

Overview

Useful For

Evaluating patients with suspected autoimmune liver disease, specifically autoimmune hepatitis or primary biliary cholangitis

Evaluating patients with liver disease of unknown etiology

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
AMA	Mitochondrial Ab, M2, S	Yes	Yes
NAIFA	Antinuclear Ab, HEp-2 Substrate, S	Yes	Yes
SMAS	Smooth Muscle Ab Screen, S	Yes	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
SMAT	Smooth Muscle Ab Titer, S	No	No

Testing Algorithm

If smooth muscle antibody (SMA) screen is positive, then the SMA titer will be performed at an additional charge.

For more information see [First-Line Screening for Autoimmune Liver Disease Algorithm](#).

Special Instructions

- [First-Line Screening for Autoimmune Liver Disease Algorithm](#)

Method Name

AMA: Enzyme Immunoassay (EIA)

NAIFA, SMAS, SMAT: Indirect Immunofluorescence

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

This test should be used for evaluating patients at-risk for antinuclear antibody-associated systemic autoimmune rheumatic disease.

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)

Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Submission Container/Tube: Plastic vial

Specimen Volume: 1.5 mL Serum

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

Forms

If not ordering electronically, complete, print, and send a [Gastroenterology and Hepatology Test Request](#) (T728) with the specimen.

Specimen Minimum Volume

Serum: 1.1 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**

Autoimmune liver disease (ALD) collectively refers to diseases that result from immune-mediated damage to hepatocytes, cholangiocytes, or both.(1-3) The disease entity includes autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and their overlaps.(1) Testing for liver-related autoantibodies is usually included in the workup of patients with hepatitis or cholestasis of unknown origin.(1-5) However, it is important to note that these autoantibodies are not specific and must be interpreted in context of risk factors. In addition, efforts

to standardize how these tests should be performed and reported are not well established.

Autoimmune hepatitis is a multifaceted liver disease characterized by the presence of diverse autoantibodies, increased concentrations of specific liver enzymes, hypergammaglobulinemia and abnormalities in liver histology.(1,2 4-6) AIH can be stratified into two main subtypes based on the presence of specific autoantibodies and patient's age, these include AIH-type 1 (AIH-1) or AIH-type 2 (AIH-2).(2,4-6) Patients with AIH-1 are positive for antinuclear autoantibodies (ANA), smooth muscles antibodies associated with anti-filamentous-actin IgG antibody, or both. While patients with AIH-2 have detectable anti-liver kidney microsomal type-1, or rarely anti-liver kidney microsomal type-3, or anti-liver cytosol type-1 antibodies. Antibodies against soluble liver antigens/liver pancreas autoantigen can also be detected in AIH-1 patients.(6) Compared to AIH-2, which generally occurs in children with a more moderated or severe disease course, AIH-1 occurs in all age groups and has a relatively mild course that is responsive to timely treatment with steroids and azathioprine.(4)

Primary biliary cholangitis is a rare cholangiopathic ALD characterized by predominantly anti-mitochondrial autoantibodies (AMA), female preponderance and progression to liver damage, if left untreated.(1,3) In some cases, patients with these diseases may present asymptotically, with increases in various liver enzymes being identified incidentally during an unrelated clinical evaluation. On the other end of the spectrum are patients who present with clinical evidence of liver disease, including fatigue, hepatomegaly, ascites, esophageal varices, and jaundice. The serological hallmark of PBC is the presence of AMA characterized by cytoplasmic reticular/AMA staining pattern on HEp-2 substrate by indirect immunofluorescence assay (IFA). In addition, autoantibodies associated with the HEp-2 IFA nuclear patterns have been reported in a subset of patients with PBC who are seronegative for AMA or may be positive for AMA but have uncertain clinical or phenotypic attributes.(1,3, 7-9) The HEp-2 IFA nuclear patterns in PBC include multiple nuclear dots (MND or AC-6) and punctate nuclear envelope (AC-12), which are associated with anti-Sp100 and anti-gp210 antibodies, respectively.(1,3) The diagnosis of PBC can be established if 2 out of the 3 following criteria are met: 1) sustained elevated levels of alkaline phosphatase (ALP); 2) evidence AMA or ANA (anti-Sp100 and anti-gp210 antibodies); and 3) diagnostic liver histology.(3) Based on these criteria, a biopsy can be avoided in case of high ALP levels and detection of these PBC-specific autoantibodies.(1,3,5) In a recent study, the prevalence of AMA and levels of ALP were both reported to vary by race/ethnicity.(10) Highlighting the need to incorporate the ANA PBC-specific autoantibodies and HEp-2 IFA in disease evaluation.

In association with chronic cholestasis after exclusion of known causes of liver disease, AMA are strongly suggestive of a diagnosis of PBC.(6) AMA have a variable prevalence in other autoimmune diseases that can also be found in some apparently healthy individuals.(7,8) AMA are found in more than 90% of patients with PBC, with a specificity of greater than 95%. AMA are included in the clinical practice guidelines for PBC, which were developed through an international collaborative effort.(9)

For more information see [First-Line Screening for Autoimmune Liver Disease Algorithm](#).

Reference Values

MITOCHONDRIAL ANTIBODIES (M2)

Negative: <0.1 Units

Borderline: 0.1-0.3 Units

Weakly positive: 0.4-0.9 Units

Positive: > or =1.0 Units

Reference values apply to all ages.

ANTINUCLEAR ANTIBODIES

Negative: <1:80

SMOOTH MUSCLE ANTIBODIES

Negative

If positive, results are titered.

Reference values apply to all ages.

Interpretation

The presence of smooth muscle antibodies (SMA) or antinuclear antibodies (ANA) is consistent with a diagnosis of chronic autoimmune hepatitis, in patients with clinical or laboratory evidence of hepatocellular damage.

A positive result for antimitochondrial antibodies (AMA) of M2 specificity in the setting of chronic cholestasis after exclusion of other causes of liver disease is highly suggestive of primary biliary cholangitis.

Negative results for SMA, ANA, or AMA does not exclude a diagnosis of an autoimmune liver disease.

This test is **not useful for** indicating the stage or prognosis of the disease or for monitoring the course of disease.

Cautions

Smooth muscle antibodies (SMA) may be found in patients with active hepatitis caused by alcohol or drug exposure.

Positive results for antimitochondrial antibodies (AMA) are found (infrequently) in patients with CREST (calcinosis, Raynaud phenomenon, esophageal hypomotility, sclerodactyly, and telangiectasia) syndrome, relatives of patients with primary biliary cholangitis, and other autoimmune diseases.

Antinuclear antibodies (ANA) occur in patients with a variety of systemic autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, Sjogren syndrome, and systemic sclerosis.

ANA may also be detectable following viral illnesses, in chronic infections, or in patients treated with many different medications.

The presence of SMA, ANA, and AMA should not be exclusively relied upon to diagnose an autoimmune liver disease. Correlation with clinical presentation and other laboratory parameters of liver disease is required.

Clinical Reference

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2. Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology.* 2008;48(1):169-176
3. Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. Primary biliary cholangitis: 2018 practice guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2019;69(1):394-419
4. Mack CL, Adams D, Assis DN, et al. Diagnosis and management of autoimmune hepatitis in adults and children: 2019 Practice Guidance and Guidelines from the American Association for the Study of Liver Diseases. *Hepatology.* 2020;72(2):671-722

5. Mieli-Vergani G, Vergani D, Czaja AJ, et al. Autoimmune hepatitis. *Nat Rev Dis Primers*. 2018;4:18017
6. Dalekos GN, Gatselis NK. Autoimmune serology testing in clinical practice: An updated roadmap for the diagnosis of autoimmune hepatitis. *Eur J Intern Med*. 2023;108:9-17
7. Colapietro F, Lleo A, Generali E. Antimitochondrial antibodies: From bench to bedside. *Clin Rev Allergy Immunol*. 2022;63(2):166-177
8. Zhang Q, Liu Z, Wu S, et al. Meta-analysis of antinuclear antibodies in the diagnosis of antimitochondrial antibody-negative primary biliary cholangitis. *Gastroenterol Res Pract*. 2019;2019:8959103
9. Dahlqvist G, Gaouar F, Carrat F, et al. Large-scale characterization study of patients with antimitochondrial antibodies but nonestablished primary biliary cholangitis. *Hepatology*. 2017;65(1):152-163
10. Caines A, Lu M, Wu T, et al. Pre-diagnosis alkaline phosphatase and antimitochondrial antibody positivity vary by race/ethnicity among patients with primary biliary cholangitis. *J Gastroenterol Hepatol*. 2025;40(9):2209-2218. doi:10.1111/jgh.17035

Performance

Method Description

Mitochondrial Antibodies:

This method is an enzyme immunosorbent assay that detects both IgG and IgM antibodies to M2 antigens. A dilution of patient serum is added to the wells coated with M2 antigen and incubated. After incubation and washing, all unbound human antibodies are washed away, and enzyme-conjugated IgG and IgM anti-human is added. The enzyme conjugate binds to the antibody complex. Excess enzyme-conjugate is washed away, and substrate is added. After incubation, the enzyme substrate reaction is stopped. The complete assay is measured on a spectrophotometer plate reader. The intensity of the color generated is proportional to the amount of IgG and IgM specific antibody in the sample. (Package insert: Kallestad Anti-Mitochondrial Kit. Bio-Rad Laboratories; 10/2014)

Antinuclear Antibodies:

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 isothiocyanate-labeled antihuman-IgG reagent. All patient specimens are initially screened at 1:80. (Package insert: NOVA Lite DAPI ANA. Inova Diagnostics; 06/2018)

Smooth Muscle Antibody:

The patient's serum in 1:20 and 1:40 dilutions is added to fresh tissue from mouse stomach/kidney and incubated; fluorescein-conjugated antiglobulin is then added. The slides are read with a fluorescence microscope. (Package insert: Kallestad Mouse Stomach/Kidney. Bio-Rad Laboratories, Inc; 06/2022)

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

See Individual Test IDs

CPT Code Information

86381
86039
86015
86015-Titer (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ALDG2	Autoimmune Liver Disease Panel, S	94700-2

Result ID	Test Result Name	Result LOINC® Value
AMA	Mitochondrial Ab, M2, S	51715-1
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
609515	Smooth Muscle Ab Screen, S	26971-2