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

Test Code: 36378

Specimen Requirements: 9 mL refrigerated serum (red-top tube [no gel]); 6 mL minimum

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; 14-3-3 eta, 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.

CLINICAL USE

- Evaluate suspected autoimmune rheumatic diseases

CLINICAL BACKGROUND

Autoimmune rheumatic diseases are conditions in which the immune system attacks the joints and certain organs. They are often difficult to diagnose, as their symptoms can be vague, vary from patient to patient, and often overlap. Diagnosis is most often based on a compilation of symptoms and signs, including clinical information and laboratory test results. Testing for antibodies associated with different conditions can provide useful information, but no single test provides a definitive diagnosis for any one rheumatic disease.

Antinuclear antibody testing

Testing for antinuclear antibodies (ANAs) using an immunofluorescence assay (IFA) is an important part of evaluating patients suspected of having certain autoimmune rheumatic diseases. ANAs are a group of autoantibodies directed against diverse nuclear and cytoplasmic antigens. They are associated with several autoimmune rheumatic diseases, but the diagnostic value of ANA testing varies with the specific clinical condition. While ANA test results are positive for most patients with certain conditions, such as mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE), or systemic sclerosis, such results may be positive or negative for patients with other common autoimmune conditions, such as Sjögren syndrome or rheumatoid arthritis (RA). Thus, a positive ANA result alone is not sufficient for diagnosis, and a negative ANA result does not definitively rule out many autoimmune rheumatic conditions.

Knowing the ANA titer and pattern can help interpret positive ANA results. A titer of at least 1:40 is considered positive, although most patients with autoimmune disease will have higher levels. Low-positive titers (eg, 1:40) are not uncommon in healthy individuals (20% to 30%), but using a threshold of 1:40 can increase sensitivity for SLE, systemic sclerosis, and Sjögren syndrome.[1,2] For patients with positive ANA screening results, nuclear and cytoplasmic antibody fluorescence patterns may inform the differential diagnosis, but they may not be specific for individual diseases.[3,4] For example, a homogeneous nuclear pattern may be associated with SLE, drug-induced SLE/vasculitis, or juvenile idiopathic arthritis, while a diffuse cytoplasmic pattern could be consistent with SLE or an inflammatory myopathy.[5]

Specific autoantibodies

ANAs are highly prevalent in many autoimmune conditions **(Table 1)**, making ANA testing sensitive but not highly specific; tests for individual antibodies offer greater specificity and are often needed to help establish the diagnosis. The relative importance of testing for ANA versus condition-specific antibodies varies and is often indicated in diagnosis or classification criteria for each condition.

For SLE, classification criteria include both ANA and SLE-specific antibodies, but the relative importance of each marker can vary by guideline.[7,25] SLE classification criteria from the European League Against Rheumatism and the American College of Rheumatology (EULAR/ACR) require ANA be present before other clinical and laboratory criteria are considered[25]; with this classification scheme, the presence of dsDNA and Sm antibodies is more heavily weighted than the presence of antiphospholipid (eg, beta-2-glycoprotein [B2GP], cardiolipin) antibodies or low complement (eg, C3, C4) levels. In contrast, SLE classification criteria from the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) places equal weight on 1) a positive ANA result; 2) a positive result for antibodies to dsDNA, Sm, or antiphospholipids; and 3) low complement levels.[7]

Table 1. Autoantibody Prevalence (%) in Rheumatic and Related Diseases[1,6-22,a]

Antibody	SLE	MCTD	SSc	SjS	PM	APS	ATD
ANA	93	100	85[b]	48	61	57	30-50
B2GP	2-29					78[c]	
C3 and C4 Complement	73[d]						
Cardiolipin	16	9	7	7	8	81[c]	
CENP-B	3-12	7[e]	27[b,f]	<2	<2		
Chromatin	37-73	>80	14	12	8		
dsDNA	57-62	0-8	8	11-20	10-43	32	
Jo-1	<2	7[g]	<2	<2	17		
RNP	22-48	>80	14	12	8		
Scl-70	2-3	7[h]	16[f]	<2	<2		
Sm	20-30	8	0	4	10		
Sm/RNP	30	54-94	4	9	9		
SS-A/Ro	33-52	13	23	>80[i]	42		
SS-B/La[i]	13-27	<2	5	>80	<2		
TPO	30		24	28			90

ANA, antinuclear antibody; APS, antiphospholipid syndrome; ATD, autoimmune thyroid disease (ie, Graves disease or Hashimoto thyroiditis); B2GP, beta-2-glycoprotein; CENP-B, centromere B; dsDNA, double-stranded DNA; Jo-1, histidyl-tRNA synthetase; MCTD, mixed connective tissue disease; PM, polymyositis; RNP, ribonucleoprotein; Scl-70, scleroderma-70 (topoisomerase 1); SLE, systemic lupus erythematosus; Sm, Smith; Sm/RNP, Smith/ribonucleoprotein; 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 do not always correspond to those with the highest prevalence in that disease.

[b] 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 66%.

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

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

[e] Typically in patients with features of PM.

[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 ("clear skin thickening of the fingers extending proximal to the metacarpophalangeal joints"[23]).

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

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

[i] Previous classification criteria from 2012 included testing for SS-B/La, rheumatoid factor (RF), and ANA titer in addition to SS-A/Ro.[35] These markers were not included in 2016 guidelines, because studies indicated the markers do not add sufficient value to SS-A/Ro testing.[24]

For some diseases, ANA is highly prevalent in patients but not included in diagnosis or classification criteria. For example, a high ANA titer is often the first indication of MCTD; however, the diagnostic criteria require a positive RNP antibody test result.[26] Similarly, a positive ANA result can indicate systemic sclerosis if clinical symptoms are consistent; however, classification criteria require a positive test result for other antibodies (eg, centromere, Scl-70).[23]

For other autoimmune rheumatic conditions, positive results for specific autoantibodies are more important than ANAs, as the prevalence of ANAs may be lower. In 2016, high-titer ANA was removed as a classification criterion for Sjögren syndrome (RF and SS-B/La were also removed), because it did not add enough value to SS-A/Ro testing.[24] Criteria for polymyositis (PM) include a positive Jo-1 antibody result; an ANA result is not required,[27] although ANAs are present in over half of PM patients **(Table 1)**.

Thus, a positive ANA test result can indicate that an autoimmune rheumatic condition is present, but testing for other antibodies may help diagnose the specific condition.

Rheumatoid arthritis biomarkers

Multiple biomarkers for the diagnosis of RA are available. Sensitivity and specificity of testing vary based on which markers are tested and which stage (early vs established) of RA. Rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) antibodies are widely used and included in the classification criteria for RA.[11] Another biomarker that can help diagnose RA is 14-3-3η protein, which improves sensitivity for diagnosis of early and established RA when used in conjunction with RF and CCP antibody testing **(Table 2)**. In one study, 21% to 67% of seronegative RA patients were positive for 14-3-3η.[28]

Since a positive result for any of these RA markers facilitates timelier diagnosis of ANA-negative RA, simultaneous testing can enable prompt initiation of disease-modifying therapy before significant joint erosion develops. Although RA is not generally associated with a high prevalence of ANA, occasional patients may present with features of RA and SLE ("rhupus") and be positive for ANA and RA analytes.[29] RA can be present as a comorbid condition in patients with other autoimmune rheumatic diseases and in patients with suspected rheumatic diseases who test negative for ANAs.

Antibody panels

Because the prevalence of ANAs varies among conditions, testing only for ANA IFA may fail to identify 1) patients with the suspected autoimmune condition, 2) patients with comorbid autoimmune diseases (eg, antiphospholipid syndrome [cardiolipin and beta-2-glycoprotein antibodies]), and 3) patients with autoimmune diseases that have similar presentations (eg, RA, autoimmune thyroiditis [TPO antibody]). Testing specific antibodies at the same time as ANA IFA may expedite evaluation. The combination of the highly sensitive ANA IFA test and specific autoantibody testing can also help maximize sensitivity and specificity.

Quest Diagnostics offers the ANAlyzeR™ ANA, IFA with Reflex Titer/Pattern, Systemic Autoimmune Panel 1. The panel simultaneously tests for ANA IFA and 24 specific biomarkers associated with rheumatic and related diseases: 14-3-3η, B2GP (IgA, IgG, IgM), C3 complement, C4 complement, cardiolipin (IgA, IgG, and IgM), CCP, centromere, chromatin, dsDNA, Jo-1, RF (IgA, IgG, IgM), ribonucleoprotein (RNP), Scl-70 (topoisomerase 1), Smith (Sm), SS-A/Ro, SS-B/La, and thyroid peroxidase (TPO). (Though not included in the SLICC classification criteria, chromatin antibodies have relatively high sensitivity [64% to 69%] and specificity [92% to 99%] for SLE).[10,30] Positive results for ANA reflex to titer and pattern; positive results for dsDNA also reflex to titer. Individual components can be ordered separately **(Table 3)**.

Tiered approaches that test ANA and reflex to specific antibodies are also available: ANA Screen, IFA, Reflex Titer/Pattern, Reflex Mplx 11 Ab Cascade with IdentRA® (test code 94954) and ANA Screen, IFA, Reflex Titer/Pattern, Reflex to Multiplex 11 Ab Cascade (test code 16814).

INDIVIDUALS SUITABLE FOR TESTING

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

Table 2. Sensitivity and Specificity of RF, CCP Antibody, and 14-3-3η for Detecting RA

Markers	Early RA[28,a,b] (n=99)		Established RA[28,a,b] (n=135)	
	Sensitivity	Specificity	Sensitivity	Specificity
RF	57	85	84	85
CCP antibody	59	99	79	99
14-3-3η	64	93	77	93
RF and CCP antibody[c]	72	84	88	84
RF, CCP antibody, and 14-3-3η[c]	78	78	90	78

CCP, cyclic citrullinated peptide; RA, rheumatoid arthritis; RF, rheumatoid factor.
[a] Early RA indicates disease history of 6 months or less. Established RA indicates disease history of >6 months.
[b] Comparison with healthy controls.
[c] Results considered positive for RA if any one of the biomarkers is positive.

METHODS

- ANA screening conducted using an IFA performed with HEp-2 cells. Positive ANA IFA results at the 1:40 dilution prompt reflex to 1) reporting of the corresponding antibody fluorescence pattern; and 2) titer, determined by serial dilution until the pattern cannot be observed or to a dilution of 1:1,280 – specimens may be titrated to endpoint upon request.
- Testing for the following antibodies is performed using immunoassay: 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.
- Complement C3 and C4 testing is performed using immunoturbidity.
- dsDNA antibody testing is performed using immunoassay (*Crithidia luciliae* substrate assay) and reflexes to titer if positive.
- 14-3-3η testing performed using an ELISA-based assay.
- Aside from ANA titer and pattern and dsDNA titer, panel components may be ordered separately **(Table 3)**.
- Reflex tests are performed at additional charge and are associated with an additional CPT code(s).

Table 3. Component Tests of ANAlyzeR™ ANA, IFA with Reflex Titer/Pattern, Systemic Autoimmune Panel[1]

Test Code	Test Name	CPT Code
91455	14.3.3 eta Protein[a]	83520
249	ANA Screen, IFA, with Reflex to Titer and Pattern[b]	86038 with reflex to 86039
36552	Beta-2-Glycoprotein I Antibody (IgA)	86146
36554	Beta-2-Glycoprotein I Antibody (IgG)	86146
36553	Beta-2-Glycoprotein I Antibody (IgM)	86146
4661	Cardiolipin Antibody (IgA)	86147
4662	Cardiolipin Antibody (IgG)	86147
4663	Cardiolipin Antibody (IgM)	86147
16088	Centromere B Antibody	86235
34088	Chromatin (Nucleosomal) Antibody	86235
351	Complement Component C3[c]	86160
353	Complement Component C4[c]	86160
11173	Cyclic Citrullinated Peptide (CCP) Antibody (IgG)	86200
37092	DNA (ds) Antibody, Crithidia IFA with Reflex to Titer[b]	86255 with reflex to 86256
5810	Jo-1 Antibody	86235
19705	Rheumatoid Factor (IgA, IgG, IgM)	83520 (3X)
19887	RNP Antibody	86235
4942	Scleroderma Antibody (Scl-70)	86235
38568	Sjögren's Antibody (SS-A)	86235
38569	Sjögren's Antibody (SS-B)	86235
37923	Sm Antibody	86235
38567	Sm/RNP Antibody	86235
5081	Thyroid Peroxidase Antibodies (TPO)	86376

[a] This test was developed and its analytical performance characteristics have been determined by Quest Diagnostics. It has not been cleared or approved by FDA. This assay has been validated pursuant to the CLIA regulations and is used for clinical purposes.
[b] Reflex tests are performed at additional charge and are associated with an additional CPT code(s).

REFERENCE RANGES

Reference ranges vary by analyte **(Table 4)**.

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 healthy individuals (particularly as they age) and those with certain infectious diseases or cancer.[7] In cases with strong clinical suspicion, specific antibody testing may be appropriate even if the ANA IFA 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 disease.

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

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 14-3-3η tests generally supports a diagnosis of early or established RA, or potential subclinical RA **(Table 2)**. In ANA-negative patients, positive results are consistent with early RA.[31] A negative result on all 3 tests is consistent with absence of RA (sensitivity=78%), although early RA cannot be ruled out **(Table 2)**.

Table 4. Reference Ranges

Test	Reference Range(s)
14-3-3η	<0.2 ng/mL
ANA IFA Screen	Negative
ANA Titer	Negative: <1:40 Low antibody level: 1:40-1:80 Elevated antibody level: >1:80
B2GP IgA	≤20 SAU
B2GP IgG	≤20 SGU
B2GP IgM	≤20 SMU

(Continued)

Table 4. Reference Ranges (Continued)

Test	Reference Range(s)
C3	<1 year old: not established 1-14 years old: 80-173 mg/dL 15-80 years old: 82-193 md/dL ≥ 80 years old: Not established
C4	<1 year old: not established 1-14 years old: 13-46 mg/dL 15-80 years old: 15-57 mg/dL ≥ 80 years old: Not established
Cardiolipin IgA	Negative: ≤11 APL Indeterminate: 12-20 APL Low- to medium-positive: 21-80 APL High positive: >80 APL
Cardiolipin IgG	Negative: ≤14 GPL Indeterminate: 15-20 GPL Low- to medium-positive: 21-80 GPL High positive: >80 GPL
Cardiolipin IgM	IgM Negative: ≤12 MPL Indeterminate: 13-20 MPL Low- to medium-positive: 21-80 MPL High positive: >80 MPL
CCP Antibody	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
RF IgA	<6 Units
RF IgG	<6 Units
RF IgM	<6 Units
RNP	<1.0 AI
Scl-70	<1.0 AI
Sm	<1.0 AI
Sm/RNP	<1.0 AI
SS-A	<1.0 AI
SS-B	<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; 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.

References

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* 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.

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