SRP | <5% | Polymyositis; does not occur with overlap syndromes |
| | Ku | <5% | Overlap syndromes |
| MCTD | RNP | ~ 100%* | Classification marker for MCTD |
| Non-connective tissue disease | DFS70 | 5-10% | Common cause of positive ANA result in healthy or non-CTD individuals; isolated presence is not associated with CTD |


**References**

- SLE – Salvador, I. et al. J Trans Med 2012; 10 (3): 41. Menendez, A. et al. Sci World 2013; article ID 832789, 8 pages.
- Systemic sclerosis – Gussin, H et al. Arth Rheum 2001; 44: 376-383. Ramos-Casals, M et al. BMJ 2012: 344:e3821. Schulte-Pelkum, J et al. Autoimm Rev 2009; 8: 632-637.
- Sjögren's syndrome – Fox, R et al. Lancet 2005; 366: 321-331. Shiboski, SC et al. Arthritis Care Res 2012; 64 (4): 475-487.
- Inflammatory myopathy – De Santis, M et al. Ann Rheum Dis 2012; 71(9): 1461-1465.
- MCTD – Gunnarsson, R et al. Ann Rheum Dis 2011; 70 (6): 1047-1051.
- DFS70 – Mahler, M et al. J Rheum 2012; 39 (11); 2104-2110.


# Autoantibodies listed reflect current or planned Inova offering

* Anti-RNP are classification criteria autoantibodies. Actual frequency of detection may be lower than 100%.

# Comprehensive offering of assays

| Method | Name |
|---|---|
| **NOVA Lite IIF** | HEp-2 |
| | DAPI ANA |
| | HEp-2 Select* |
| | dsDNA Crithidia luciliae |
| | dsDNA Crithidia luciliae DAPI conjugate |
| **QUANTA Flash CIA** | ENA 7 |
| | Jo-1 |
| | RNP |
| | Sm |
| | Ro60 (SSA) |
| | Ro52 |
| | SSB |
| | Scl-70 |
| | CTD Screen Plus* |
| | Centromere |
| | RiboP* |
| | DFS70* |
| | dsDNA |

| Method | Name |
|---|---|
| **QUANTA Lite ELISA** | ANA |
| | dsDNA |
| | dsDNA SC |
| | HA dsDNA |
| | Chromatin |
| | C1q* |
| | C1q CIC |
| | Centromere |
| | Histone |
| | HMGCR* |
| | Jo-1 |
| | Ribosome P |
| | RNA Polymerase III |
| | RNP |
| | Scl-70 |
| | Sm |
| | SSA |
| | SSA 52 |
| | SSB |
| | ssDNA |
| | ENA 4 |
| | ENA 5 |
| | ENA 6 |
| | ENA Profile* |

*Available outside the USA only

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