MAYO CLINIC LABORATORIES

Test Definition: SSCP

Systemic Sclerosis Criteria Panel, Serum

Overview

Useful For

Evaluating patients with antinuclear antibody-associated connective tissue disease, specifically systemic sclerosis

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
NAIFA	Antinuclear Ab, HEp-2 Substrate, S	Yes	Yes
SCL70	Scl 70 Ab, IgG, S	Yes	Yes
RNAP	RNA Polymerase III Ab, IgG, S	Yes	Yes

Highlights

This test is the recommended first-line autoantibody panel for evaluating patients at risk for systemic sclerosis.

Method Name

NAIFA: Indirect Immunofluorescence
SCL70: Multiplex Flow Immunoassay
RNAP: Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 0.7 mL
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Specimen Minimum Volume

0.5 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK
Heat-treated specimens	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	21 days	
	Frozen	21 days	

Clinical & Interpretive

Clinical Information

Systemic sclerosis (SSc) is a multisystem autoimmune connective tissue disease (CTD) characterized by vascular dysfunction, fibrotic changes in the skin, organ involvement, and autoimmune response manifested by the production of diverse antibodies.(1,2) The clinical manifestations in SSc may overlap with other systemic autoimmune rheumatic diseases (SARD), complicating early diagnosis and appropriate management.(1,3,4) Clinically, early diagnosis and risk stratification of patients with SSc for specific clinical manifestations is important for appropriate management.(1,3,4) A characteristic clinical feature of SSc is Raynaud phenomenon's vasculopathy characterized by SSc capillaroscopic changes on nailfold capillaroscopy.(3,4) Fibrosis and vasculopathy can extend to internal organs, causing severe complications such as interstitial lung disease (ILD), pulmonary arterial hypertension (PAH), and scleroderma renal crisis (SRC).(2,4-6) Although SSc has traditionally been categorized into two main subsets (limited cutaneous [lcSSc] and diffuse cutaneous [dcSSc]), the clinical manifestations and severity of disease are highly variable.(3-7) Of the two forms, the dcSSc has the worse prognosis and survival rates compared to the lcSSc subset.(6,7)

Immunologically, SSc may be characterized by the presence of mutually exclusive disease-specific or SSc-associated autoantibodies, which could be helpful in the diagnosis, risk stratification, or management of patients.(1-8).Of the described autoantibodies in SSc, the 2013 American College of Rheumatology/European League against Rheumatism classification criteria for SSc recommend testing for centromere (ACA), topoisomerase I (topo I or Scl-70), and RNA polymerase III (RNAP) autoantibodies.(4) Antibodies to Scl-70 and RNAP are generally associated with dcSSc while ACA is typically correlates with the lcSSc form of the disease.(1-3,5) In addition to their correlations with lcSSc or dcSSc, these antibodies may be useful in predicting certain clinical manifestations, disease severity, and have varied prevalence in different racial/ethnic populations.(2,5,7,8) Depending on the racial/ethnic cohort, after ACA and anti-Scl-70 antibodies, autoantibodies to RNAP are the most frequent antinuclear antibodies in SSc.(7,8) In a reported meta-analysis of 30 peer-reviewed studies, the overall pooled prevalence of anti-RNAP antibodies was 11% with a 95% confidence interval of 8% to 14%.(5)

Other noncriteria autoantibodies specific or associated with potential for diagnosis or phenotypic stratification in SSc

have been described.(2,5,7,8) The two best described SSc-specific antibodies include those targeting Th/To and U3RNP antigens expressed as antinuclear antibody (ANA) nucleolar pattern when detected using HEp-2 substrate by indirect immunofluorescence assay (HEp-2 IFA).(2,5,8) Anti-Th/To and anti-U3RNP antibodies are associated with unique clinical features (lcSSc or dcSSc) and are useful in predicting certain clinical manifestations in patients with SSc.(2,5,8) The SSc-associated antibodies useful diagnosis and risk stratification includes anti-U1RNP, anti-PM/Scl, anti-Ku, anti-Ro52, anti-Nor90 autoantibodies. These autoantibodies may be used to identify SSc with overlap syndromes (OS) or patients at risk for certain clinical manifestations such as ILD, and myositis.(1,2,5-7) Except for anti-Ro52 and anti-U1RNP autoantibody tests that are widely available, the routine use of the noncriteria antibodies is restricted to a few specialty laboratories with limited availability of commercial tests due technical issues that limit their sensitivities and specificities in methods typically used in the clinical laboratories.(5,8,9)

Antinuclear antibody detected using HEp-2 IFA is present in most patients with SSc.(2,5,8) The most unique SSc pattern identified by HEp-2 IFA is ACA with the nucleolar pattern suggesting the presence of anti-U3 RNP, anti-Th/To, anti-NOR 90, or anti-PM/Scl antibodies.(2,5,8). In addition, the presence of anti-Scl70, anti-RNAP, anti-Ro52, anti-U1RNP, anti-Ku, or the other recently reported antibodies (anti-U11/U12 RNP, and anti-RuvBL1/2) may be characterized by composite or unique nuclear (nucleolar, speckled, homogeneous nuclear), cytoplasmic patterns and metaphase staining (for examples see www.anapatterns.org), confirming that the ANA test using HEp-2 IFA is important for the evaluation of SSc and SSc/OS.(2,5) Due to the complexity of diagnosing SSc including its clinical overlap with other ANA-CTD and high prevalence of ANA, the use of HEp-2 IFA in addition to criteria antibody tests is optimal for the initial evaluation of at-risk patients. The use of the HEp-2 IFA is also optimal in the interpretation of specific antibodies and may be clinically relevant in the absence of defined autoantibody specificities. ACA is optimally detected with HEp-2 IFA while anti-Scl-70 and anti-RNAP antibodies are routinely detected or measured with diverse solid-phase immunoassays (SPAs) such as the line immunoblot (LIA), enzyme-linked immunosorbent assay (ELISA), multiplex bead immunoassay (MBIA), chemiluminescence immunoassay (CIA), and fluorescence enzyme immunoassay (FEIA).(2,5-9) These SPAs have been reported to be less specific than the classical methods, especially in distinguishing SSc patients from those with other SARD, with ACA, anti-Scl70 and anti-RNAP antibodies generally demonstrating moderate to excellent correlations (Cohen’s kappa: 0.53 to 0.97).(9) However, in routine laboratory evaluation, tests for anti-Scl-70 antibodies have been reported to lack diagnostic specificity.(10,11) Therefore, results for these and other autoantibodies must be interpreted in the appropriate clinical context taking into consideration the presence of a positive ANA test using HEp-2 IFA, the degree of antibody positivity, and the method of their detection.(1,2,5,10)

Reference Values

ANTINUCLEAR ANTIBODIES, HEp-2 SUBSTRATE, IgG
<1:80 (Negative)

Scl 70 ANTIBODIES, IgG
<1.0 U (negative)
> or = 1.0 U (Positive)

RNA POLYMERASE III ANTIBODIES, IgG
<20.0 U (Negative)
20.0-39.9 U (Weak positive)
40.0-80.0 U (Moderate positive)
>80.0 U (Strong positive)

Interpretation

Presence of anti-cellular antibody (also known as antinuclear antibody) is a characteristic feature of systemic autoimmune rheumatic diseases such as systemic lupus erythematosus, mixed connective tissue disease, Sjogren syndrome and systemic sclerosis (SSc), and inflammatory myopathies (dermatomyositis, anti-synthetase syndrome and necrotizing autoimmune myopathy). It may also be of diagnostic relevance in patients with autoimmune liver diseases.

Patients' sera are screened at 1:80. The following nuclear patterns and their titers are reported: centromere, homogeneous, nuclear dots, nucleolar, speckled, fine dense speckled (also referred to as DFS70), and proliferating cell nuclear antigen (PCNA). If observed, the following cytoplasmic patterns are reported: reticular/AMA (antimitochondrial antibody), cytoplasmic speckled, fibrillar, polar/Golgi-like, or rods and rings. The spindle fiber and centrosome mitotic patterns are also reported if observed. Reported patterns may help guide differential diagnosis, although they may not be specific for individual antibodies or diseases. Negative results do not necessarily rule out systemic autoimmune rheumatic disease.

The antinuclear antibody test lacks diagnostic specificity and is associated with some cancers, infectious, and inflammatory conditions, with variable prevalence in healthy individuals. The lack of diagnostic specificity requires confirmation of positive results using associated antibody tests such as those targeting extractable nuclear antigens.

A positive test result for Scl-70 antibodies may be consistent with a diagnosis of systemic sclerosis in the appropriate clinical context.

A positive result for RNA polymerase III antibody may support a diagnosis of SSc in the appropriate clinical context. Anti-RNA polymerase III autoantibody in patients with SSc is associated with the diffuse cutaneous form of disease and an increased risk of sclerodermal renal crisis.

A negative result indicates no detectable IgG antibodies to RNA polymerase III and does not rule out a diagnosis of SSc. The RNA polymerase III IgG enzyme-linked immunosorbent assay tests only for the RP155 dominant epitope, other epitopes in the antigenic complex are absent and cannot be detected.(6) The overall pooled prevalence of anti-RNA polymerase III antibody is reported to be 11%, 95% CI: 8 to 14, range of 0% to 41% in published studies.(4)

Cautions

Some patients without clinical evidence of systemic autoimmune rheumatic disease (SARD) may be positive for antinuclear antibody. This occurs at variable prevalence depending on the patient demographics. A positive result may also precede clinical manifestation of SARD or be associated with some viral or chronic infections, cancers, or use of certain medications. All results must be reported in the appropriate clinical context as the performance of the test can be variable.

Low positive Scl-70 antibody results should be interpreted with a high degree of suspicion. Anti-Scl-70 antibodies have been reported in some inflammatory conditions and other connective tissue diseases, especially in patients with systemic lupus erythematosus.

A positive result indicates detectable anti-RNA polymerase III above assay cutoff and does not unequivocally establish a diagnosis of systemic sclerosis (SSc).(6,7)

Enzyme immunoassay to detect anti-RNA polymerase III antibody uses an immunodominant epitope as antigen. Negative result does not also rule out the presence of antibodies targeting other epitopes in the RNA polymerase complex.

The level of RNA polymerase III autoantibodies does not indicate the severity of disease in patients with SSc. However, patients with high positive anti-RNA polymerase III antibody titers are more likely to have SSc compared to those with low antibodies.(7)

Anti-RNA polymerase III antibodies may occur prior to clinical onset of SSc.(7)

The presence of immune complexes or other immunoglobulin aggregates in the patient specimen may cause an increased level of nonspecific binding and produce false-positive results with this assay.

Clinical Reference

1. Abraham DJ, Black CM, Denton CP, et al. An international perspective on the future of systemic sclerosis research. Nat Rev Rheumatol. 2025;21:174-187
2. Chepy A, Collet A, Launay D, Dubucquoi S, Sobanski V. Autoantibodies in systemic sclerosis: From disease bystanders to pathogenic players. J Transl Autoimmun. 2025;10:100272
3. LeRoy EC, Medsger TA Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol. 2001;28(7):1573-1576
4. 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
5. Cavazzana I, Vojinovic T, Airo P, et al. Systemic sclerosis-specific antibodies: Novel and classical biomarkers. Clin Rev Allergy Immunol. 2023;64(3):412-430
6. Pokeerbux MR, Giovannelli J, Dauchet L, et al. Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. Arthritis Res Ther. 2019;21(1):86
7. Kuchinad KE, Kim JS, Woods A, et al. Racial variability in immune responses only partially explains differential systemic sclerosis disease severity. Ann Rheum Dis. 2024;83(11):1513-1521
8. Nandiwada SL, Peterson LK, Mayes MD, et al. Ethnic differences in autoantibody diversity and hierarchy: More clues from a US cohort of patients with systemic sclerosis. J Rheumatol. 2016; 43(10):1816-1824
9. Alkema W, Koenen H, Kersten BE, et al. Autoantibody profiles in systemic sclerosis; a comparison of diagnostic tests. Autoimmunity. 2021;54(3):148-155
10. Tebo AE, Schmidt RL, Frech TM. Presence of anti-topoisomerase I antibody alone may not be sufficient for the diagnosis of systemic sclerosis. J Rheumatol. 2019;46(4):440-442

Performance

Method Description

Antinuclear Antibodies, HEp-2 Substrate:
Antibodies to nuclear antigens in a human epithelial type 2 (HEp-2) cell line by an indirect immunofluorescent technique. Commercial slides prepared from HEp-2 cells are used as a substrate. IgG antibodies in serum samples are detected after incubation of serum with the commercial slides by the addition of a fluorescein isothiocyanate (FITC)-labeled antihuman-IgG reagent. All patient samples are initially screened at 1:80.(Package insert: NOVA Lite DAPI ANA. Inova Diagnostics; 06/2018)

Scl 70 Antibodies:
Recombinant Scl-70 antigen is bound to polystyrene microspheres, which are impregnated with fluorescent dyes to create a unique fluorescent signature. Scl-70 antibodies, if present in diluted serum, bind to the Scl-70 antigen on the

microspheres. The microspheres are washed to remove extraneous serum proteins. Phycoerythrin-conjugated antihuman IgG antibody is then added to detect IgG anti-Scl-70 bound to the microspheres. The microspheres are washed to remove unbound conjugate, and bound conjugate is detected by laser photometry. A primary laser reveals the fluorescent signature of each microsphere to distinguish it from microspheres that are labeled with other antigens, and a secondary laser reveals the level of PE fluorescence associated with each microsphere. Results are calculated by comparing median fluorescence response for Scl-70 microspheres to a 4-point calibration curve.(Package insert: Bioplex 2200 ANA Screen. Bio-Rad Laboratories; 02/2019)

RNA Polymerase III Antibodies:
The immunodominant fragment of RNA polymerase III antigen is derived from recombinant DNA technology. Purified RNA polymerase III antigen is adsorbed to the wells of a polystyrene microtiter plate under conditions that preserve the antigen in its antigenic state. Prediluted controls and diluted patient sera are added to separate wells. Unbound sample is washed away, and an enzyme-labeled antihuman IgG conjugate is added to each well. After incubation and washing away of unbound enzyme-labeled antihuman IgG, the bound conjugate is measured by adding a chromogenic substrate. The intensity of the absorbance produced is measured with an automated microwell plate reader. Results are calculated by comparison to a single-point calibrator.(Package insert: QUANTA Lite RNA Pol III. INOVA Diagnostics; 05/2019)

PDF Report
No

Day(s) Performed
Tuesday, Thursday

Report Available
2 to 7 days

Specimen Retention Time
14 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

Test Definition: SSCP

Systemic Sclerosis Criteria Panel, Serum

CPT Code Information

86039
86235
83516

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
SSCP	Systemic Sclerosis Panel, S	In Process

Result ID	Test Result Name	Result LOINC® Value
SCL70	Scl 70 Ab, IgG, S	47322-3
RNAP	RNA Polymerase III Ab, IgG, S	79182-2
ANAH	Antinuclear Ab, HEp-2 Substrate, S	59069-5
1TANA	ANA Titer:	33253-6
1PANA	ANA Pattern:	49311-4
2TANA	ANA Titer 2:	33253-6
2PANA	ANA Pattern 2:	49311-4
CYTQL	Cytoplasmic Pattern:	55171-3
LCOM	Lab Comment:	77202-0