Test Definition: RAIFA

Antinuclear Antibodies, HEp-2 Substrate, IgG, with Reflex, Serum

Overview

Useful For

Evaluation of patients suspected of having systemic autoimmune rheumatic disease (antinuclear antibody-associated rheumatic diseases or connective tissue diseases), especially systemic lupus erythematosus, mixed connective tissue disease and Sjogren syndrome

Reflex Tests

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

Testing Algorithm

If human epithelial type 2 (HEp-2) indirect immunofluorescence assay (IFA) result is positive with a titer of 1:80 or greater, then a titer and pattern will be reported.

If positive for a homogeneous, speckled, or dense fine speckled pattern, then reflex confirmatory testing for double-stranded DNA antibodies (Ab), ribonucleoprotein Ab, Scl-70 Ab, Sm Ab, SS-A/Ro Ab, or SS-B/La Ab will be performed at an additional charge. If confirmatory tests are negative, consideration for other ANA-associated antibodies may be required for evaluation. Other confirmatory autoantibodies may be performed based on reported patterns or clinical suspicion.

Highlights

This test ensures that testing proceeds in an algorithmic fashion based on the presence of homogeneous, speckled or dense fine speckled patterns.

Method Name

Indirect Immunofluorescence Assay (IFA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

This algorithm is recommended for the initial evaluation of patients at risk for systemic lupus erythematosus, mixed connective tissue disease, and Sjogren syndrome.

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 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 reproducibilities 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

<1:80 (negative)

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

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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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7. Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Arthritis Rheumatol. 2019;71(9):1400-1412
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
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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
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 specimens are detected after incubation of serum with the commercial slides by the addition of a fluorescein isothiocyante (FITC)-labeled antihuman-IgG reagent. All patient specimens are initially screened at 1:80.(Package insert: NOVA Lite DAPI ANA. Inova Diagnostics; 06/2018)

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 Definition: RAIFA

Antinuclear Antibodies, HEp-2 Substrate, IgG, with Reflex, Serum

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.

CPT Code Information

86039

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
RAIFA	Antinuclear Ab, HEp-2,with reflex,S	59069-5

Result ID	Test Result Name	Result LOINC® Value
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
IM_04	Antinuclear Ab,HEp-2,reflex Comment	77202-0