MAYO CLINIC LABORATORIES

Test Definition: RFLXP

Antinuclear Antibodies, HEp-2, Reflex Panel

Overview

Useful For

Confirmatory testing after a positive homogenous, speckled, or dense fine speckled pattern identified in the human epithelial type 2 antinuclear antibody indirect immunofluorescence assay

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
ADNA1	dsDNA Ab, IgG, S	Yes	Yes
RNP	RNP Ab, IgG, S	Yes	Yes
SCL70	Scl 70 Ab, IgG, S	Yes	Yes
SM	Sm Ab, IgG, S	Yes	Yes
SSA	SS-A/Ro Ab, IgG, S	Yes	Yes
SSB	SS-B/La Ab, IgG, S	Yes	Yes
IM_04	Antinuclear Ab,HEp-2,reflex Comment	No	No

Method Name

Only orderable as a reflex. For more information see RAIFA / Antinuclear Antibodies, HEp-2 Substrate, IgG, with Reflex, Serum.

ADNA1: Enzyme-Linked Immunosorbent Assay (ELISA)
RNP, SCL70, SM, SSA, SSB: Multiplex Flow Immunoassay
IM_04: Technical Interpretation

NY State Available

Yes

Specimen

Specimen Type

Serum

Specimen Required

Only orderable as a reflex. For more information see RAIFA / Antinuclear Antibodies, HEp-2 Substrate, IgG, with Reflex, Serum.

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 Serum
Collection Instructions: Centrifuge and aliquot serum into a plastic vial.

Specimen Minimum Volume

Serum: 0.5 mL

Reject Due To

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

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Autoantibodies targeting antigens in the nuclear region in the human epithelial type 2 (HEp-2) cell line substrate using the indirect immunofluorescence assay (IFA) have traditionally been called antinuclear antibodies (ANA). ANA is a commonly performed antibody test in the initial evaluation of patients with systemic autoimmune rheumatic diseases (also referred to as connective tissue disease). Classic ANA-associated rheumatic diseases include systemic lupus erythematosus (SLE), mixed connective tissue disease, Sjogren syndrome (Sjs), and systemic sclerosis (SSc) including CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia), and inflammatory myopathies (IM) such as dermatomyositis.(1-4) Testing for ANA may also be of diagnostic relevance in the differential evaluation of autoimmune liver diseases (ALD).(5-6)

Classical ANA patterns (antibodies targeting the nuclear region) include homogeneous, speckled, centromere, nuclear dots, and nucleolar. These patterns are routinely reported by most clinical laboratories. Patients with SLE, SSc, Sjs, IM (such as anti-synthetase syndrome and necrotizing autoimmune myopathy), or ALD have also been shown to have clinically significant antibodies that react with antigens in other cellular compartments such as the cytoplasm and structures associated mitosis or mitotic patterns with HEp-2 substrate.(1-3) Based on the increasing recognition of these non-nuclear antigenic targets and their documented clinical relevance, the First International Consensus on ANA Patterns established a classification tree for ANA with alpha-numeric anti-cell (AC) code for each pattern with a recommendation for a change in terminology from antinuclear antibody to anti-cellular antibody.(2) These changes are

relevant as, in addition to the nuclear patterns, the classification includes cytoplasmic and mitotic patterns with descriptions for their interpretation, associated antibody targets, and clinical associations when available.(4)

The diagnosis of ANA-associated rheumatic diseases is usually based on a set of criteria of which the presence of anti-cellular antibody or specific associated antibodies may be components. Of all ANA-associated rheumatic diseases, the presence of anti-cellular antibodies is considered a mandatory entry criterion by the 2019 European League Against Rheumatism and the American College of Rheumatology classification criteria for SLE.(7) Since cytoplasmic staining patterns may be reported as "ANA negative" or as a comment with no quantitative or titer result, some patients with clinicopathological symptoms consistent with neuropsychiatric SLE would not qualify for entry based on where testing is performed.(8-10) This limitation may therefore exclude patients who may meet the clinical and other laboratory criteria for disease but are not reported as "ANA positive" due to the use of the current terminology. In an international inception cohort of newly diagnosed SLE patients, 6.2% were anti-cellular antibody-negative with 1.5% testing positive for isolated cytoplasmic or mitotic pattern.(11) In addition, a recent investigation of various HEp-2 IFA kits showed variabilities in the expression of specific patterns with high reproducibility between tests for centromere, multiple nuclear dots, nuclear coarse speckled, nuclear homogeneous and cytoplasmic reticular AMA (antimitochondrial antibody) patterns.(12)

Overall, the anti-cellular antibody is a good screening test for ANA-associated rheumatic diseases with variable sensitivities in the different clinical subsets but lacks diagnostic specificity.(1-4) Therefore, positive results require confirmation with the use of specific ANA-associated antibody tests except for the centromere pattern, which is very characteristic for patients with limited diffuse SSc. Confirmation of a positive anti-cellular antibody test result may be guided by HEp-2 IFA patterns or titer, patient's clinical presentation, or, in some cases, the patient's demographic.(13)

Reference Values

Only orderable as a reflex. For more information see RAIFA / Antinuclear Antibodies, HEp-2 Substrate, IgG, with Reflex, Serum.

DOUBLE-STRANDED DNA (dsDNA) ANTIBODIES, IGG, SERUM
<100 IU/mL (negative)
> or =100 IU/mL (positive)
Negative is considered normal.
Reference values apply to all ages.

RNP ANTIBODIES, IGG, SERUM
<1.0 U (negative)
> or =1.0 U (positive)
Reference values apply to all ages.

SCL 70 ANTIBODIES, IGG, SERUM
<1.0 U (negative)
> or =1.0 U (positive)
Reference values apply to all ages.

SMITH (Sm) ANTIBODIES, IGG, SERUM
<1.0 U (negative)

> or =1.0 U (positive)
Reference values apply to all ages.

SS-A/RO ANTIBODIES, IGG, SERUM
<1.0 U (negative)
> or =1.0 U (positive)
Reference values apply to all ages.

SS-B/LA ANTIBODIES, IGG, SERUM
<1.0 U (negative)
> or =1.0 U (positive)
Reference values apply to all ages.

Interpretation

Presence of anti-cellular antibody (also known as antinuclear antibody) is a feature of systemic autoimmune rheumatic diseases such as systemic lupus erythematosus, mixed connective tissue disease, Sjogren syndrome, and systemic sclerosis and some 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 anti-cellular 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.

Cautions

Some patients without clinical evidence of systemic autoimmune rheumatic disease (SARD) may be positive for anti-cellular antibodies. 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.

Reflex testing is limited to specimens with three patterns namely, homogeneous, speckled or dense fine speckled. Not all patients with these three patterns will test positive in the confirmatory tests. Negative results do not rule out the presence of disease.

For individuals positive for other HEp-2 indirect immunofluorescence assay (IFA) patterns, additional testing may be available based on the pattern present, clinical suspicion, or availability of reliable antibody tests. In patients with certain autoimmune diseases such as myositis and Sjogren syndrome, testing for specific antibodies may be indicated in the absence of antinuclear antibody positivity using HEp-2 IFA.

Clinical Reference

1. 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
2. Chan EK, Damoiseaux J, Carballo OG, et al. Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014-2015. Front Immunol. 2015;6:412
3. Bossuyt X, De Langhe E, Borghi MO, Meroni PL. Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases. Nat Rev Rheumatol. 2020;16(12):715-726
4. International Consensus on ANA Patterns. Nomenclature and Classification Tree. ICAP; 2021 Accessed April 11, 2025. Available at www.anapatterns.org/trees.php
5. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol. 2017;67(1):145-172
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8. Naides SJ, Genzen JR, Abel G, Bashleben C, Ansari MQ. Antinuclear antibodies testing method variability: A survey of participants in the College of American Pathologists' Proficiency Testing Program. J Rheumatol. 2020;47(12):1768-1773
9. Van Hoovels L, Broeders S, Chan EKL, et al. Current laboratory and clinical practices in reporting and interpreting anti-nuclear antibody indirect immunofluorescence (ANA IIF) patterns: results of an international survey. Auto Immun Highlights. 2020;11(1):17
10. Tebo AE, Schmidt RL, Kadkhoda K, et al. The antinuclear antibody HEp-2 indirect immunofluorescence assay: a survey of laboratory performance, pattern recognition and interpretation. Auto Immun Highlights. 2021;12(1):4
11. Choi MY, Clarke AE, St Pierre Y, et al. Antinuclear antibody-negative systemic lupus erythematosus in an international inception cohort. Arthritis Care Res (Hoboken). 2019;71(7):893-902
12. 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
13. Silva MJ, Dellavance A, Baldo DC, et al. Interkit Reproducibility of the Indirect Immunofluorescence Assay on HEp-2 Cells Depends on the Immunofluorescence Reactivity Intensity and Pattern. Front Immunol. 2022;12:798322

Performance

Method Description

Double-Stranded DNA (dsDNA) IgG Antibodies

The test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with double-stranded DNA (dsDNA). In the first reaction step, diluted patient samples, calibrators and controls are incubated in the wells. Anti-dsDNA antibodies will bind to the antigens coated in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, rabbit anti-human IgG-HRP enzyme conjugate is added to each well. The enzyme conjugate will bind to any wells that have human IgG binding to the dsDNA antigen. The wells are washed to remove any unbound HRP enzyme conjugate. 3,3,5,5, tetramethylbenzidine (TMB) enzyme substrate is added. If the HRP enzyme is present in the well (positive reaction), the HRP enzyme will react with the TMB substrate and produce a blue color. After an additional incubation time to allow the color development, a stop solution is added which turns the blue color yellow and inhibits further color development to allow for stable spectrophotometric reading.

The test strips are placed in a microplate reader and the optical density of the color is measured. The amount of antigen specific bound antibody is proportional to the color intensity.(Package insert: Anti-dsDNA-NcX ELISA (IgG). EUROIMMUN; 7/8/2020)

RNP IgG Antibodies
Recombinant ribonucleoprotein particle (RNP)-68 and RNP-A antigens are bound to polystyrene microspheres, which are impregnated with fluorescent dyes to create a unique fluorescent signature. RNP antibodies, if present in diluted serum, bind to the RNP antigens on the microspheres. The microspheres are washed to remove extraneous serum proteins. Phycoerythrin (PE)-conjugated antihuman IgG antibody is then added to detect IgG anti-RNP 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 the median fluorescence response for RNP microspheres to a 4-point calibration curve.(Package insert: Bioplex 2200 ANA Screen. Bio-Rad Laboratories; 02/2019)

Scl 70 IgG 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)

Smith (Sm) IgG Antibodies
Affinity-purified Smith (Sm) antigens are bound to polystyrene microspheres, which are impregnated with fluorescent dyes to create a unique fluorescent signature. Sm antibodies, if present in diluted serum, bind to the Sm antigens on the microspheres. The microspheres are washed to remove extraneous serum proteins. Phycoerythrin (PE)-conjugated antihuman IgG antibody is then added to detect IgG anti-Sm 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 the median fluorescence response for Sm microspheres to a 4-point calibration curve.(Package insert: Bioplex 2200 ANA Screen. Bio-Rad Laboratories; 02/2019)

SS-A/Ro IgG Antibodies
Recombinant SS-A/Ro 52 kD and affinity-purified SS-A/Ro 60 kD antigens are coupled covalently to polystyrene microspheres, which are impregnated with fluorescent dyes to create a unique fluorescent signature. SS-A/Ro antibodies, if present in diluted serum, bind to the SS-A/Ro antigens on the microspheres. The microspheres are washed to remove extraneous serum proteins. Phycoerythrin (PE)-conjugated antihuman IgG antibody is then added to detect IgG anti-SS-A/Ro 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 the median fluorescence response for SS-A/Ro microspheres to a 4-point calibration curve.(Package insert: Bioplex 2200 ANA Screen. Bio-Rad Laboratories, 02/2019)

SS-B/La IgG Antibodies
Affinity-purified SS-B antigen is coupled covalently to polystyrene microspheres, which are impregnated with fluorescent dyes to create a unique fluorescent signature. SS-B/La antibodies, if present in diluted serum, bind to the SS-B antigen on the microspheres. The microspheres are washed to remove extraneous serum proteins. Phycoerythrin (PE)-conjugated antihuman IgG antibody is then added to detect IgG anti-SS-B/La 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 the median fluorescence response for SS-B/La microspheres to a 4-point calibration curve.(Package insert: Bioplex 2200 ANA Screen. Bio-Rad Laboratories; 02/2019)

Technical Interpretation
An interpretation based on the test results is generated by the laboratory information system.

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
3 to 4 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
Not Applicable

CPT Code Information

86225
86235 x5

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
RFLXP	ANA Ab HEp-2 Reflex Panel	97564-9

Result ID	Test Result Name	Result LOINC® Value
RNP	RNP Ab, IgG, S	29958-6
SCL70	Scl 70 Ab, IgG, S	47322-3
SM	Sm Ab, IgG, S	18323-6
SSA	SS-A/Ro Ab, IgG, S	33610-7
SSB	SS-B/La Ab, IgG, S	33613-1
ADNA1	dsDNA Ab, IgG, S	33799-8
IM_04	Antinuclear Ab,HEp-2,reflex Comment	77202-0