Quest Diagnostics™

IdentRA® test panel with 14-3-3eta

A clinically proven biomarker for earlier, accurate RA diagnosis—and now, prognosis and monitoring

Did you know there are more than **100 forms of arthritis?**

Every type of arthritis is a challenge

Differential diagnosis and early diagnosis of rheumatoid arthritis (RA) are difficult, as patients present with nonspecific symptoms involving pain and stiffness, and symptoms may involve joints that have similar swelling and fluid dynamics.

Who should be tested for RA? The ACR target population

The American College of Rheumatology (ACR) guideline for patient testing to screen for rheumatoid arthritis:

1 joint with definitive clinical synovitis (swelling), not explained by another disease[3]

More than **1** in **5** adults has arthritis (53 million)[1]

1 in **162** has rheumatoid arthritis (1.5 million)[1]

30 million have osteoarthritis[1]

7.5 million have psoriasis, and 30% of these will develop psoriatic arthritis[2]

2010 ACR-EULAR* Classification Criteria for RA[3]

Score of ≥ 6/10 needed for "definite RA"	
Joint involvement	
1 large joint	0
2-10 large joints	1
1-3 small joints	2
4-10 small joints	3
> 10 joints (≥ 1 small joint)	5
Serology (≥ 1 test result needed)	
Negative RF *and* negative ACPA†	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
Acute phase reactants (≥ 1 test result needed)	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
Duration of symptoms	
< 6 weeks	0
≥ 6 weeks	1

Lab testing matters, but current markers are sub-optimal

Among ACR-EULAR classification criteria, 40% are based on serology. However, current markers have limitations.

- C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are general inflammatory markers, but not specific to RA
- Rheumatoid factor (RF) has low sensitivity and low specificity
- Cyclic citrullinated peptide (CCP) has low sensitivity but high specificity for RA, but is underutilized in primary care
- RF and CCP together demonstrate low sensitivity in early RA, not optimal for screening
- 28% to 44% of patients with RA are seronegative for RF[4] and CCP, which means RA patients can be overlooked

*American College of Rheumatology-European League Against Rheumatism

†ACPA is an anti-citrullinated peptide antibody, commonly called Cyclic citrullinated peptide (CCP) antibody in the U.S.

14-3-3eta enables an **earlier, more accurate** diagnosis for RA[4-6]

The IdentRA panel includes 14-3-3eta, a novel, **mechanistic** biomarker for early RA.*

Mechanistic biomarker is directly involved in the pathogenesis of RA disease

What it is:
14-3-3 proteins are molecular chaperones critical to the regulation of intracellular functions.

What it does:
14-3-3eta is released extracellularly in the surrounding synovial fluid and peripheral blood when there is joint inflammation. The "eta" isoform is found in significantly higher amounts in the serum and synovial fluid of individuals with inflamed joints compared with healthy subjects.

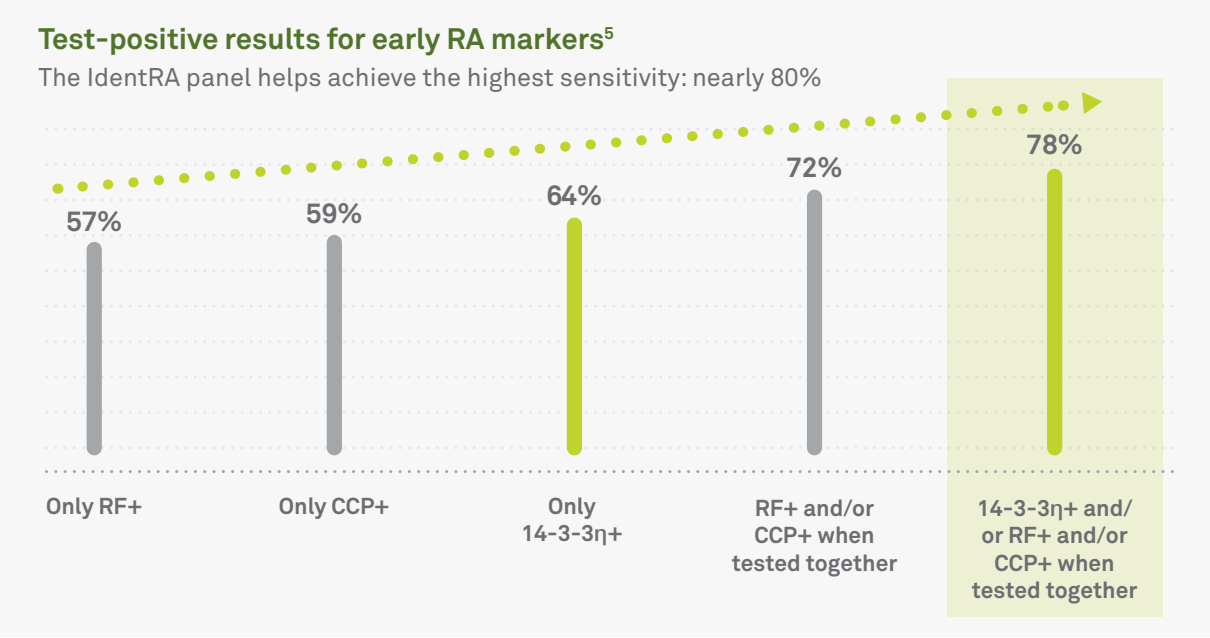
What makes it specific to RA:
Blood levels of 14-3-3eta are significantly higher in patients with early and established RA, but not in healthy individuals or those with other inflammatory diseases, including:

Crohn's disease • gout • lupus • multiple sclerosis • osteoporosis • psoriasis†
Sjögren's syndrome • systemic sclerosis • type 1 diabetes • ulcerative colitis

93%

specificity of 14-3-3eta[5]

14-3-3eta outperforms both RF and CCP:
RF and CCP demonstrate 57% and 59% sensitivity, respectively, vs. 64% for 14-3-3eta.[5]



14-3-3eta identifies patients who are RF and CCP seronegative

Seronegativity in early and established RA‡ is a major limitation of the traditional markers, RF and CCP, and can delay proper diagnosis and treatment.

- Seronegative rates for RF and CCP in patients with RA range from 28% to 44%[4]

14-3-3eta positively identifies RA in RF/CCP seronegative patients[6]

- 21% of patients with early RA
- 67% with established RA

*Defined as symptoms lasting fewer than 6 months.
‡Defined as symptoms lasting longer than 6 months.

†In psoriasis, 14-3-3eta is a marker that signals the onset of erosive psoriatic arthritis.

Early RA screen, diagnosis, and care: **the RA patient care continuum**

Modifiable biomarker—measurable level declines when patient is responding to therapy

A RA Screen [Differential diagnosis of RA vs. OA]

- 14-3-3eta ≥ 0.19 ng/mL
- RF ≥ 14 IU/mL
- CCP antibody IgG
  - Negative: < 20 units
  - Weak positive: 20–39 units
  - Moderate positive: 40–59 units
  - Strong positive: > 59 units
- C-reactive protein (CRP)
- Erythrocyte Sedimentation Rate (ESR)
- 14-3-3eta level is not elevated in OA

B RA Prognosis[7,8] 14-3-3eta ≥ 0.50 ng/mL:

- Predicts poorer clinical and radiographic outcomes at baseline
- Predicts high risk of clinically refractory RA
- Predicts significant joint damage over next 5 years

C RA Management and Care: disease progression

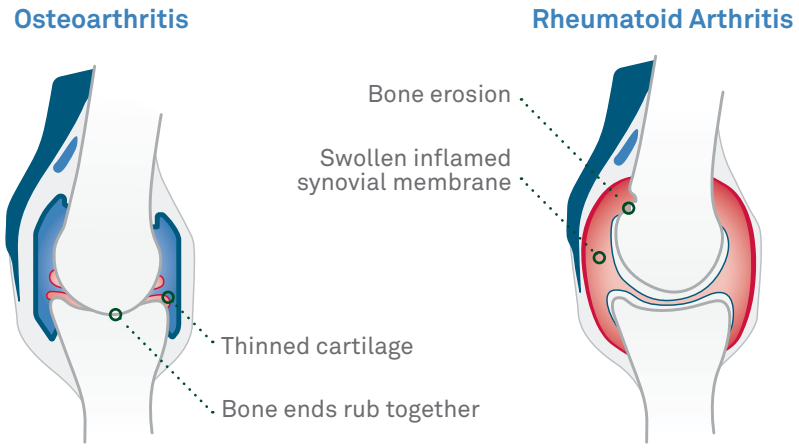
- Test CRP, ESR, 14-3-3eta
- Others: TB, HBV, HCV, CVD

D Treatment Response and Monitoring[9–11]

- 14-3-3eta is a **modifiable** marker
- Serial 14-3-3eta is useful for monitoring
- A decrease in response to DMARDs and anti-TNF drugs indicates better clinical outcomes
- An increase in response to DMARDs and anti-TNF drugs is associated with worse outcomes

14-3-3eta can aid differential diagnosis of RA vs. OA[5]

- Positive/elevated result for one or more markers (RF, CCP antibody, and 14-3-3eta) is suggestive of inflammatory arthritis
- Median serum levels elevated in RA patients but not OA patients
- Median serum levels are elevated in erosive PsA vs. nonerosive PsA
- RA and erosive PsA may coexist with OA



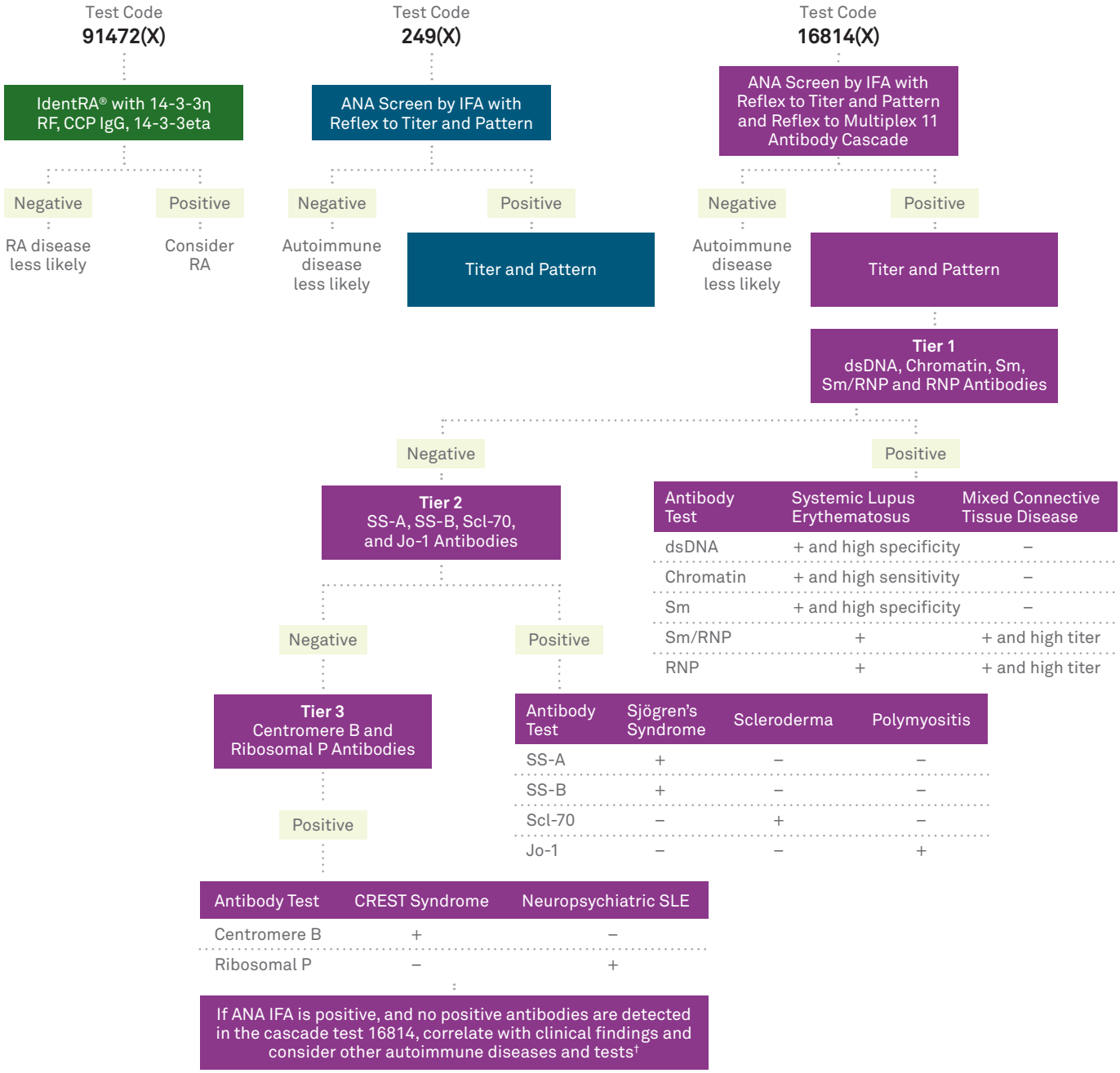
14-3-3eta is prognostic[7,8]

- Decreased levels are associated with better clinical outcomes
- An increase implies a worse prognosis

14-3-3eta can be tested serially for monitoring[9,10]

- A decrease marks response to DMARDs and anti-TNF drugs

Algorithm for **screening and diagnosis** of patients with suspected autoimmune disease*



The acronym CREST refers to a syndrome defined by the presence of calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia. dsDNA indicates double-stranded DNA; Sm/RNP, Smith/ribonucleoprotein; SS-A and SS-B, Sjögren's Syndrome A and B; Scl-70, topoisomerase; Jo-1, histidyl-tRNA synthetase; and SLE, systemic lupus erythematosus.

This figure was developed by Quest Diagnostics® based on references 12-16. It is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient-management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

*Laboratory testing should be directed by careful history and examination of the patient.

†Drug-induced lupus, polymyositis/dermatomyositis, rheumatoid arthritis, oligoarticular juvenile chronic arthritis, polyarteritis nodosa. Patients with organ-specific autoimmune diseases may also have a positive test for ANA. These diseases include: thyroid diseases (Hashimoto's thyroiditis, Grave's disease), gastrointestinal diseases (autoimmune hepatitis, primary biliary cholangitis [also known as primary biliary cirrhosis], inflammatory bowel disease), pulmonary diseases (idiopathic pulmonary fibrosis). Patients with infectious diseases may also test positive for ANA. These diseases include: viral infections (hepatitis C, parvovirus), bacterial infections (tuberculosis), parasitic infections (schistosomiasis).

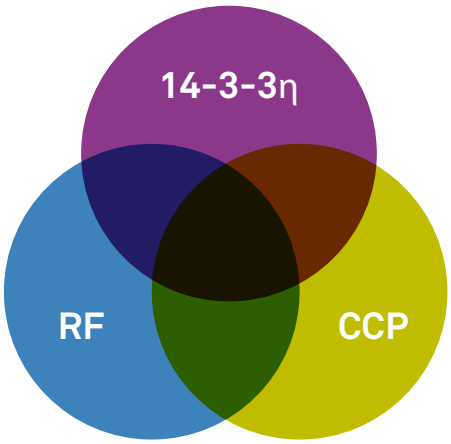
14-3-3eta in diagnosis, prognosis, care, and monitoring: **key findings from recent publications**

1 Serum 14-3-3eta is a novel marker that complements current serological measurements to enhance detection of patients with RA[5]

- Improves diagnostic capacity for early RA by 6 points, from 72% to 78%
- Is elevated in RA and erosive psoriatic arthritis (PsA) but not in other inflammatory diseases
- High specificity of 14-3-3eta at 93%
- Differentiates RA vs. osteoarthritis (OA), since it is positive only in RA patients or RA/OA patients
- Informs response to therapy because it is a modifiable marker; a decrease marks response to the disease-modifying antirheumatic drug (DMARD) or biologic drug used to treat RA[9,10]

2 14-3-3eta in "seronegative" RA[6]

- 14-3-3eta has utility beyond RF and CCP because it confirms joint-specific inflammation in the absence of the traditional markers
- 28% to 44% of patients with early RA test negative for RF and CCP[4]
- 14-3-3eta identifies 21% of RF/CCP seronegative patients in early RA and 67% of RF/CCP seronegative patients in established RA[6]



For early RA diagnosis, prognosis, and monitoring, consider IdentRA® with 14-3-3eta protein—the only biomarker produced exclusively in the joint.

3 Serum levels of 14-3-3eta protein supplement C-reactive protein and rheumatoid arthritis-associated antibodies to predict clinical and radiographic outcomes in a prospective cohort of patients with recent-onset inflammatory polyarthritis[7]

- Elevation of 14-3-3eta greater than 0.19 ng/mL should be considered positive
- Levels greater than or equal to 0.50 ng/mL predict even poorer clinical and radiographic outcomes, a high risk of clinically refractory RA disease, and significant joint damage over the next 5 years
- Helps primary care providers prioritize patients for rapid referral to rheumatologists and facilitate early intervention with biological therapies
- High levels of 14-3-3eta and CRP at baseline and with patients under treatment independently represent poor prognosis that likely results in significant joint deterioration

4 Serum 14-3-3eta level is associated with severity and clinical outcomes of rheumatoid arthritis, and its pretreatment level is predictive of DAS28 remission with tocilizumab[11]

- 14-3-3eta is modifiable; a decrease in 14-3-3eta in response to therapy is associated with better outcomes, while an increase implies worse prognosis
- Baseline 14-3-3eta measurement is an independent predictor of remission (as defined by DAS28-ESR) in patients treated with the biologic tocilizumab

5 A prospective cohort study of 14-3-3eta in ACPA and/or RF-positive patients with arthralgia[8]

- 14-3-3eta is detected up to 5 years prior to onset of clinical arthritis and is associated with arthritis development in arthralgia subjects pre-selected by RF and ACPA criteria

Get more than test results from **Quest Diagnostics**

Highly accurate results and deeper insights into your patient's health status are just the beginning. Quest also offers tools and services to help streamline your practice so you can focus on your patients.

- Interactive Insights™-enhanced results reporting
- Point-of-care analytics
- Seamless EHR integration
- Hundreds of MDs and PhDs available for consultation
- 2,000+ Patient Service Centers
- Financial assistance programs
- Patient engagement tools such as MyQuest™

We're here to help you diagnose and manage your patients with RA and other autoimmune diseases

For more information, contact your Quest Diagnostics sales representative at 1.866.MYQUEST (1.866.697.8378) or visit QuestDiagnostics.com/RA

References

1. Centers for Disease Control and Prevention. Arthritis-related statistics. Available at www.cdc.gov/arthritis/data_statistics/arthritis-related-stats.htm. Accessed September 19, 2016. **2.** National Psoriasis Foundation. Psoriasis fact sheet. Available at www.psoriasis.org/sites/default/files/psoriasis_fact_sheet.pdf. Accessed September 19, 2016. **3.** Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria. *Arthritis Rheum*. 2010;62(9):2569–2581. **4.** Jansen AL, van der Horst-Bruinsma I, van Schaardenburg D, *et al*. Rheumatoid factor and antibodies to cyclic citrullinated peptide differentiate rheumatoid arthritis from undifferentiated polyarthritis in patients with early arthritis. *J Rheumatol*. 2002;29:2074–2076. **5.** Maksymowych WP, Naides SJ, Bykerk V, *et al*. Serum 14-3-3η is a novel marker that complements current serological measurements to enhance detection of patients with rheumatoid arthritis. *J Rheumatol*. 2014;41;2104–2113. **6.** Naides SJ, Marotta A. 14-3-3η in "seronegative" rheumatoid arthritis. *J Rheumatol*. 2015;42(10):1995. **7.** Carrier N, Marotta A, de Brum-Fernandes AJ, *et al*. Serum levels of 14-3-3η protein supplement C-reactive protein and rheumatoid arthritis-associated antibodies to predict clinical and radiographic outcomes in a prospective cohort of patients with recent-onset inflammatory polyarthritis. *Arthritis Res Ther*. 2016;18:37. **8.** van Beers-Tas MH, Marotta A, Boers M, *et al*. A prospective cohort study of 14-3-3η in ACPA and/or RF-positive patients with arthralgia. *Arthritis Res Ther*. 2016;18:76. **9.** Britsemmer K, Maksymowych WP, van Schaardenburg D, *et al*. FRI0059 14-3-3eta is an early RA biomarker that is modifiable with standard DMARDs and corresponds with improvement in clinical variables. *Ann Rheum Dis*. 2013;72:A388. **10.** Maksymowych WP, *et al*. *Arthritis Rheum*. 2013: poster presentation. **11.** Hirata S, Marotta A, Gui Y, *et al*. Serum 14-3-3η level is associated with severity and clinical outcomes of rheumatoid arthritis, and its pretreatment level is predictive of DAS28 remission with tocilizumab. *Arthritis Res Ther.* 2015;17:280. **12.** Kavanaugh A, Tomar R, Reveille J, *et al*. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. *Arch Pathol Lab Med*. 2000;124:71–81. **13.** Satoh M, Chan EKL, Sobel ES, *et al*. Clinical implication of autoantibodies in patients with systemic rheumatic diseases. *Expert Rev Clin Immunol*. 2007;3:721–738. **14.** Stinton LM, Fritzler MJ. A clinical approach to autoantibody testing in systemic autoimmune rheumatic disorders. *Autoimmun Rev*. 2007;7:77–84. **15.** Petri M, Orbai AM, Alarcón GS, *et al*. Derivation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum*. 2012 Aug;64(8):2677–2686. **16.** Cappelli S, Randone SB. "To be or not to be," ten years after: evidence for mixed connective tissue disease as a distinct entity. *Semin Arthritis Rheum*. 2012;41:589–598.

We're here to help you **diagnose and manage** your patients with RA and other autoimmune diseases

Test Code	Test Name
Antinuclear Antibody (ANA) IFA and Related Specific Antibodies	
249	ANA Screen, IFA, with Reflex to Titer and Pattern
16814	ANA Screen, IFA, Reflex to Titer/Pattern, and Reflex to Multiplex 11 Ab Cascade ANA Screen, IFA with reflex to titer/pattern and reflex to multiplex cascade: dsDNA, Sm, Sm/RNP, RNP, and Chromatin; if all 5 antibodies are negative, reflex to Sjögren's (SS-A and SS-B), Scl-70, and Jo-1; if all 4 of these antibodies are negative, reflex to Ribosomal P and Centromere B antibodies.
Rheumatoid Arthritis/Psoriatic Arthritis	
17669	Rheumatoid Arthritis Diagnostic Panel 1 Rheumatoid factor, Cyclic citrullinated peptide (CCP) IgG.
91472	Rheumatoid Arthritis Diagnostic IdentRA® Panel 2 Rheumatoid factor, Cyclic citrullinated peptide (CCP) IgG and 14-3-3eta.
19878	Rheumatoid Arthritis Diagnostic Panel 3 Rheumatoid factor IgG, IgA, and IgM; Cyclic citrullinated peptide (CCP) IgG; Sjögren's (SS-A and SS-B).
92812	Rheumatoid Arthritis Diagnostic IdentRA® Panel 4 Rheumatoid factor, IgG, IgA, and IgM; Cyclic citrullinated peptide (CCP) IgG; Sjögren's (SS-A and SS-B), and 14-3-3eta.
90071	ANA Screen, IFA, with Reflex to Titer and Pattern/Rheumatoid Arthritis Panel 1 ANA screen IFA with reflex to titer/pattern, Rheumatoid factor, Cyclic citrullinated peptide (CCP) IgG.
92813	ANA Screen, IFA, with Reflex to Titer and Pattern/Rheumatoid Arthritis Panel 2 ANA screen IFA, Rheumatoid factor, Cyclic citrullinated peptide (CCP) IgG, and 14-3-3eta.
Systemic Lupus Erythematosus	
90072	ANA Screen, IFA, with Reflex to Titer and Pattern/Lupus Panel 1 ANA screen IFA with reflex to titer/pattern, dsDNA, Sm, and Chromatin (nucleosomal) antibodies.
29839	ANA Screen, IFA, with Reflex to Titer and Pattern/Lupus Panel 2 ANA screen, IFA with reflex to titer/pattern; dsDNA, Sm, Sm/RNP, Sjögren's (SS-A and SS-B), Scl-70.
19881	ANA Screen, IFA, with Reflex to Titer and Pattern/Lupus Panel 3 ANA screen IFA with reflex to titer/pattern, dsDNA, Chromatin (nucleosomal), Sm, RNP, Sjögren's (SS-A and SS-B), and Complement components C3c, C4c, CH50.
10716	ANA Screen, IFA, with Reflex to Titer and Pattern/Lupus Panel 4 ANA screen, IFA with reflex to titer/pattern; Complement C3+C4, dsDNA, Ribosomal P, Sm, Sm/RNP, Sjögren's (SS-A and SS-B), TPO, Scl-70, Rheumatoid factor.
37491	ANA Screen, IFA, with Reflex to Titer and Pattern/Lupus Panel 5 ANA screen, IFA with reflex to titer/pattern; Rheumatoid factor, Sjogren's (SS-A and SS-B), Sm, Sm/RNP, Scl-70, Ribosomal P, Reticulin IgA screen w/reflex to titer, Mitochondrial w/reflex to titer, Actin (smooth muscle) (IgG), dsDNA, Crithidia, IFA w/reflex, Complement C3 + C4, Thyroid peroxidase (TPO), Striated muscle w/reflex to titer, Myocardial screen w/reflex to titer, Gastric parietal cell.
19874	ANA Screen, IFA with Reflex to Titer and Pattern/Lupus Drug Induced Panel 1 ANA screen IFA with reflex to titer/pattern and Histone antibody.
Connective Tissue Disease	
10547	ANA Multiplex, with Reflex to dsDNA ANA multiplex with reflex to dsDNA.
Mixed Connective Tissue Disease	
19875	ANA Screen, IFA, with Reflex to Titer and Pattern/Mixed Connective Panel 1 ANA screen IFA with reflex to titer/pattern and RNP antibody.
90074	ANA Screen, IFA, with Reflex to Titer and Pattern/Mixed Connective Panel 2 ANA screen IFA with reflex to titer/pattern, dsDNA, RNP, and Scl-70.
Systemic Sclerosis (Scleroderma)	
90073	ANA Screen, IFA, with Reflex to Titer and Pattern/Systemic Sclerosis Panel 1 ANA screen IFA with reflex to titer/pattern, Scl-70, and Centromere B antibodies.
TBD	New Systemic Sclerosis Panel
Sjögren's Syndrome	
90077	ANA Screen, IFA, with Reflex to Titer and Pattern/Sjögren's Panel 1 ANA screen IFA with reflex to titer/pattern, Sjögren's (SS-A and SS-B), and Rheumatoid factor.
19880	ANA Screen, IFA, with Reflex to Titer and Pattern/Sjögren's Panel 2 ANA screen IFA with reflex to titer/pattern, Sjögren's (SS-A and SS-B), Rheumatoid factor, AMA screen with reflex to titer, Thyroid peroxidase (TPO).
93748	New Early Sjögren's Syndrome Panel Carbonic anhydrase VI IgG, IgA, IgM; Parotid specific protein IgG, IgA, IgM; Salivary protein 1 IgG, IgA, IgM.
Myositis/Polymyositis/Dermatomyositis	
10185	Myositis AssessR™ plus Jo-1 Antibodies PL-7, PL-12, Mi-2, Ku, EJ, OJ, SRP, Jo-1 antibodies.
TBD	Myositis Specific Panel, Myositis Associated Panel, and Comprehensive Panel

* This test was developed and its performance characteristics have been determined by Quest Diagnostics Nichols Institute. Performance characteristics refer to the analytical performance of the test.

These tests are available to order individually: dsDNA (255); Sm (37923); Sm/RNP (38567); RNP (19887); Chromatin (34088); SS-A (38568); SS-B (38569); Scl-70 (4942); Jo-1 (5810); Cen B (16088); Rib P (34283); 14-3-3eta Protein (91455); RF (4418); CCP IgG (11173); C3 (351); C4 (353); CH50 (618); TPO (5081); Reticulin IgA screen with reflex to titer (37520); AMA with reflex to titer (259); ASMA IgG (50430); Crithidia IFA with reflex to titer (37092); Striated muscle with reflex to titer (266); Myocardial screen with reflex to titer (261); Gastric parietal cell (15114); Histone (37056); ANA multiplex cascade (19946); PL-7 (1226); PL-12 (1224); Mi-2 (17172); Ku (18855); EJ (1225); OJ (1223); SRP (16318).

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