Quest Diagnostics™

# Autoimmune Rheumatic and Related Diseases

## Laboratory Support for Classification and Diagnosis

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## CLINICAL BACKGROUND

Autoimmune rheumatic diseases (ARDs) are diseases in which the immune system attacks the joints and certain systems. The cause of many of these diseases is unknown. ARDs are sometimes difficult to distinguish owing to overlapping signs and symptoms: joint pain, diminished joint mobility, rash, fever, malaise, fatigue, and weight loss. Laboratory testing may be useful for the differential diagnosis and classification.

This Clinical Focus provides background on the available laboratory tests and their use in diagnosis and classification of the following autoimmune rheumatic and related diseases: gout and pseudogout, juvenile idiopathic arthritis (JIA), mixed connective tissue disease (MCTD), polymyositis and dermatomyositis (PM/DM), rheumatoid arthritis (RA), sarcoidosis, Sjögren syndrome, spondyloarthropathies (SpA), systemic lupus erythematosus (SLE) and neuropsychiatric lupus, systemic sclerosis (SSc), and systemic vasculitis. It does not cover laboratory testing as it relates to prognosis or treatment. It also does not comprehensively cover nonrheumatic autoimmune diseases (ie, Crohn disease, ulcerative colitis, autoimmune hepatitis) or nonautoimmune rheumatic diseases (ie, osteoarthritis), though some conditions that fit in these categories are covered (eg, gout, PM).

## INDIVIDUALS SUITABLE FOR TESTING

Individuals who have signs and symptoms consistent with 1 or more ARD **(Table 1)**.

## TEST AVAILABILITY

Quest Diagnostics offers many tests and panels that may be useful for classifying or diagnosing ARDs **(Appendix Table)**.

## TEST SELECTION AND INTERPRETATION

### Gout and Pseudogout

The American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for gout **(Table 2)** include laboratory testing for monosodium urate (MSU) crystals and serum urate. However, a patient should only be tested if he or she has had at least 1 episode of pain, swelling, or tenderness in peripheral joints. If the patient has had an episode, the presence of MSU crystals in symptomatic joints indicates gout. If MSU crystals are absent, other criteria, including serum urate levels, are needed for a diagnosis. A high titer of serum urate is consistent with gout if other clinical or imaging criteria are met.[1]

Pseudogout, also known as calcium pyrophosphate dihydrate (CPPD) crystal deposition disease, is definitively diagnosed by detection of CPPD crystals in synovial fluid or biopsy; diagnosis depends upon exclusion of other causes of arthritis.[2,3]

**Table 1. Common Signs and Symptoms of Autoimmune Rheumatic and Related Diseases[a]**

| Sign or Symptom | Gout | JIA | MCTD | PM/DM | Pseudogout | RA | Sarcoidosis |
|---|---|---|---|---|---|---|---|
| **Joint-/muscle-related** | | | | | | | |
| Joint pain, stiffness, or inflammation | X | X | X | X | X | X | X |
| Muscle weakness | | | X | X | | | |
| Myalgia | | | | | | | |
| **Skin-/hair-related** | | | | | | | |
| Alopecia | | | | | | | |
| Rash | | X | X | X | | | X |
| Raynaud phenomenon | | | X | X | | O | |
| Skin lesions | | X | | O[b] | | | |
| **General** | | | | | | | |
| Anorexia | | | | | | X | X |
| Cough | | | | | | | X |
| Ear involvement | O[c] | | | | | | |
| Eye involvement | | O | | | | | X |
| Fatigue | | | | X | | X | X |
| Fever | X | X | X | X | | X | X |
| GI involvement | | | | | | | |
| Malaise | X | | | | | X | X |
| Nasal symptoms | | | | | | | O |
| Nervous system involvement | | | | | | | O |
| Respiratory involvement | | X | | | | | X |
| Weight loss | | | | X | | X | X |
| **Other** | | | | | | | |
| Adenopathy | | X | | | | | |
| Anemia | | | | | | O | |
| Dysphagia | | | | X | | | |
| Swelling of hands | | | X | O | | | O |

*(Continued)*

**Table 1. Common Signs and Symptoms of Autoimmune Rheumatic and Related Diseases[a] *(Continued)***

| Sign or Symptom | SjS | SLE | SpA | | | | SSc | Systemic Vasculitis | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | AS | ReA | PsA | EA | | GPA | EGPA | MPA |
| **Joint-/muscle-related** | | | | | | | | | | |
| Joint pain, stiffness, or inflammation | X | X | X | X | X | X | X | X | X | X |
| Muscle weakness | | O | | | | | | | | |
| Myalgia | | O | | | | | | X | X | X |
| **Skin-/hair-related** | | | | | | | | | | |
| Alopecia | X | X | | | | | | | | |
| Rash | X | X | | | | | | | X | X |
| Raynaud phenomenon | X | X | | | | | X | | | |
| Skin lesions | | O | | O | X | | | O | | O |
| **General** | | | | | | | | | | |
| Anorexia | | | X | | | | | | | |
| Cough | | | | | | | | X | X | |
| Ear involvement | | | | | | | | X[c] | | |
| Eye involvement | X | X | X | X | | | | X | | X |
| Fatigue | | X | X | X | | | | | X | X |
| Fever | | X | X | X | | | | X | X | X |
| GI involvement | | | | | | X | X | | X | X |
| Malaise | | X | | | | | | | X | X |
| Nasal symptoms | | | | | | | | X | X | |
| Nervous system involvement | | X | | | | | | X | X | X |
| Respiratory involvement | | | | | | | | X | X | |
| Weight loss | | | X | X | | | | | X | X |
| **Other** | | | | | | | | | | |
| Adenopathy | X | X | | | | | | | | |
| Anemia | | X | X | | | | | | | |
| Dysphagia | X | | | | | | X | | | |
| Swelling of hands | | | | | X | | X | | | |

X indicates common; O indicates less common but not rare. AS, ankylosing spondylitis; EA, enteropathic (inflammatory bowel disease-associated) arthritis; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; JIA, juvenile idiopathic arthritis; MCTD, mixed connective tissue disease; MPA, microscopic polyangiitis; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; PsA, psoriatic arthritis; ReA, reactive arthritis; SjS, Sjögren syndrome; SLE, systemic lupus erythematosus; SpA, spondyloarthropathies; SSc, systemic scleroderma.

[a] This is not a complete list of signs and symptoms; some conditions have more signs and symptoms than could be presented here.

[b] In dermatomyositis.

[c] External ear in gout; middle ear in GPA.

**Table 2. Gout Classification Criteria[1]**

Classify a patient as having gout if:

1. Patient has had ≥1 episode of pain, swelling, or tenderness in peripheral joint or bursa *and* monosodium urate crystals are present in symptomatic joint or bursa or tophus *or*
2. Patient has had ≥1 episode of pain, swelling, or tenderness in peripheral joint or bursa *and* sum of points for criteria below is ≥8

| Criteria | Score |
|---|---|
| **Clinical** | |
| 3. Pattern of joint involvement in monoarticular or oligoarticular episode | |
| – Involving ankle or midfoot (without involvement of first metatarsophalangeal joint) | 1 |
| – Involving first metatarsophalangeal joint | 2 |
| 4. Characteristics of symptomatic episode | |
| – Erythema on affected joint<br>– Touch or pressure on affected joint unbearable<br>– Inability to use affected joint | 1 per characteristic |
| 5. Time course of typical episodes[a] | |
| – 1 typical episode | 1 |
| – Recurrent typical episodes | 2 |
| 6. Presence of tophus[b] | 4 |
| **Laboratory** | |
| 1. Serum uric acid: <4 mg/dL | -4 |
| 2. Serum uric acid: ≥4 to <6 mg/dL | 0 |
| 3. Serum uric acid: 6 to 8 mg/dL | 2 |
| 4. Serum uric acid: 8 to <10 mg/dL | 3 |
| 5. Serum uric acid: ≥10 mg/dL | 4 |
| 6. Monosodium urate negative in synovial fluid of symptomatic joint or bursa | -2 |
| **Imaging** | |
| 7. Ultrasound or dual-energy computed tomography (DECT) evidence of urate deposition in symptomatic joint or bursa | 4 |
| 8. Radiographic evidence of gout-related joint damage (ie, erosion) | 4 |

[a] Typical episode is defined by ≥2 of the following, regardless of anti-inflammatory treatment: 1) maximal pain occurs in <24 hours; 2) symptoms resolve in ≤14 days; 3) symptoms completely resolve between symptomatic episodes.
[b] Clinical evidence of tophus includes draining or chalk-like nodule under transparent skin, usually located in joints, ears, olecranon bursae, finger pads, or tendons.

## Juvenile Idiopathic Arthritis

The International League of Associations for Rheumatology (ILAR) classification criteria define JIA as arthritis that begins before 16 years of age, persists for ≥6 weeks, and has unknown etiology.[4] Although diagnosis of JIA is primarily clinical, ILAR recommends laboratory testing to distinguish between the forms of JIA **(Table 3)**. For example, human leukocyte antigen (HLA)-B27 antigen testing and rheumatoid factor (RF) testing can help distinguish forms of JIA. A positive HLA-B27 antigen test result is consistent with enthesitis-related arthritis. A positive RF test result differentiates RF-positive and RF-negative forms of polyarthritis. Both of these test results also serve as exclusion criteria for other forms of JIA.

## Mixed Connective Tissue Disease

Patients with MCTD can present with a wide range of signs and symptoms, most of which overlap with other ARDs. Four sets of MCTD classification criteria exist: Sharp, Alarcón-Segovia, Kasukawa, and Kahn.[5] The different sets require a variety of clinical and serological criteria be met, but all 4 require either a positive result or a high titer for RNP antibody. For example, the Kasukawa criteria require a positive anti-RNP test result, whereas the Alarcón-Segovia criteria require a high RNP antibody titer **(Table 4)**. The other sets of criteria factor in different laboratory test results, including a negative result for Smith antibody (Sm) and high titers of RNP or extractable nuclear antigen antibodies.

The first indication of MCTD is often a high antinuclear antibody (ANA) titer, which occurs in 94% to 97% of MCTD patients (see Appendix for more information about ANA testing).[6] This test result should be followed by testing for antibodies to RNP, Sm, SS-A, SS-B, histone, and dsDNA.[7] Over 90% of MCTD patients are positive for antibodies to RNP, while the other antibodies occur less frequently (<20% of patients).[6,8] Antibodies to dsDNA, Sm, and SS-A can be seen transiently in MCTD, but consistent presence of these antibodies may indicate SLE.[7]

## Polymyositis and Dermatomyositis

PM and DM are subgroups of idiopathic inflammatory myopathies (IIMs), which can be identified using the EULAR/ACR classification criteria for IIMs.[9] These criteria include laboratory testing, including testing for Jo-1 antibody, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase. A web-based calculator (http://www.imm.ki.se/biostatistics/calculators/iim/) that incorporates both clinical and laboratory criteria is available to estimate the probability that a patient has IIM. The

**Table 3. Juvenile Idiopathic Arthritis Classification Criteria[4]**

**General**

1. Arthritis begins before 16 years of age
2. Arthritis persists ≥6 weeks
3. Other potential causes of arthritis are excluded

| JIA Form | Inclusion Criteria | Exclusion Criteria |
|---|---|---|
| Systemic arthritis | 1. Arthritis in ≥1 joint *and*<br>2. Fever for ≥2 weeks that is daily for ≥3 days *and*<br>3. ≥1 of the following:<br>1. Evanescent erythematous rash<br>2. Generalized adenopathy<br>3. Hepatomegaly, splenomegaly, or both<br>4. Serositis | See footnotes a, b, c, and d. |
| Oligoarthritis | *Persistent form*: Arthritis in 1 to 4 joints during first 6 months of disease *and* in ≤4 joints during disease course<br>*Extended form*: Arthritis in 1 to 4 joints during first 6 months of disease *and* in >4 joints after first 6 months | See footnotes a, b, c, d, and e. |
| Polyarthritis (RF-negative) | Arthritis in ≥5 joints during first 6 months of disease *and* negative results in RF test | See footnotes a, b, c, d, and e. |
| Polyarthritis (RF-positive) | Arthritis in ≥5 joints during first 6 months of disease *and* positive results in ≥2 RF tests run ≥3 months apart during first 6 months | See footnotes a, b, c, and e. |
| Psoriatic arthritis | 1. Arthritis and psoriasis *or*<br>2. Arthritis and ≥2 of the following:<br>1. Dactylitis<br>2. Nail pitting or onycholysis<br>3. First-degree relative with psoriasis | See footnotes b, c, d, and e. |
| Enthesitis-related arthritis | 1. Arthritis and enthesitis *or*<br>2. Arthritis or enthesitis *and* ≥2 of the following:<br>1. Sacroiliac joint tenderness and/or inflammatory lumbosacral pain<br>2. Positive for HLA-B27 antigen<br>3. Arthritis onset in male >6 years of age<br>4. Acute anterior uveitis<br>5. First-degree relative with ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with IBD, reactive arthritis, *or* acute anterior uveitis | See footnotes a, d, and e. |
| Undifferentiated arthritis | Arthritis that fulfills criteria for none of the above or ≥2 of the above types | NA |

IBD, inflammatory bowel disease; JIA, juvenile idiopathic arthritis; NA, not applicable; RF, rheumatoid factor.

[a] Psoriasis in patient or first-degree relative.

[b] Arthritis beginning after 6th birthday in HLA-B27-positive male.

[c] First-degree relative with ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with IBD, reactive arthritis syndrome, or acute anterior uveitis.

[d] Positive results in ≥2 RF tests run ≥3 months apart.

[e] Presence of systemic JIA.

**Table 4. Mixed Connective Tissue Disease Diagnostic Criteria[5,a]**

| Kasukawa Criteria | Alarcón-Segovia Criteria |
|---|---|
| **Diagnose MCTD if:** | **Diagnose MCTD if:** |
| 1. RNP antibody test is positive *and* | 1. RNP antibody titer >1:1,600 and |
| 2. ≥1 common symptom is present *and* | 2. ≥3 clinical criteria present, including synovitis or myositis |
| 3. ≥1 mixed symptom in ≥2 disease categories | *Clinical criteria* |
| *Common symptoms* | 1. Edema in hands |
| 1. Raynaud phenomenon | 2. Synovitis |
| 2. Swollen fingers or hands | 3. Myositis |
| *Mixed symptoms* | 4. Raynaud phenomenon |
| 1. SLE-like symptoms (polyarthritis, lymphadenopathy, facial erythema, pericarditis or pleuritis, leukothrombocytopenia) | 5. 5Acrosclerosis |
| 2. SSc-like findings (sclerodatyly, pulmonary fibrosis, restrictive changes of lung, reduced diffusion capacity, hypomotility or dilation of esophagus) | |
| 3. PM-like findings (muscle weakness, elevated serum levels of muscle enzymes [creatinine phosphokinase], myogenic pattern on electromyogram) | |

MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; PM, polymyositis.
[a] Two of 4 existing MCTD criteria are shown here; they were selected because of their higher reported sensitivity and specificity (though the Kahn criteria perform similarly to Alarcón-Segovia criteria).[5,6]

calculator also indicates type: PM, DM, amyopathic myositis, inclusion body myositis, and juvenile dermatomyositis. Criteria relevant to subclassifying PM and DM are summarized in **Table 5**.

The classical myositis-specific antibodies include Jo-1, EJ, OJ, PL-7, and PL-12 synthetase antibodies, as well as Mi-2 and anti-signal recognition particle (SRP) antibodies. Of these, only Jo-1 antibody is currently included in the EULAR/ACR criteria.[9] Others are anticipated to be included in future updates.[9] Together, these antibodies are found in about 50% of patients with PM/DM and are often mutually exclusive.[10]

Jo-1 antibody is observed in 21% of patients with PM and in 11% of those with DM.[10] It is 100% specific for PM/DM.[11,12] The remaining classical myositis-specific antibodies occur less frequently than Jo-1[10] but are also highly specific (97% to 100%) for PM/DM.[11,13,14] Accordingly, a positive test result for any of the classical myositis-specific antibodies is highly suggestive of PM or DM (with rash). A negative test result does not rule out either PM or DM, however, as classical myositis-specific antibodies are not detected in 50% of patients with these IIMs.

"Antisynthetase syndrome" is commonly observed in PM and DM patients. Arthralgia and interstitial lung disease are the

**Table 5. PM and DM Classification Criteria[9]**

| Classify patient as having PM (rash[a] absent) or DM (rash[a] present) if |
|---|
| • No other cause is present |
| • Sum of EULAR/ACR classification points is ≥5.5 (≥6.7 with biopsy) |
| • Age of onset is ≥18 years |
| • Muscle weakness[b] is present |

DM, dermatomyositis; PM, polymyositis.
[a] Heliotrope rash, Gottron sign, or Gottron papules.
[b] Objective symmetric weakness, usually progressive of the upper or lower extremities; neck flexors weaker than extensors; or proximal leg muscles weaker than distal.

most prevalent extramuscular symptoms in patients with the syndrome.[10] These patients typically have a moderate response to standard immunosuppressive therapy.[15] Depending on the type of synthetase antibody, symptoms vary. A meta-analysis of 3,487 patients (27 studies) indicated that[10]

- Jo-1 antibody is associated with a significantly higher risk of myositis, arthralgia, and mechanic's hand compared with non-Jo-1 antibodies.
- Non–Jo-1 antibodies (eg, EJ, OJ, PL-7, PL-12) are associated with a significantly higher risk of fever and interstitial lung disease compared with Jo-1 antibody.
- The risk of Raynaud phenomenon does not differ significantly between patients with Jo-1 antibodies and those with non-Jo-1 antibodies.

## Rheumatoid Arthritis

### Rheumatoid Factor and Cyclic Citrullinated Peptide

The ACR/EULAR classification criteria for RA **(Table 6)** include testing for autoantibodies to RF and cyclic citrullinated peptide (CCP).[16] RF is a widely used laboratory marker of RA. The reported sensitivity of RF is 57% for early RA[17] and ranges from 60% to 86% for established RA.[18,19] Positive RF results are suggestive of RA, but the relatively low specificity (70%-85%) precludes a definitive diagnosis for either early or established disease.[17,19-23] Negative RF results are consistent with conditions other than RA but do not rule out RA; 14% to 43% of patients with RA are seronegative.[17-19]

The sensitivity of CCP antibody is comparable to that of RF in early (59%)[17] and established RA (64%-88%).[18,21] Unlike RF, CCP antibody is highly specific (90%-98%) for early and established RA.[17,20,22,24] Most side-by-side comparisons demonstrate that CCP antibody is at least as sensitive as and more specific than RF in various clinical situations.[18-21,23,25] Thus, positive CCP antibody results are highly suggestive of RA[17,20,22,24]; however, patients with other rheumatic diseases may also have elevated titers. Negative results are consistent with RA and other rheumatic diseases. They do not rule out a diagnosis of RA; 12% to 41% of patients with RA are CCP seronegative.[17,21] In RF-positive patients with chronic HCV or other infections associated with polyarticular arthritis, a positive CCP antibody result suggests a likely diagnosis of coexisting RA; HCV patients with cryoglobulinemia, but not RA, typically have negative CCP antibody results.[26]

**Table 6. Rheumatoid Arthritis Classification Criteria[16]**

Classify a patient as having RA if sum of points is ≥6.

| Criteria | Points |
|---|---|
| **Joint involvement** | |
| 1. 1 large joint | 0 |
| 2. 2-10 large joints | 1 |
| 3. 1-3 small joints, with or without large joint | 2 |
| 4. 4-10 small joints, with or without large joint | 3 |
| 5. >10 joints with ≥1 small joint | 5 |
| **Symptom duration** | |
| 1. <6 weeks | 0 |
| 2. ≥6 weeks | 1 |
| **RF and CCP antibody** | |
| 1. Normal RF and CCP antibody | 0 |
| 2. Low-positive RF or CCP antibody | 2 |
| 3. High-positive RF or CCP antibody | 3 |
| **CRP and ESR** | |
| 1. Normal CRP and ESR | 0 |
| 2. Elevated CRP or ESR | 1 |

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

The combination of RF and CCP antibodies provides greater sensitivity than either assay alone[18,19,23] and is commonly used in the diagnostic evaluation of suspected RA. The combination of a positive IgM RF *and* CCP antibody result is highly suggestive of RA (~90%-100%). However, this test result may be found in some patients with other rheumatic diseases such as SLE, sclerosis, and psoriatic arthritis. Patients with negative RF and positive CCP antibody results are also likely to have RA. Patients with positive RF and negative CCP results are less likely to have RA, but RA remains a possibility. Negative results on both assays indicate a low likelihood of RA but do not exclude the diagnosis. Between 28% and 44% of patients with early disease test negative for both RF and CCP antibodies,[27,28] which has led to the search for other RA markers.

### 14-3-3η

The 14-3-3η protein is elevated in serum and synovial fluid during joint inflammation and is a relatively new RA marker.[29] Although it is not yet included in the ACR/EULAR classification criteria, 14-3-3η antibody test sensitivity (64%) was higher than that of RF (57%) or CCP antibody (59%) in early RA patients in a side-by-side comparison.[17] In patients with established RA, the sensitivity of 14-3-3η (77%) was comparable to that of RF or CCP antibody and specificity (93%) fell between that of RF and CCP antibody.[17] Thus, positive 14-3-3η results are suggestive of RA.[17] However, negative results do not rule out RA; 23% to 36% of patients with RA are 14-3-3η seronegative.[12] In a patient with a personal or family history of psoriasis, nail changes, and back or heel pain along with peripheral joint polyarthritis, a positive 14-3-3η result is consistent with a diagnosis of psoriatic arthritis.

Adding 14-3-3η testing to RF and CCP antibody testing provides greater sensitivity for early RA: 78% with 14-3-3η versus 72% without 14-3-3η.[17] In seronegative patients, 14-3-3η detects 21% of patients with early RA and 67% of patients with established RA.[17] This increased sensitivity may translate into treatment earlier in the course of disease, which can minimize irreversible joint damage. In patients with suspected RA, a positive/elevated result of RF, CCP antibody, and/or 14-3-3η protein suggests an RA diagnosis. Negative/normal results for all 3 markers indicate that an RA diagnosis is less likely.

### C-reactive Protein and Erythrocyte Sedimentation Rate

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) measurements are also included in the ACR/EULAR classification criteria for RA **(Table 6)**. Elevated levels are consistent with an RA diagnosis if other laboratory and clinical criteria are met **(Table 6)**. In patients with RA, elevated levels of CRP or ESR indicate heightened disease activity. However, elevations may also be due to other inflammatory conditions. Normal CRP and ESR results indicate relatively low disease activity. In patients with discordant CRP and ESR results, CRP levels may be the more reliable marker of RA disease activity.[30]

## Sarcoidosis

According to the American Thoracic Society (ATS), diagnosis of sarcoidosis requires 3 criteria **(Table 7)**.[31] The identification of noncaseating granulomas via biopsy is a strong indication of sarcoidosis; however, other causes of granulomatous disease must be excluded.[31] Because other diseases must be excluded, laboratory tests may indirectly help with diagnosis.

**Table 7. Sarcoidosis Diagnostic Criteria[31]**

| Diagnose sarcoidosis if patient has all of the following criteria: |
|---|
| 1. Clinical or radiological presentation[a] |
| 2. Presence of noncaseating granulomas |
| 3. Absence of alternative diseases[b] |

[a] Chest x-ray or CT can identify hilar or mediastinal lymphadenopathy, upper lobe disease, subpleural reticulonodular infiltrates, peribronchial thickening, or traction bronchiectasis of the upper lobe.[33]

[b] Sarcoidosis shares signs and symptoms with other conditions including tuberculosis, prescription drug use (eg, methotrexate), granulomatous lesions of unknown significance (GLUS) syndrome, lymphoma, idiopathic pulmonary fibrosis, berylliosis, and multiple other infectious diseases.[31,32]

Laboratory tests are not diagnostic for sarcoidosis, because they lack specificity, but some tests can support diagnosis. The following lab results are consistent with sarcoidosis: leukopenia, anemia, thrombocytopenia, elevated urinary calcium:creatinine ratio, and elevated serum levels of calcium, blood urea nitrogen (BUN), liver enzymes, immunoglobulins, or angiotensin-converting enzyme (ACE).[32] The ATS recommends including peripheral blood counts, urine analysis, and serum levels of calcium, liver enzymes, creatinine, and BUN as part of a work-up once a diagnosis is confirmed.[31]

## Sjögren Syndrome

The ACR and EULAR updated classification criteria for Sjögren syndrome in 2016.[34] The updated criteria involve inclusion criteria, exclusion criteria, and a scoring system based on 5 items of different weights **(Table 8)**.The 2 most heavily weighted items are 1) SS-A/Ro testing and 2 ) labial salivary gland biopsy, which is a relatively invasive procedure that may be unnecessary if the other results are consistent with Sjögren syndrome. An initial validation indicates that sensitivity of the 2016 criteria is 96% (95% CI 92%-98%) and specificity is 95% (95% CI 92%-97%).[34]

Previous classification criteria from 2012 included testing for SS-B/La, RF, and ANA titer in addition to SS-A/Ro.[35] Those criteria indicated that testing for SS-A or SS-B antibody has a sensitivity of 84% and specificity of 92%, and that a positive RF result (sensitivity, 72%; specificity, 86%) in addition to an ANA titer ≥1:320 (sensitivity, 73%; specificity, 80%) was consistent with the Sjögren syndrome. These markers were not included in 2016 guidelines, because studies indicated the markers do not add sufficient value to SS-A/Ro testing.[34]

**Table 8. Sjögren Syndrome Classification Criteria[34]**

| Classify as Sjögren Syndrome if |
|---|
| Patient meets ≥1 of the inclusion criteria<br>1. Ocular or oral dryness[a]<br>2. Suspicion of Sjögren syndrome based on EULAR Disease Activity Index questionnaire[36] |
| Patient does *not* meet any of the exclusion criteria[b]<br>1. Radiation treatment of head and neck<br>2. Active hepatitis C infection (confirmed by PCR)<br>3. AIDS<br>4. Sarcoidosis<br>5. Amyloidosis<br>6. Graft-versus-host disease<br>7. IgG4-related disease |
| Patient scores ≥4 based on the weights of the following items (weights in parentheses)<br>1. Labial salivary gland biopsy exhibits focal lymphocytic sialadenitis with focus score ≥1 focus/4 $mm^2$ (3 points)<br>2. SS-A/Ro test is positive (3 points)<br>3. Ocular staining score is ≥5 (or van Bijsterveld score ≥4) in ≥1 eye<br>4. Schirmer's test ≤5 mm/5 minutes in ≥1 eye<br>5. Unstimulated whole saliva flow rate ≤0.1 mL/min |

EULAR, European League Against Rheumatism; PCR, polymerase chain reaction.

[a] Defined as having or doing ≥1 of the following: dry eyes for >3 months; recurrent sensation of sand or gravel in eyes; use of tear substitutes ≥3 times per day; feeling of dry mouth every day for >3 months; often drink to help swallow food.

[b] Clinical features of these conditions overlap with SS and interfere with criteria tests.

## Spondyloarthropathies

The term "spondyloarthropathies" (SpA) encompasses a group of inflammatory rheumatic diseases that cause arthritis; these include ankylosing spondylitis, reactive arthritis, psoriatic arthritis, and inflammatory bowel disease-associated arthritis. SpA can be subdivided into axial SpA, which involves the spine and sacroiliac joints, and peripheral SpA, which involves peripheral arthritis, enthesitis, and dactylitis.[37,38] Overlapping presentations involving both axial and peripheral joints may occur.

The Assessment of SpondyloArthritis International Society (ASAS) has released classification criteria for axial SpA and peripheral SpA.[37,38] Laboratory testing for HLA-B27 can help identify individuals with SpA.[37,38] The axial SpA criteria have radiographic (imaging) and clinical arms, both of which incorporate HLA-B27 testing **(Table 9)**; these criteria (ie, fulfilling criteria of either arm) have a sensitivity of 83% and a specificity of 84%.[38] The peripheral SpA criteria, which also incorporate HLA-B27 testing, have a sensitivity of 78% and a specificity of 82%.[37]

A positive HLA-B27 result is consistent with any type of SpA (ankylosing spondylitis, reactive arthritis, psoriatic arthritis, or inflammatory bowel disease-associated arthritis), acute anterior uveitis, or JIA. However, most people who are HLA-B27 positive do not develop an associated condition. Thus, diagnosis and classification should be based on multiple criteria.[37,38] A negative HLA-B27 result does not rule out conditions associated with HLA-B27; the allele is not always present in patients with these conditions.

CRP levels can also assist with classification of axial SpA. Elevated levels are consistent with axial SpA in the presence of other criteria. A negative result does not rule out a classification of SpA since other criteria may be met.

**Table 9. Spondyloarthropathy Classification Criteria**

| Axial SpA[38] | | Peripheral SpA[37] |
|---|---|---|
| Patients with back pain for ≥3 months who are <45 years at onset and meet criteria in clinical or imaging arm | | Patients with peripheral manifestations only |
| **Clinical Arm** | **Imaging Arm** | |
| HLA-B27 and ≥2 other SpA features from footnote a | Sacroiliitis on imaging *and* ≥1 SpA feature from footnote a | Arthritis, enthesitis, or dactylitis *and* ≥1 SpA feature from footnote b *or* ≥2 other SpA features from footnote c |

[a] HLA-B27, inflammatory back pain, arthritis, enthesitis, uveitis, dactylitis, psoriasis, Crohn disease or ulcerative colitis, response to NSAIDs, family history of SpA, elevated C-reactive protein levels.

[b] HLA-B27, uveitis, psoriasis, Crohn disease or ulcerative colitis, preceding infection, sacroiliitis on imaging.

[c] Arthritis, enthesitis, dactylitis, inflammatory back pain, family history of SpA.

## Systemic Lupus Erythematosus and Neuropsychiatric Lupus

Classification of SLE according to the Systemic Lupus International Collaborating Clinics (SLICC) criteria requires a combination of clinical and immunologic evaluation **(Table 10)**.[39] Laboratory testing can help assess some clinical criteria such as the presence of proteinuria, anemia, leukopenia, lymphopenia, and thrombocytopenia. It can also help assess immunologic criteria such as the presence of antibodies to DNA, Sm, antiphospholipids, and ANA, as well as levels of complement proteins (C3, C4, and total).

When considered individually, most of the laboratory tests included in the SLICC classification criteria provide high specificity (86% to 99%) and low to medium sensitivity for SLE (7% to 59%). The exception is ANA testing, which has medium specificity (45%) and high sensitivity (96%)[39]; a negative ANA test can help rule out SLE because ANA-negative SLE is rare.[40]

Though not included in the SLICC classification criteria, chromatin antibodies have relatively high sensitivity (64% to 69%) and specificity (92% to 99%) for SLE, [41,42] and may provide value when diagnosing SLE. RNP antibodies are also present in SLE patients, but are not specific to SLE; RNP antibodies are more useful for identifying MCTD.[43,44]

Neuropsychiatric lupus has a broad range of symptoms in the central or peripheral nervous systems. Validated diagnostic criteria do not exist, but diagnosis usually involves a clinical evaluation that is supported by results from brain MRI, serology and cerebrospinal fluid (CSF) testing, and neuropsychiatric assessment; diagnosis also requires the exclusion of other potential causes of neuropsychiatric signs and symptoms.[40] Laboratory testing can indirectly help diagnose neuropsychiatric lupus by excluding other causes, including infectious diseases. In addition, compared to SLE patients without neuropsychiatric disease, patients with neuropsychiatric lupus often have higher serum levels of neuronal antibodies or antibodies to ribosomal P, cardiolipin, lupus anticoagulants, or phospholipids.[45]

**Table 10. SLE Classification Criteria[39]**

Classify a patient as having SLE if:

1. 4 criteria are met, including ≥1 clinical and ≥1 immunologic criterion *or*
2. Biopsy-proven nephritis compatible with SLE *and* ANA *or* dsDNA antibodies are present

**Clinical Criteria**

1. Acute cutaneous lupus in the absence of dermatomyositis *or* subacute cutaneous lupus
2. Chronic cutaneous lupus
3. Oral ulcers[a] *or* nasal ulcers[a]
4. Nonscarring alopecia[a]
5. Synovitis of ≥2 joints *or* tenderness of ≥2 joints and >30 minutes morning stiffness
6. Pleurisy (>1 day), pleural effusion, or pleural rub[a] *or* pericardial pain (>1 day), pericardial effusion, pericardial rub, or pericarditis by ECG[a]
7. Urine protein-to-creatinine ratio indicates 500 mg protein/24 hours *or* red blood cell casts
8. Seizures, psychosis, myelitis, mononeuritis multiplex,[a] peripheral or cranial neuropathy,[a] or acute confusional state[a]
9. Hemolytic anemia
10. Leukopenia (<4,000/mm$^3$)[a] *or* lymphopenia (<1,000/mm$^3$)[a]
11. Thrombocytopenia (<100,000/mm$^3$)[a]

**Immunologic Criteria**

1. ANA level above reference range
2. dsDNA antibody level above reference range (or > twice reference range if tested by ELISA)
3. Sm antibody positive
4. Antiphosopholipid antibody positive[b]
5. Low C3, C4, or CH50
6. Direct Coombs test if hemolytic anemia is absent

[a] If no other cause is present.

[b] As determined by positive result for lupus anticoagulant; false-positive result for rapid plasma reagin; medium- to high-titer of cardiolipin antibody; or positive results for β2-glycoprotein I antibody.

## Systemic Sclerosis

The EULAR/ACR classification criteria for SSc **(Table 11)** comprise mainly clinical criteria, but also include laboratory testing for autoantibodies.[46] A positive result for centromere, Scl-70 (topoisomerase I), or RNA polymerase III antibodies is consistent with SSc but is not diagnostic. A positive ANA result suggests the need for testing for specific autoantibodies if clinical symptoms are consistent with SSc; 85% to 97% of patients with SSc are ANA-positive (see Appendix for more information about ANA testing).[47,48]

The 2 most common types of SSc are diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc), also called CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) syndrome. The type of SSc can affect prognosis and treatment. Differentiation may

be possible based on location of skin fibrosis (proximal vs distal extremities) and clinical manifestations, but laboratory test results can support differentiation. Scl-70 antibody is found in approximately 40% of patients with dcSSc, whereas centromere antibody is found in up to 90% of patients with lcSSc.[49] When detected by indirect immunofluorescence, immunoprecipitation, or immunodiffusion, Scl-70 and centromere antibodies are almost always mutually exclusive in SSc patients: only 0.5% test positive for both.[49] Thus, a positive test result for Scl-70 antibody is consistent with dcSSc if clinical symptoms are present, and a positive test result for centromere antibody is consistent with lcSSc if clinical symptoms are present.

## Systemic Vasculitis

The ACR has created classification criteria for 2 autoimmune systemic vasculitis disorders **(Table 12)**: granulomatosis with polyangiitis (GPA, formerly Wegener granulomatosis)[50] and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome).[51] Laboratory tests are included in both sets of criteria. If GPA is suspected, testing for microhematuria can assist with classification. If EGPA is suspected, testing for eosinophilia can assist with classification. Other routine laboratory test results can suggest systemic vasculitis. Anemia, leukocytosis, thrombocytosis, and elevated ESR and CRP levels are consistent with an acute phase response.[52]

Diagnosis should be confirmed via biopsy of the affected tissue when possible; this is true for GPA, EGPA, and another autoimmune systemic vasculitis disorder, microscopic polyangiitis (MPA). Classification criteria for MPA are not published, but clinical and histological findings in patients with MPA include fibrinoid necrotizing vasculitis of predominantly small vessels without immune deposits, focal segmental necrotizing glomerulonephritis, pulmonary capillaritis, and neutrophilic infiltration of the alveolar wall.[52]

**Table 11. Systemic Sclerosis Classification Criteria[46]**

Classify a patient as having systemic sclerosis if sum of points is ≥9.

| Criteria | Points |
|---|---|
| Skin thickened on fingers of both hands, extending proximal to the metacarpophalangeal joints | 9 |
| Skin on fingers thickened (only count highest score) | |
| – Puffy fingers | 2 |
| – Sclerodactyly[a] | 4 |
| Lesions on fingertips (only count highest score) | |
| – Ulcers on tip of digits | 2 |
| – Pitting scars on fingertips | 3 |
| Telangiectasia | 2 |
| Abnormal nailfold capillaries | 2 |
| Pulmonary arterial hypertension and/or interstitial lung disease (max score is 2) | |
| – Pulmonary arterial hypertension | 2 |
| – Interstitial lung disease | 2 |
| Raynaud phenomenon | 3 |
| Presence of any of the following SSc-related autoantibodies[b]: centromere, Scl-70, or RNA polymerase III | 3[b] |

[a] Distal to metacarpophalangeal joints but proximal to proximal interphalangeal joints.

[b] 3 points for 1 or more of the antibodies; maximum score is 3.

**Table 12. Granulomatosis with Polyangiitis and Eosinophilic Granulomatosis with Polyangiitis Classification Criteria**

| Classify patient as having **granulomatosis with polyangiitis** if ≥2 of the following are present[50]: | Classify patient as having **eosinophilic granulomatosus with polyangiitis** if ≥4 of the following are present[51]: |
|---|---|
| 1. Oral ulcers or bloody or purulent nasal discharge | 1. Asthma |
| 2. Nodules, fixed infiltrates, or cavities on chest radiograph | 2. Eosinophilia >10% |
| 3. Microhematuria or red cell casts in urine sediment | 3. Mono- or polyneuropathy |
| 4. Granulomatous inflammation in wall of artery or peri- or extra-vascular area | 4. Nonfixed pulmonary infiltrates |
| | 5. Paranasal sinus abnormality |
| | 6. Extravascular esoinophils |

Differential diagnosis of GPA, EGPA, and MPA can be aided by testing for specific antineutrophil cytoplasmic antibodies (ANCA).[53] Each disorder is associated with predominance of a specific ANCA type.[54] The ANCA types are revealed by fluorescent patterns obtained in an indirect immunofluorescence ANCA screen. For example, the cytoplasmic pattern (C-ANCA) is very common in GPA, but not MPA or EGPA. The perinuclear pattern (P-ANCA), on the other hand, is rare in GPA, common in MPA, and moderately common in EGPA cases. The atypical P-ANCA pattern is rare in all 3 of these; it is usually associated with nonvasculitic conditions such as inflammatory bowel disease.[55] The sensitivity and specificity of these markers for the various disorders are summarized in **(Table 13)**.[56-58]

**Table 13. Diagnostic Accuracy of Antibodies for Systemic Vasculitis**

| Markers | % Sensitivity (Specificity) | | |
|---|---|---|---|
| | GPA[58,59,a] | MPA[58,59,a] | EGPA[56] |
| ANCA | 85 (93) | 68 (87) | 31 |
| C-ANCA | 81 (100) | 3 (93) | 5 |
| C-ANCA+/PR3+ | 69 (100) | 0 | 1 |
| PR3+ | 77-81 (98-99) | 9-12 (96-99) | |
| P-ANCA | 4 (94) | 65 (94) | 21 |
| P-ANCA+/ MPO+ | 2 (99) | 48 (100) | 20 |
| MPO+ | 5-9 (98-99) | 71-88 (96-99) | |

ANCA, antineutrophil cytoplasmic antibodies; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis.

[a] Sensitivity and specificity based on patients tested for ANCA in a rheumatology clinic.

An international consensus group recommends improving the diagnostic accuracy of the ANCA screen by combining it with immunoassays specific for myeloperoxidase (MPO) and proteinase-3 (PR3) antibodies **(Table 13)**.[54] The P-ANCA pattern predominantly reflects MPO specificity. Similarly, the C-ANCA pattern typically reflects specificity to PR3; however, concordance between C-ANCA and PR3 antibody is not 100% because C-ANCA has multiple targets.

Alternatively, a more recent international consensus statement recommends that high quality MPO- and PR3-immunoassays be used as a primary screening method for GPA and MPA, based on the improved performance of these assays **(Table 13)**.[59] Positive PR3 assay results are highly suggestive of GPA and positive MPO results are highly suggestive of MPA. The group proposes that indirect immunofluorescence only be used in patients with negative MPO- and PR3-assay results and a high clinical suspicion of disease.[59]

A positive ANCA screen supports a diagnosis of autoimmune-related systemic vasculitis in a symptomatic patient **(Table 13)**. Positive results are also seen in inflammatory bowel disease (ulcerative colitis) and occasionally in other autoimmune diseases (SLE, RA, autoimmune hepatitis). Exposure to certain drugs (eg, propylthiouracil, hydralazine, methimazole) and infectious agents (eg, hepatitis C virus) can result in secondary vasculitis and an ANCA-positive screen result.[60,61] A negative ANCA, MPO antibody, and/or PR3 antibody result does not rule out systemic vasculitis.

Owing to limitations in sensitivity and specificity, ANCA, MPO antibody, and PR3 antibody test results should be interpreted carefully in light of clinical and other laboratory data.

## APPENDIX
## Antinuclear Antibody Testing

The laboratory work-up for patients with suspected rheumatic disease often begins with an antinuclear antibody (ANA) screen. The classic ANA testing approach uses HEp-2 human tissue culture cells in an immunofluorescence assay (IFA) to detect autoantibodies directed against antigens in the cell cytoplasm and nucleus. Because it is highly sensitive, this method is considered by the American College of Rheumatology (ACR) to be the current gold standard.[43]

The high sensitivity of IFA stems from inclusion of a large number of antigens; however, these antigens are not very disease specific. Analyte-specific immunoassays are more disease specific owing to the use of antigens strongly associated with particular rheumatic diseases. Multiplex immunoassays can identify multiple autoantibodies simultaneously, but the number of antibodies detected is fewer than in the IFA; this difference results in lower sensitivity. Thus, the IFA and immunoassay methods complement each other for the initial evaluation of suspected rheumatic disorders. When ANA is positive by IFA, especially with high titer, testing for disease-specific antibodies can help with differential diagnosis.

Samples with an IFA titer <1:40 are considered negative for ANA antibodies; follow-up with more specific testing is not needed. Higher titers are generally associated with greater likelihood of rheumatic disease, but do not reflect disease activity. When results are positive, various fluorescent staining patterns are observed in the nucleus or the cytoplasm. These patterns can aid in the differential diagnosis of rheumatic disease and guide selection of further testing for specific autoantibodies.

## Table. Laboratory Tests for Classification and Diagnosis of Autoimmune Rheumatic and Related Diseases[a]

| Test Code | Test Name | Clinical Use |
|---|---|---|
| **Gout and Pseudogout** | | |
| 4563 | Crystals, Synovial Fluid | Diagnose gout and pseudogout |
| **Juvenile Idiopathic Arthritis** | | |
| 4420 | C-Reactive Protein (CRP) | Diagnose axial spondyloarthritis and assess disease activity |
| 528 | HLA-B27 Antigen | Diagnose spondyloarthropathies |
| **Mixed Connective Tissue Disease** | | |
| 19875[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Mixed Connective Panel 1<br>Includes ANA screen (IFA) with a reflex to titer and pattern and RNP antibody. | Diagnose MCTD |
| 90074[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Mixed Connective Panel 2<br>Includes ANA screen (IFA) with reflexes to titer and pattern; dsDNA, RNP, and Scl-70 antibodies. | Diagnose MCTD |
| 19887 | RNP Antibody | Diagnose SLE or MCTD |
| 38567 | Sm/RNP Antibody | Diagnose SLE or MCTD |
| **Polymyositis and Dermatomyositis** | | |
| 823 | Alanine Aminotransferase (ALT) | Diagnose PM/DM |
| 227 | Aldolase | Diagnose PM/DM |
| 38075 | Antisynthetase Antibody Panel 1<br>Includes Jo-1 (test code 5810[X]), EJ (test code 90998), OJ (test code 90999), PL-7 (test code 90996), and PL-12 (test code 90997) antibodies. | Diagnose antisynthetase syndrome in a symptomatic patient |
| 822 | Aspartate Aminotransferase (AST) | Diagnose PM/DM |
| 374 | Creatine Kinase (CK), Total | Diagnose PM/DM |
| 5810(X) | Jo-1 Antibody | Diagnose PM/DM |
| 593 | Lactate Dehydrogenase (LD) | Diagnose PM/DM |
| 90995 | Myositis AssessR™<br>Includes EJ (test code 90998), OJ (test code 90999), PL-7 (test code 90996), PL-12 (test code 90997), Mi-2 (test code 17172), Ku (test code 18855), and SRP (test code 16318) antibodies. | Diagnose PM/DM |
| 10185(X) | Myositis AssessR™ plus Jo-1 Antibodies<br>Includes Antisynthetase Antibody Panel 1 (test code 38075), Mi-2 (test code 17172), Ku (test code 18855), and SRP (test code 16318) antibodies. | Diagnose PM/DM (Jo-1 provides a more definitive diagnosis) |
| 94777[c] | Myositis Specific 11 Antibodies Panel<br>Includes Jo-1, EJ, OJ, PL-7, PL-12, SRP, Mi-2α, Mi-2β, MDA-5, NXP-2, and TIF1-γ antibodies. | Diagnose PM/DM (Jo-1 provides a more definitive diagnosis)<br>Diagnose NM<br>Diagnose cancer-associated DM<br>Diagnose juvenile DM<br>Diagnose amyopathic DM |
| **Rheumatoid Arthritis** | | |
| 91455[c] | 14.3.3 eta Protein | Diagnose RA; more sensitive than either RF or CCP for early RA[17] |

*(Continued)*

## Table. Laboratory Tests for Classification and Diagnosis of Autoimmune Rheumatic and Related Diseases[a] *(Continued)*

| Test Code | Test Name | Clinical Use |
|---|---|---|
| 90071[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Rheumatoid Arthritis Panel 1<br>Includes ANA screen (IFA) with reflex to titer and pattern; cyclic citrullinated peptide (CCP) IgG; rheumatoid factor. | Diagnose RA |
| 92813[b,c] | ANA Screen, IFA, with Reflex to Titer and Pattern/ (Rheumatoid Arthritis Panel 2)<br>Includes ANA screen (IFA) with reflex to titer and pattern, 14-3-3 eta protein; cyclic citrullinated peptide (CCP) IgG; rheumatoid factor. | Diagnose RA |
| 4420 | C-Reactive Protein (CRP) | Diagnose RA and assess disease activity |
| 11173 | Cyclic Citrullinated Peptide (CCP) Antibody (IgG) | Diagnose and determine prognosis of RA; more specific than RF |
| 809 | Sed Rate b Modified Westergren | Diagnose RA and assess disease activity |
| 657 | Mucin Clot, Synovial Fluid | Differential diagnosis of diseases of joints and joint fluid |
| 91472[c] | Rheumatoid Arthritis Diagnostic IdentRA® Panel 2<br>Includes 14-3-3 eta protein, cyclic citrullinated peptide (CCP) IgG, and rheumatoid factor. | Diagnose RA; provides additional diagnostic and prognostic value relative to each assay alone |
| 92812[c] | Rheumatoid Arthritis Diagnostic IdentRA® Panel 4<br>Includes 14-3-3 eta protein, cyclic citrullinated peptide (CCP) IgG, rheumatoid factor antibodies (IgA, IgG, IgM), and SS-A and SS-B antibodies. | Diagnose RA; may help differentiate RA from RA with secondary Sjögren syndrome |
| 17669 | Rheumatoid Arthritis Diagnostic Panel 1<br>Includes cyclic citrullinated peptide (CCP) IgG and rheumatoid factor. | Diagnose RA |
| 19878 | Rheumatoid Arthritis Diagnostic Panel 3<br>Includes cyclic citrullinated peptide (CCP) IgG; rheumatoid factor (IgA, IgG, and IgM); and SS-A and SS-B antibodies. | Diagnose RA; may help differentiate RA from RA with secondary Sjögren syndrome |
| 4418 | Rheumatoid Factor | Diagnose and determine prognosis of RA; detects primarily IgM RF |
| 15682 | Rheumatoid Factor (IgA) | Diagnose RA; provides added specificity when used in combination with other RF or CCP antibody assays; may help predict severity of disease course |
| 19705 | Rheumatoid Factor (IgA, IgG, IgM) | Diagnose RA; detecting all 3 isotypes improves specificity and predictive value |
| 15683 | Rheumatoid Factor (IgG) | Diagnose RA; provides added specificity when used in combination with other RF or CCP antibody assays |
| 15384[b] | Rheumatoid Factor Screen with Reflex to Titer, Synovial Fluid | Diagnose RA and determine prognosis |
| **Sarcoidosis** | | |
| 8561 | Absolute Lymphocyte Count | Support diagnosis of sarcoidosis |
| 683 | Angiotensin Converting Enzyme | Support diagnosis of sarcoidosis |
| 303 | Calcium | Support diagnosis of sarcoidosis |
| 1635 | Calcium, 24-Hour Urine with Creatinine | Support diagnosis of sarcoidosis |
| 6399 | CBC (Includes Differential and Platelets) | Support diagnosis of sarcoidosis |
| 375 | Creatinine | Support diagnosis of sarcoidosis and determine extent of organ involvement |

*(Continued)*

## Table. Laboratory Tests for Classification and Diagnosis of Autoimmune Rheumatic and Related Diseases[a] *(Continued)*

| Test Code | Test Name | Clinical Use |
|---|---|---|
| 7083 | Immunoglobulins Panel, Serum<br>Includes IgA, IgG, and IgM. | Support diagnosis of sarcoidosis |
| 294 | Urea Nitrogen (BUN) | Support diagnosis of sarcoidosis and determine extent of organ involvement |
| **Sjögren Syndrome** | | |
| 90077[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Sjögren's Panel 1<br>Includes ANA screen (IFA) with reflex to titer and pattern; rheumatoid factor; and SS-A and SS-B antibodies. | Diagnose Sjögren syndrome |
| 19880[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Sjögren's Panel 2<br>Includes ANA screen (IFA) with reflex to titer and pattern (test code 249); mitochondrial antibody screen with reflex to titer (test code 259); rheumatoid factor (test code 4418); and SS-A (test code 38568), SS-B (test code 38569), and thyroid peroxidase (test code 5081) antibodies. | Diagnose Sjögren syndrome |
| 4418 | Rheumatoid Factor | Diagnose Sjögren syndrome |
| 38568 | Sjögren's Antibody (SS-A) | Diagnose Sjögren syndrome |
| 38569 | Sjögren's Antibody (SS-B) | Diagnose Sjögren syndrome |
| 7832 | Sjögren's Antibodies (SS-A, SS-B) | Diagnose Sjögren syndrome |
| **Spondyloarthropathies** | | |
| 4420 | C-Reactive Protein (CRP) | Diagnose axial spondyloarthritis and assess disease activity |
| 528 | HLA-B27 Antigen | Diagnose SpA |
| 15584 | HLA-B27 DNA Typing | Diagnose SpA |
| **Systemic Lupus Erythematosus** | | |
| 8561 | Absolute Lymphocyte Count | Detect lymphopenia |
| 90072[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Lupus Panel 1<br>Includes ANA screen (IFA) with reflex to titer and pattern and chromatin (nucleosomal), dsDNA, and Sm antibodies. | Diagnose SLE |
| 29839[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Lupus Panel 2<br>Includes ANA screen (IFA) with reflex to titer and pattern; also includes dsDNA, scleroderma (Scl-70), Sm, Sm/RNP, SS-A, and SS-B antibodies. | Diagnose SLE |
| 19881[b] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Lupus Panel 3<br>Includes ANA screen (IFA) with reflex to titer and pattern; also includes chromatin (nucleosomal), dsDNA, RNP, Sm, SS-A, and SS-B antibodies; complement components C3 and C4 and total complement (CH50). | Diagnose SLE |
| 10716 | ANA Screen, IFA, with Reflex to Titer and Pattern/ Lupus Panel 4<br>Includes ANA screen (IFA) with reflex to titer and pattern; dsDNA, rheumatoid factor, ribosomal P, Scl-70, Sm, Sm/RNP, SS-A, SS-B, and thyroid peroxidase antibodies; and complement components C3 and C4. | Diagnose SLE |

*(Continued)*

## Table. Laboratory Tests for Classification and Diagnosis of Autoimmune Rheumatic and Related Diseases[a] *(Continued)*

| Test Code | Test Name | Clinical Use |
|---|---|---|
| 37491[b,c] | ANA Screen, IFA, with Reflex to Titer and Pattern/ Lupus Panel 5<br>Includes ANA screen (IFA) with reflex to titer and pattern (test code 249); also includes actin (IgG, test code 15043), gastric parietal cell (test code 15114), rheumatoid factor (test code 4418), ribosomal P (test code 34283), Scl-70 (test code 4942), Sm (test code 37923), Sm/RNP (test code 38567), SS-A (test code 38568), SS-B (test code 38569), and thyroid peroxidase (test code 5081) antibodies; dsDNA (Crithidia, test code 37092); mitochondrial (test code 259), myocardial (test code 261), reticulin (test code 37520), and striated muscle antibody (test code 266) screens with reflex to titers; and C3 and C4 complement components (test code 5704). | Diagnose SLE |
| 30340 | Beta-2-Glycoprotein I Antibodies (IgG, IgA, IgM) | Diagnose SLE |
| 7352 | Cardiolipin Antibodies (IgA, IgG, IgM) | Diagnose SLE |
| 34088 | Chromatin (Nucleosomal) Antibody | Diagnose SLE |
| 37859 | Complement Component C3, C4, CH50 | Diagnose SLE |
| 361 | Direct Antiglobulin Test (DAT) | Determine presence of autoimmune hemolytic anemia |
| 255 | DNA (ds) Antibody | Diagnose SLE |
| 427 | Erythropoietin | Determine presence of hemolytic anemia |
| 19654 | Lupus Anticoagulant and Antiphospholipid Confirmation (non-Coumadin) with Consultation | Diagnose SLE |
| 17725(X) | Lupus Activity Panel 2<br>Includes complement component C3 and C4 and high avidity dsDNA antibody. | Diagnose SLE |
| 91740 | Platelet Antibody, Direct (IgG) | Detect autoimmune thrombocytopenia |
| 34283 | Ribosomal P Antibody | Diagnose neuropsychiatric SLE |
| 19887 | RNP Antibody | Diagnose SLE or MCTD |
| 38567 | Sm/RNP Antibody | Diagnose SLE or MCTD |
| 37923 | Sm Antibody | Diagnose SLE |
| 937 | White Blood Cell Count (WBC) | Determine presence of leukopenia |
| **Systemic Sclerosis** | | |
| 90073[b] | ANA Screen, IFA with Reflex to Titer and Pattern/ Systemic Sclerosis Panel 1<br>Includes ANA screen (IFA) with reflexes to titer and pattern and centromere B and Scl-70 antibodies. | Diagnose systemic sclerosis |
| 94646[d] | Anti-PM/Scl-100 Antibody, EIA | Determine prognosis |
| 16088 | Centromere B Antibody | Diagnose limited cutaneous systemic sclerosis (CREST) |
| 18855 | Ku Autoantibodies | Predict muscle and joint involvement and digital vasculopathy-related complications |
| 19899 | RNA Polymerase III Antibody | Diagnose systemic sclerosis |
| 4942 | Scleroderma Antibody (Scl-70) | Diagnose systemic sclerosis |

*(Continued)*

## Table. Laboratory Tests for Classification and Diagnosis of Autoimmune Rheumatic and Related Diseases[a] *(Continued)*

| Test Code | Test Name | Clinical Use |
|---|---|---|
| 94685[c] | Systemic Sclerosis 12 Antibodies Panel 2<br>Includes centromere protein (CENP)-A, CENP-B, PM-Scl100, PM-Scl75, RP11 (RNA polymerase III), RP155 (RNA polymerase III), Scl-70, Th/To, U1 snRNP RNP70k, U1 snRNP RNP A, U1 snRNP RNP C, and U3-snRNP (fibrillarin) antibodies. | Diagnose systemic sclerosis |
| **Systemic Vasculitis** | | |
| 10547[b] | ANA Multiplex with Reflex to dsDNA | Differentially diagnose ARDs |
| 70159[b] | ANCA Screen with MPO and PR3, with Reflex to ANCA Titer | Differentiate types of systemic vasculitis |
| 4420 | C-Reactive Protein (CRP) | Identify inflammatory conditions |
| 375 | Creatinine | Assess renal function |
| 425 | Eosinophil Count, Blood | Assess eosinophilia |
| 427 | Erythropoietin | Determine presence of hemolytic anemia |
| 809 | Sed Rate by Modified Westergren | Determine disease severity |
| 8796 | Myeloperoxidase Antibody (MPO) | Diagnose ANCA-associated vasculitides |
| 723 | Platelet Count, EDTA | Assess thrombocytopenia |
| 34151 | Proteinase-3 Antibody | Diagnose ANCA-associated vasculitides |
| 294 | Urea Nitrogen (BUN) | Assess renal function |
| 8563 | Urinalysis, Microscopic | Determine presence of microhematuria |
| 937 | White Blood Cell Count (WBC) | Assess leukopenia |
| **ANA Screening Panels** | | |
| 249[b] | ANA Screen, IFA, with Reflex to Titer and Pattern | Differentially diagnose ARDs |
| 16814[b] | ANA Screen, IFA, with Reflex Titer and Pattern, and Reflex to Multiplex 11 Ab Cascade<br>Antibody cascade includes chromatin, dsDNA, RNP, Sm, Sm/RNP antibodies; if all 5 antibodies are negative, reflex to SS-A, SS-B, Scl-70, and Jo-1 antibodies; if all 4 of these antibodies are negative, reflex to ribosomal P and centromere B antibodies. | Differentially diagnose ARDs |
| 19946[b] | ANA Multiplex with Reflex to 11 Antibody Cascade<br>Includes ANA multiplex test with reflex to chromatin, dsDNA, RNP, Sm, Sm/RNP antibodies; if all 5 antibodies are negative, reflex to SS-A, SS-B, Scl-70, and Jo-1 antibodies; if all 4 of these antibodies are negative, reflex to ribosomal P and centromere B antibodies. | Differentially diagnose ARDs |
| 94954[b] | ANA Screen, IFA, Reflex Titer/Pattern, Reflex Mplx 11 Ab Cascade with IdentRA® | Differentially diagnose ARDs |

[a] Panel components may be ordered separately.
[b] Reflex tests are performed at an additional charge and are associated with an additional CPT code.
[c] This test was developed and its analytical performance characteristics have been determined by Quest Diagnostics. It has not been cleared or approved by the US Food and Drug Administration. This assay has been validated pursuant to the CLIA regulations and are used for clinical purposes.
[d] This test was developed and its performance characteristics validated by Rheumatology Diagnostic Laboratory. There is no FDA approved assay for this test. As a lab developed test (LDT), approval or clearance by the FDA is not required. This test may be used for clinical purposes and should not be regarded as investigational or for research.

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