MAYO CLINIC LABORATORIES

Test Definition: AMA

Mitochondrial Antibodies (M2), Serum

Overview

Useful For

Establishing the diagnosis of primary biliary cholangitis

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

Method Name

Enzyme Immunoassay (EIA)

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

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-General Request (T239)
-Gastroenterology and Hepatology Test Request (T728)

Specimen Minimum Volume

Serum: 0.4 mL

Reject Due To

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

specimens	

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Primary biliary cholangitis (PBC) is a cholestatic, chronic autoimmune liver disease with a wide individual variation in disease progression, characterized by progressive destruction of the small intrahepatic bile ducts leading to the buildup of bile and liver damage. Untreated patients with PBC have a high risk of liver cirrhosis and related complications, liver failure and death.(1) Key clinical features include fatigue, itchy skin, sicca syndrome, jaundice, hyperpigmentation, and xanthomas in later stages. The serological hallmark of PBC is the presence of antimitochondrial antibody (AMA) characterized by cytoplasmic reticular/AMA (anti-cell 21 [AC-21] based on the International Consensus on Antinuclear Antibody Patterns [ICAP] nomenclature) staining pattern on HEp-2 substrate by indirect immunofluorescence assay (IFA).(2) 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.(1,3,4) 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.(2) The diagnosis of PBC can be established if 2 out of the 3 following criteria are met: sustained elevated levels of alkaline phosphatase (ALP), evidence AMA or specific antinuclear antibody (ANA) (anti-Sp100 and anti-gp210 antibodies), and diagnostic liver histology.(1) Based on these criteria, a biopsy can be avoided in case of high ALP levels and detection of these PBC-specific autoantibodies.(1) Therefore, reliable and accurate serologic determination of PBC-specific autoantibodies play a critical role in disease evaluation.

Positivity of AMA ranges from 90% to 95% in patients with PBC, while the PBC-specific ANA (anti-Sp100 and anti-gp210 antibodies) may occur in approximately 30% of all patients with PBC, and up to 50% of patients who are AMA-negative.(5) The M2-type AMA (AMA-M2) is the dominant target of the 9 subunits of the mitochondrial antigenic complex.(1) AMA-M2 target components of the 2-oxo-acid dehydrogenase complex: pyruvate dehydrogenase complex (PDC), 2-oxoglutarate dehydrogenase complex (OGDC), and branched-chain 2-oxoacid dehydrogenase complex (BCOADC). Specifically, autoantibodies mainly recognize the E2 subunits of these complexes: PDC-E2 (80%-90% of cases), BCOADC-E2 (50%-80% of cases) and OGDC-E2 (20%-60% of cases), and to a lesser extent, the E1 and E3 subunits.(1) Although the sensitivities of the anti-Sp100 and anti-gp210 antibodies are low, their specificities for PBC are excellent; therefore, both tests have been reported to be useful in confirming a diagnosis of PBC or predicting development of disease in nonestablished PBC cases with positive AMA.(3,4) In addition to the diagnostic relevance of anti-gp210 IgG antibody, a few studies have suggested a role for their use in the risk stratification and prognosis in PBC; however, the significance of these remain contentious. In one study, the presence of anti-gp210 antibodies was reported to pose a significant risk for hepatic failure type progression, more severe interface hepatitis, and lobular inflammation, compared to those with centromere antibodies who had relatively higher ductular reaction.(6) In addition to MND and punctate nuclear envelope, the anticentromere (AC-3) and the speckled (AC-4 and AC-5) patterns can be found in variable prevalence in patients with PBC with overlapping connective tissue diseases (systemic sclerosis [SSc] and Sjogren

syndrome).(7) In the context of other liver diseases, the cytoplasmic fibrillar linear (AC-15) HEp-2 IFA pattern associated with autoimmune hepatitis (AIH) may also be seen when PBC overlaps in patients with AIH or other liver diseases, such as hepatitis B virus infection, hepatitis C virus infection, and hepatic carcinoma.(8) In general, a mixed pattern composed of at least two HEp-2 IFA patterns is mostly found in patients with PBC rather than in other liver diseases.(8)

Traditionally, the IFA method was used for the detection of AMA; however, antigen-specific solid-phase immunoassays (SPAs), such as enzyme-linked immunosorbent assay (ELISA), line blot immunoassay (LIA), and dot immunoassay (DIA) have been developed and are increasingly being used in the laboratory evaluation of PBC.(3,4,6-9) The AMA SPAs use a variety of M2 antigens, including fusion protein combining the three E2 subunits, a mixture of recombinant E2 subunits, or the three E2 recombinant subunits isolated, among others.(3,4,5,6-9) The anti-Sp100 and anti-gp210 antibodies can also be determined using analyte-specific ELISA, LIA and DIA. In addition to the SPAs for detecting antibodies to AMA, Sp100, and gp210, the use HEp-2 substrate by IFA provides a simple and strategic approach for confirming the presence of AMA cytoplasmic staining if positive by enzyme immunoassay (EIA) with the possibility of identifying patients who may be AMA-negative but positive to nuclear antibodies. In PBC patients, the nuclear envelope pattern is associated with anti-gp210 antibody while the multiple nuclear dot pattern is specific for anti-Sp100 antibodies. However, expression of the MND and nuclear envelope patterns may not be easily identified in the presence of other antibodies. Testing for these antibodies is indicated in patients who are AMA positive by EIA as well as patients at risk for PBC but are AMA negative. In addition to providing additional support for PBC diagnosis in AMA-positive and AMA-negative patients, the use of HEp-2 substrate offers the possibility to identify patients at-risk for PBC who may present with coexisting systemic autoimmune rheumatic diseases (systemic lupus erythematosus, systemic sclerosis, and Sjogren syndrome) or autoimmune liver disease (autoimmune hepatitis) through additional pattern recognition. The use of SPAs for ANA testing does not provide these additional diagnostic insights. While the HEp-2 IFA is useful for detecting patterns associated with PBC, the predictive value of AMA-positivity in asymptomatic individuals including those with normal liver enzymes remains a diagnostic dilemma.(5) In a recent study, Yahia and colleagues reported that AMA-positive individuals with normal liver enzymes typically experience a benign clinical course. However, high AMA titers identify a subgroup at increased risk for progression to PBC and advanced liver disease.(10)

Reference Values

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.

Interpretation

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

Cautions

Positive results are found (infrequently) in patients with systemic sclerosis, relatives of patients with primary biliary cholangitis (PBC), and other autoimmune diseases. If negative, and clinical suspicion for PBC is strong, patients should be tested for antinuclear antibodies using HEp-2 substrate by indirect immunofluorescence assay, anti-SP100 IgG, and anti-gp210 IgG.

Supportive Data

Testing performed in the Antibody Immunology Laboratory of the antimitochondrial antibody-M2 by enzyme

immunoassay (EIA) revealed a false-positive rate of less than 2% in 196 normal samples, and overall concordance compared with indirect immunofluorescence (IFA) of 90% on sera from the Mayo primary biliary cholangitis (PBC) Serum Bank. Ten discordant results were obtained (negative by EIA and positive by IFA). Seven of the 10 patients had no histologic evidence of PBC on liver biopsy.

Clinical Reference

1. Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. Primary biliary cholangitis: 2018 practice guidance update from the American Association for the Study of Liver Diseases. Hepatology. 2019;69(1):394-419
2. International Consensus on ANA Patterns. AC-20 Cytoplasmic fine speckled. ICAP; 2015. Accessed November 16, 2023. Available at www.anapatterns.org/view_pattern.php?pattern=20
3. 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
4. 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
5.Trivella J, John BV, Levy C. Primary biliary cholangitis: Epidemiology, prognosis, and treatment. Hepatol Commun. 2023;7(6):e0179
6. Nakamura M, Kondo H, Mori T, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. Hepatology. 2007;45(1):118-127
7. Favoino E, Grapsi E, Barbuti G, et al. Systemic sclerosis and primary biliary cholangitis share an antibody population with identical specificity. Clin Exp Immunol. 2023;212(1):32-38
8. Wei Q, Jiang Y, Xie J, et al. Investigation and analysis of HEp 2 indirect immunofluorescence titers and patterns in various liver diseases [published correction appears in Clin Rheumatol. 2021;40(4):1667]. Clin Rheumatol. 2020;39(8):2425-2432
9. Munoz-Sanchez G, Perez-Isidro A, Ortiz de Landazuri I, et al. Working algorithms and detection methods of autoantibodies in autoimmune liver disease: A nationwide study. Diagnostics (Basel). 2022;12(3):697
10. Yahia A, Baker FA, Tatour M, Hazzan R. Reevaluating the clinical course of AMA-positive patients with normal liver enzymes: A large retrospective cohort study. Ann Hepatol. 2025;31(1):102147. doi:10.1016/j.aohep.2025.102147

Performance

Method Description

A recombinant pyruvate dehydrogenase complex-E2 (M2) antigen for detection of antibodies against M2 is attached to the surface of a microplate. Diluted patient serum, standards, or controls are added to the wells, and the M2 specific IgG and IgM antibodies, if present, bind to the antigen. All unbound human antibodies are washed away, and a conjugate of enzyme-labeled polyclonal antibody to human IgG and IgM is added. The enzyme conjugate binds to the antibody complex. Excess enzyme-conjugate is washed away, and substrate is added. After a specified time, the enzyme reaction is stopped. The intensity of the color generated is proportional to the amount of anti-M2 IgG and/or IgM antibody in the sample. The results are read by a spectrophotometer producing a direct measurement of the anti-M2 IgG and IgM antibodies in the serum. Testing is performed on the Agility instrument by Dynex.(Package insert: Kallestad Anti-Mitochondrial Kit. Bio-Rad Laboratories, Inc; 10/2014)

PDF Report

No

Day(s) Performed
Monday through Saturday

Report Available
2 to 3 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.

CPT Code Information
86381

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
AMA	Mitochondrial Ab, M2, S	51715-1

Result ID	Test Result Name	Result LOINC® Value
AMA	Mitochondrial Ab, M2, S	51715-1