ANAlyzeR™ ANA, IFA With Reflex Titer/Pattern, Systemic Autoimmune Panel 1

Test code: 36378

Specimen requirements: 9 mL refrigerated serum from blood drawn to fill 3×8.5 mL serum separator tubes (preferred).

Collection instructions: Refer to ANAlyzeR™ ANA, IFA with Reflex Titer/Pattern, Systemic Autoimmune Panel 1 | Test Detail | Quest Diagnostics Test Directory for detailed collection instructions.

CPT® codes: 86038 (ANA; if positive, reflex testing to titer and pattern [CPT code 86039]); 86235 (9X; centromere B, chromatin, Jo-1, RNP, Scl-70, Sm, Sm/RNP, SS-A, SS-B); 83520 (4X; MCV, RF IgA, IgG, IgM); 86146 (3X; beta-2-glycoprotein IgA, IgG, IgM); 86147 (3X; cardiolipin IgA, IgG, IgM); 86160 (2X; C3, C4); 86376 (thyroid peroxidase); 86255 (dsDNA; if positive, reflex testing to titer [CPT code 86256]); 86200 (CCP). Reflex tests are performed at additional charge with additional CPT codes as indicated.

CLINICAL USE

- Evaluate suspected systemic autoimmune rheumatic diseases (SARDs) and autoimmune thyroid disease

CLINICAL BACKGROUND

SARDs are a group of related conditions in which the immune system attacks the joints, muscles, and connective tissue. Many distinct SARDs have been identified, but some of the most common include

- Rheumatoid arthritis (RA)
- Systemic sclerosis (SSc)
- Sjögren syndrome (SS)
- Systemic lupus erythematosus (SLE)
- Antiphospholipid syndrome (APS)
- Idiopathic inflammatory myopathies (polymyositis [PM] and dermatomyositis [DM])
- Mixed connective tissue disease (MCTD)

SARDs are often difficult to diagnose because their symptoms can be vague, vary from patient to patient, and often overlap. However, differentiation is important to help inform prognosis and treatment strategy.[1] Diagnosis is based on clinical information, presentation, and laboratory test results. Although no single test can provide a definitive diagnosis for any one SARD, testing for autoantibodies—a hallmark of SARDs—is often one of the first steps in establishing a diagnosis.[2,3]

SARDs are associated with a high number of circulating autoantibodies, and many SARDs have distinct autoantibody profiles. Consequently, panels that simultaneously test for the presence or absence of numerous antibodies can efficiently help rule in or out specific conditions. To aid in differential diagnosis, autoantibody testing for SARDs typically includes testing for antinuclear antibodies (ANAs), which are found in most SARDs, as well as individual autoantibodies specific to particular SARDs.

ANA testing

ANAs are antibodies directed against nuclear and cytoplasmic antigens, which serve as markers of autoimmune processes. A high concentration of ANAs may indicate an autoimmune disease. Therefore, screening for ANAs is often the first test for suspected SARDs.[2] The American College of Rheumatology[4] and the European Autoimmunity Standardization Initiative[2] recommend testing for ANAs using an immunofluorescence assay (IFA) with human epithelial (HEp-2) cells, which are sensitive substrates containing up to 150 autoantigens.[5]

The diagnostic value of ANA testing varies by condition. For example, ANAs are found in most patients with MCTD, SLE, and SSc but may or may not be found in patients with SS or RA. Thus, a positive ANA result alone is not sufficient for diagnosis and a negative result cannot definitively rule out certain conditions.[5]

For patients who test positive for ANAs, the titer and pattern can provide further information. A titer of at least 1:40 is considered positive, but most patients with SARD have higher concentrations. Low-positive titers (ie, 1:40 to 1:80) are not uncommon in healthy individuals (up to 25%),[2] but using a threshold of 1:40 increases the test's sensitivity for SLE, SSc, and SS.[5] Nuclear and cytoplasmic staining patterns can inform the differential diagnosis because certain patterns tend to be associated with certain diseases.[6] For example, a homogeneous nuclear pattern is associated with SLE, chronic

autoimmune hepatitis, or juvenile idiopathic arthritis, while a speckled cytoplasmic pattern is associated with SLE or an inflammatory myopathy (eg, PM or DM).[7]

Specific antibody testing

Because ANA testing is sensitive but not highly specific, tests for individual antibodies are often needed to help establish the diagnosis. The relative importance of testing for ANA versus condition-specific antibodies varies by condition and is often indicated in the diagnostic or classification criteria for each condition (**Table 1**).

- **SLE**: Classification criteria for SLE include both ANA and SLE-specific antibodies, but the relative importance of each marker varies by guideline.[8,9]
  - Criteria from the European League Against Rheumatism and the American College of Rheumatology require ANAs be present before other clinical and laboratory criteria are considered; under this classification scheme, the presence of double-stranded DNA (dsDNA) and Smith (Sm) antibodies is more heavily weighted than that of antiphospholipid (eg, beta-2-glycoprotein [B2GP], cardiolipin) antibodies or low complement (eg, C3, C4) levels.[8]
  - In contrast, criteria from the Systemic Lupus Erythematosus International Collaborating Clinics place equal weight on (1) a positive ANA result; (2) a positive result for antibodies to dsDNA, Sm, or antiphospholipids; and (3) low complement levels.[9]

  Though not included in either set of criteria, chromatin antibodies also have relatively high sensitivity (48%-100%) and specificity (90%-99%) for SLE.[10]
- **MCTD and SSc**: ANAs are commonly found in patients with MCTD and SSc but are not included in diagnostic or classification criteria. Although a high ANA titer is often the first laboratory indication of MCTD, the diagnostic criteria require a positive ribonucleoprotein (RNP) antibody test result.[11] Similarly, a positive ANA result can indicate SSc if clinical symptoms are consistent, but classification criteria include a positive test result for other antibodies, including centromere and scleroderma-70 (Scl-70).[12]
- **SS and PM/DM**: ANAs are less prevalent in SS and PM/DM, so the presence of specific antibodies is more important. Although high-titer ANA was previously included in the SS classification criteria, this criterion was removed in 2016 (along with rheumatoid factor [RF] and Sjögren antibody B [SS-B/La]) for lack of added value. Sjögren antibody A (SS-A/Ro) positivity remains the only serologic criterion for SS.[13] ANA positivity is also not required for classification of PM/DM; these criteria include a positive histidyl-tRNA synthetase (Jo-1) antibody result.[14]
- **APS and autoimmune thyroid disease (ATD)**: Though ANAs may be found in patients with APS or ATD, ANA screening has no proven value for diagnosis of these conditions.[15] Instead, classification criteria for APS include a positive test result for antiphospholipid antibodies.[16] ATDs (namely, Graves disease and Hashimoto thyroiditis) are classified as organ-specific autoimmune diseases but often have extrathyroidal manifestations[17] and are related to certain SARDs.[18] No criteria for diagnosis or classification of ATDs have been established, but testing for thyroid peroxidase (TPO) antibodies is useful because these antibodies are prevalent in both conditions.[17,18]
- **RA**: Although RA is not generally associated with a high level of ANAs,[15] patients occasionally present with features of both RA and SLE ("rhupus") and test positive for ANA and analytes associated with RA.[19] RA can also be a comorbid condition in patients with other SARDs, as well as in patients with suspected SARDs who test negative for ANAs. Multiple biomarkers are used for the diagnosis of RA. Sensitivity and specificity vary based on the marker and the stage (early vs established) of RA. RF and cyclic citrullinated peptide (CCP) antibodies are the most widely used and are included in the RA classification criteria.[20] However, 20% or more of patients with RA are seronegative (negative for both RF and CCP).[21] For these patients, another antibody that can help diagnose RA is anti-mutated citrullinated vimentin (MCV).[22] Although not yet included in the classification criteria, MCV has demonstrated similar utility to the established markers and improves sensitivity for early RA when tested in conjunction with CCP.[22] A large meta-analysis found similar diagnostic performance for all 3 biomarkers for detecting all RA (**Table 2**), although the included studies varied in design and criteria used for RA diagnosis.[23]

Quest Diagnostics offers the ANAlyzeR™ ANA, IFA With Reflex Titer/Pattern, Systemic Autoimmune Panel 1. The panel simultaneously tests for ANA and 24 specific biomarkers associated with rheumatic and related diseases (**Table 2**). Positive results for ANA reflex to titer and pattern; positive results for dsDNA also reflex to titer. Individual components can be ordered separately. Tiered approaches that test ANA and reflex to specific antibodies are also available, including ANA, IFA, Cascade and Rheumatoid Arthritis Panel 2, With

Table 1. Antibody Prevalence in Rheumatic and Related Diseases and Healthy Individuals,[a] %

Analyte	SLE[8,9]	MCTD[11]	SSc[12]	SS[13]	PM/DM[14]	APS[16]	ATD	RA[20]		HI
								Early[b]	All	
ANA[5,24,25]	90-95	90-100	85-95[c]	50-60	50-60	40-70	10-20	20	38	20-30
B2GP[26-29]	7-22		7	5		78[d]				5
C3 and C4 Complement[30]	73[e]									
Cardiolipin[11,26-29]	7-21	15	10-14	4		81[d]				1
CCP[22,23,31]	1		3	1				62	71	1
CENP-B[24]	2-5	2-5	20-40[c,f]	5-10	1-3					<3
Chromatin[24]	40-70	5-18	<3	<3	<3					<3
dsDNA[24]	40-70	<3	<3	<3	<3					<3
Jo-1[24]	1-3	<2[g]	1-3	<2	15-30					<1
MCV[22,23,32]	12-36		10	10		27		78	71	≤5
RF[22,23,31,33]	15-35	50-60	20-30	75-95	20			72	77	5-25
RNP[24]	10-40	100	5-15	<3	5-15					<3
Scl-70[24]	0-5	<3[h]	20-40[f]	<3	<3					<1
Sm[24]	5-20	<2	<2	<1	<1					<1
Sm/RNP[6,34]	30	100	4	9	5					
SS-A/Ro[24]	40-70	<3	3-10	60-90	<3					<3
SS-B/La[24]	15-30	<3	1-5	60-80	5-15					<3
TPO[18,35,36]	4-13		5-21	10			>90			

ANA, antinuclear antibody; APS, antiphospholipid syndrome; ATD, autoimmune thyroid disease (ie, Graves disease or Hashimoto thyroiditis); B2GP, beta-2-glycoprotein; CCP, cyclic citrullinated peptide; CENP-B, centromere B; dsDNA, double-stranded DNA; HI, healthy individuals; Jo-1, histidyl-tRNA synthetase; MCTD, mixed connective tissue disease; MCV, mutated citrullinated vimentin; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; RF, rheumatoid factor; RNP, ribonucleoprotein; Scl-70, scleroderma-70 (topoisomerase 1); SLE, systemic lupus erythematosus; Sm, Smith; Sm/RNP, Smith/ribonucleoprotein; SS, Sjögren syndrome; SS-A, SS-B, Sjögren antibodies A and B; SSc, systemic sclerosis; TPO, thyroid peroxidase.

[a] Highlighted antibodies represent classification or diagnostic criteria for the disease. Note that antibodies included in criteria are not always those with the highest prevalence in that disease.

[b] Early RA defined as disease history <1 year.

[c] CREST syndrome is a variant of systemic sclerosis defined by the presence of calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia. Also known as limited cutaneous scleroderma. ANA is present in 70% of CREST patients, and CENP-B is present in 90% to 95%.[31]

[d] IgG type; 49% for cardiolipin IgM and 40% for B2GP IgM.[29]

[e] Low levels of both C3 and C4 are required for the prevalence of 73% of patients with SLE.[30]

[f] The presence of scleroderma-related antibodies (centromere, Scl-70, or RNA polymerase III antibodies) is not necessary or sufficient for diagnosis, but is useful for classification in the absence of diagnostic clinical findings (ie, "skin thickening of the fingers extending proximal to the metacarpophalangeal joints"[12]).

[g] Especially in patients with features of muscle inflammation.

[h] Especially in patients with features of systemic sclerosis.

Table 2. Component Tests of ANAlyzeR™ ANA, IFA With Reflex Titer/Pattern, Systemic Autoimmune Panel 1[a]

Test code	Test name
249	ANA Screen, IFA, With Reflex to Titer and Pattern[b,c]
36552	Beta-2-Glycoprotein I Antibody (IgA)
36554	Beta-2-Glycoprotein I Antibody (IgG)
36553	Beta-2-Glycoprotein I Antibody (IgM)
4661	Cardiolipin Antibody (IgA)
4662	Cardiolipin Antibody (IgG)
4663	Cardiolipin Antibody (IgM)
16088	Centromere B Antibody
34088	Chromatin (Nucleosomal) Antibody
351	Complement Component C3c
353	Complement Component C4c
11173	Cyclic Citrullinated Peptide (CCP) Antibody (IgG)
37092	DNA (ds) Antibody, Crithidia IFA With Reflex to Titer[b,d]
5810	Jo-1 Antibody
13238	Mutated Citrullinated Vimentin (MCV) Antibody
19705	Rheumatoid Factor (IgA, IgG, IgM)
19887	RNP Antibody
4942	Scleroderma Antibody (Scl-70)
38568	Sjögren's Antibody (SS-A)
38569	Sjögren's Antibody (SS-B)
37923	Sm Antibody
38567	Sm/RNP Antibody
5081	Thyroid Peroxidase Antibodies (TPO)

[a] Panel components can be ordered separately except as noted.
[b] Reflex tests are performed at additional charge and are associated with an additional CPT code(s).
[c] ANA titer and pattern cannot be ordered separately.
[d] dsDNA titer and pattern cannot be ordered separately.

Reflexes and ANA Cascade (ANA, IFA w/Reflex Titer/Pattern and Reflex to 11 Ab Cascade). Details for these panels can be found in the Test Directory at https://testdirectory.questdiagnostics.com/test/home.

INDIVIDUALS SUITABLE FOR TESTING

- Individuals with signs and symptoms associated with autoimmune disease(s)

METHODS

- ANA screening performed with IFA using HEp-2 cells; positive ANA IFA results at the 1:40 dilution prompt reflex to
  - Reporting of the corresponding antibody fluorescence pattern
  - Titer, determined by serial dilution until the pattern cannot be observed or to a dilution of 1:1,280
- B2GP (IgA, IgG, and IgM), cardiolipin (IgA, IgG, and IgM), CCP, centromere B, chromatin, Jo-1, RF (IgA, IgG, IgM), RNP, Scl-70, SS-A, SS-B, Sm, Sm/RNP, and TPO antibody testing performed with immunoassay
- C3 and C4 complement testing performed by immunoturbidity
- dsDNA antibody testing performed by immunoassay (*Crithidia luciliae* substrate assay) with reflex to titer if positive
- MCV testing performed with an enzyme-linked immunosorbent assay

REFERENCE RANGES

Reference ranges vary by analyte (**Table 3**).

INTERPRETIVE INFORMATION

A positive ANA result in conjunction with clinical suspicion suggests, but does not necessarily confirm, the presence of an autoimmune disease. Positive results are not uncommon in individuals who are healthy (particularly with increased age) and those with certain infectious diseases or cancer.[5,24] In cases with strong clinical suspicion, specific antibody testing may be appropriate even if the ANA result is negative. A positive ANA result reflexes to titer and pattern. High titers and certain nuclear and cytoplasmic patterns are associated with, but do not confirm, certain autoimmune diseases.

A negative ANA result suggests the absence of many autoimmune diseases but does not rule them out. Thus, specific antibody results may be more informative.

Table 3. Reference Ranges

Test	Reference range(s)	
ANA IFA Screen	Negative	
ANA Titer	Negative: <1:40 Low antibody level: 1:40-1:80 Elevated antibody level: >1:80	
B2GP IgA, IgG, IgM	<20.0 U/mL	
C3	Female: <1 year old: not established 1-14 years old: 82-173 mg/dL 15-80 years old: 83-193 mg/dL ≥81 years old: not established	Male: <1 year old: not established 1-14 years old: 80-170 mg/dL 15-80 years old: 82-185 mg/dL ≥81 years old: not established
C4	Female: <1 year old: not established 1-14 years old: 13-46 mg/dL 15-80 years old: 15-57 mg/dL ≥81 years old: not established	Male: <1 year old: not established 1-14 years old: 14-44 mg/dL 15-80 years old: 15-53 mg/dL ≥81 years old: not established
Cardiolipin IgA	<20.0 APL-U/mL	
Cardiolipin IgG	<20.0 GPL-U/mL	
Cardiolipin IgM	<20.0 MPL-U/mL	
CCP	Negative: <20 Units Weak positive: 20-39 Units Moderate positive: 40-59 Units Strong positive: >59 Units	
Centromere B	<1.0 AI	
Chromatin	<1.0 AI	
dsDNA	Negative	
dsDNA Titer	<1:10	
Jo-1	<1.0 AI	
MCV	<20 U/mL	
RF IgA, IgG, IgM	≤6 Units	
RNP	<1.0 AI	
Scl-70	<1.0 AI	
Sm	<1.0 AI	
Sm/RNP	<1.0 AI	
SS-A/Ro	<1.0 AI	
SS-B/La	<1.0 AI	
TPO	<9 IU/mL	

ANA, antinuclear antibody; B2GP, beta-2-glycoprotein; CCP, cyclic citrullinated peptide; dsDNA, double-stranded DNA; IFA, immunofluorescence assay; MCV, mutated citrullinated vimentin; RF, rheumatoid factor; RNP, ribonucleoprotein; Scl-70, scleroderma-70 (topoisomerase 1); Sm, Smith; Sm/RNP, Smith/ribonucleoprotein; SS-A, SS-B, Sjögren antibodies A and B; TPO, thyroid peroxidase.

A positive result for 1 or more of the specific antibodies may suggest the presence of a certain autoimmune disease (**Table 1**). If the ANA result is positive but the tests for specific antibodies are negative, the patient may still have an autoimmune disease other than those typically associated with the antibodies tested. Tests for other autoimmune diseases may be considered if clinically indicated; these include autoimmune hepatitis, primary biliary cholangitis, Addison disease, pernicious anemia, autoimmune neuropathies, vasculitis, celiac disease, and bullous disease.

A positive result on the RF, CCP antibody, or MCV tests generally supports a diagnosis of early or established RA, or potential subclinical RA. A negative result on all 3 tests is consistent with absence of established RA, though early RA cannot be ruled out.

References

1. Goldblatt F, O'Neill SG. Clinical aspects of autoimmune rheumatic diseases. *Lancet*. 2013;382(9894):797-808. doi:10.1016/S0140-6736(13)61499-3
2. Agmon-Levin N, Damoiseaux J, Kallenberg C, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis*. 2014;73(1):17-23. doi:10.1136/annrheumdis-2013-203863
3. Bizzaro N. Autoantibody profiles in autoimmune rheumatic diseases. *Mediterr J Rheumatol*. 2019;30(2):86-89. doi:10.31138/mjr.30.2.86
4. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis*. 2010;69(8):1420-1422. doi:10.1136/ard.2009.127100
5. 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: American College of Pathologists. *Arch Pathol Lab Med*. 2000;124(1):71-81. doi:10.1043/0003-9985(2000)124<0071:GFCUOT>2.0.CO;2
6. Satoh M, Vázquez-Del Mercado M, Chan EK. Clinical interpretation of antinuclear antibody tests in systemic rheumatic diseases. *Mod Rheumatol*. 2009;19(3):219-228. doi:10.1007/s10165-009-0155-3
7. Damoiseaux J, Andrade LEC, Carballo OG, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA Patterns (ICAP) perspective. *Ann Rheum Dis*. 2019;78(7):879-889. doi:10.1136/annrheumdis-2018-214436
8. Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis*. 2019;78(9):1151-1159. doi:10.1136/annrheumdis-2018-214819
9. Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum*. 2012;64(8):2677-2686. doi:10.1002/art.34473
10. Gómez-Puerta JA, Burlingame RW, Cervera R. Anti-chromatin (anti-nucleosome) antibodies: diagnostic and clinical value. *Autoimmun Rev*. 2008;7(8):606-611. doi:10.1016/j.autrev.2008.06.005
11. Ortega-Hernandez OD, Shoenfeld Y. Mixed connective tissue disease: an overview of clinical manifestations, diagnosis and treatment. *Best Pract Res Clin Rheumatol*. 2012;26(1):61-72. doi:10.1016/j.berh.2012.01.009
12. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum*. 2013;65(11):2737-2747. doi:10.1002/art.38098
13. Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis*. 2017;76(1):9-16. doi:10.1136/annrheumdis-2016-210571
14. Lundberg IE, Tjärnlund A, Bottai M, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis*. 2017;76(12):1955-1964. doi:10.1136/annrheumdis-2017-211468
15. Solomon DH, Kavanaugh AJ, Schur PH, et al. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. *Arthritis Rheum*. 2002;47(4):434-444. doi:10.1002/art.10561
16. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4(2):295-306. doi:10.1111/j.1538-7836.2006.01753.x
17. Marinò M, Chiovato L, Pinchera A. Graves' Disease. In: Jameson JL, De Groot LJ, eds. *Endocrinology.* 6th ed. Saunders Elsevier; 2010:1527-1558:chap 80.
18. Lazarus JH. Chronic (Hashimoto's) thyroiditis. In: Jameson JL, De Groot LJ, eds. *Endocrinology*. 6th ed. Saunders Elsevier; 2010:1583-1594:chap 83.
19. Amezcua-Guerra LM, Springall R, Marquez-Velasco R, et al. Presence of antibodies against cyclic citrullinated peptides in patients with 'rhupus': a cross-sectional study. *Arthritis Res Ther*. 2006;8(5):R144. doi:10.1186/ar2036
20. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*. 2010;62(9):2569-2581. doi:10.1002/art.27584
21. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet*. 2010;376(9746):1094-1108. doi:10.1016/S0140-6736(10)60826-4
22. Liu X, Jia R, Zhao J, et al. The role of anti-mutated citrullinated vimentin antibodies in the diagnosis of early rheumatoid arthritis. *J Rheumatol.* 2009;36(6):1136-1142. doi:10.3899/jrheum.080796
23. Zhu JN, Nie LY, Lu XY, et al. Meta-analysis: compared with anti-CCP and rheumatoid factor, could anti-MCV be the next biomarker in the rheumatoid arthritis classification criteria? *Clin Chem Lab Med.* 2019;57(11):1668-1679. doi:10.1515/cclm-2019-0167

24. Mahler M, Meroni PL, Bossuyt X, et al. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *J Immunol Res.* 2014;2014:315179. doi:10.1155/2014/315179

25. Rönnelid J, Turesson C, Kastbom A. Autoantibodies in rheumatoid arthritis - laboratory and clinical perspectives. *Front Immunol*. 2021;12:685312. doi:10.3389/fimmu.2021.685312

26. Marchetti T, Ribi C, Perneger T, et al. Prevalence, persistence and clinical correlations of classic and novel antiphospholipid antibodies in systemic lupus erythematosus. *Rheumatology (Oxford)*. 2018;57(8):1350-1357. doi:10.1093/rheumatology/key095

27. Morrisroe KB, Stevens W, Nandurkar H, et al. The association of antiphospholipid antibodies with cardiopulmonary manifestations of systemic sclerosis. *Clin Exp Rheumatol.* 2014;32(6) (suppl 86):S133-S137.

28. Pasoto SG, Chakkour HP, Natalino RR, et al. Lupus anticoagulant: a marker for stroke and venous thrombosis in primary Sjögren's syndrome. *Clin Rheumatol*. 2012;31(9):1331-1338. doi:10.1007/s10067-012-2019-z

29. Rodríguez-Pintó I, Moitinho M, Santacreu I, et al. Catastrophic antiphospholipid syndrome (CAPS): descriptive analysis of 500 patients from the International CAPS Registry. *Autoimmun Rev.* 2016;15(12):1120-1124. doi:10.1016/j.autrev.2016.09.010

30. Li H, Lin S, Yang S, et al. Diagnostic value of serum complement C3 and C4 levels in Chinese patients with systemic lupus erythematosus. *Clin Rheumatol.* 2015;34(3):471-477. doi:10.1007/s10067-014-2843-4

31. van Paassen P, Damoiseaux J, Tervaert JWC. Laboratory assessment in musculoskeletal disorders. *Best Pract Res Clin Rheumatol*. 2003;17(3):475-494.

32. Alessandri C, Agmon-Levin N, Conti F, et al. Anti-mutated citrullinated vimentin antibodies in antiphospholipid syndrome: diagnostic value and relationship with clinical features. *Immunol Res.* 2017;65(2):524-531. doi:10.1007/s12026-017-8899-x

33. Ingegnoli F, Castelli R, Gualtierotti R. Rheumatoid factors: clinical applications. *Dis Markers*. 2013;35(6):727-734. doi:10.1155/2013/726598

34. Scholz J, Grossmann K, Knütter I, et al. Second generation analysis of antinuclear antibody (ANA) by combination of screening and confirmatory testing. *Clin Chem Lab Med*. 2015;53(12):1991-2002. doi:10.1515/cclm-2015-0083

35. Posselt RT, Coelho VN, Pigozzo DC, et al. Prevalence of thyroid autoantibodies in patients with systematic autoimmune rheumatic diseases: cross-sectional study. *Sao Paulo Med J*. 2017;135(6):535-540. doi:10.1590/1516-3180.2017.0089110617

36. Koszarny A, Majdan M, Dryglewska M, et al. Prevalence of selected organ-specific autoantibodies in rheumatoid arthritis and primary Sjögren's syndrome patients. *Reumatologia*. 2015;53(2):61-68. doi:10.5114/reum.2015.51504

The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payer being billed.