Test Definition: NSFIB

Nonalcoholic Steatohepatitis (NASH)-FibroTest, Serum and Plasma

Overview

Useful For

Diagnosis and the follow-up of liver fibrosis, steatosis, and inflammation

Estimating hepatic fibrosis

Assessing inflammation for metabolic diseases

Assessing severity of nonalcoholic steatohepatitis (NASH) in patients with nonalcoholic fatty liver disease with steatosis (NAFLD)

Assessing steatosis or fatty liver

Reassuring patients with steatosis only, without fibrosis

Managing patients with severe injuries such as advanced fibrosis and NASH

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
INTNS	NASH-FibroTest, Interpretation	No	Yes
APOAF	Apolipoprotein A1, S	No	Yes
A2MF	Alpha-2-Macroglobulin, S	Yes, (Order A2M)	Yes
HAPTF	Haptoglobin, S	Yes, (Order HAPT)	Yes
ALTF	Alanine Aminotransferase (ALT), S	Yes, (Order ALT)	Yes
GGTF	Gamma Glutamyltransferase (GGT), S	Yes, (Order GGT)	Yes
TBILF	Bilirubin, Total, S	Yes, (Order BILIT)	Yes
ASTF	Aspartate Aminotransferase (AST), S	Yes, (Order AST)	Yes
CHOLF	Cholesterol, Total, S	Yes, (Order CHOL)	Yes
TRIGF	Triglycerides, S	Yes, (Order TRIG)	Yes
GLURF	Glucose, Fasting, P	No	Yes

Testing Algorithm

This test is a patented test algorithm developed by BioPredictive. It combines 10 standard biomarkers: gamma-glutamyltransferase, total bilirubin, alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, alanine

aminotransferase, aspartate aminotransferase, total cholesterol, triglycerides, and fasting glucose. These markers are weighted depending on the patient's age and sex.

Testing is compliant with BioPredictive's technical recommendations and approvals.

Method Name

INTNS: Algorithm and Interpretation Provided through BioPredictive
APOAF: Turbidimetric Immunoassay
A2MF, HAPTF: Nephelometry
ALTF, ASTF, GLURF: Photometric
CHOLF, GGTF, TBILIF, TRIGF: Enzymatic Colorimetric

NY State Available

Yes

Specimen

Specimen Type

Plasma NaFl-KOx
Serum

Ordering Guidance

This test is not offered for patients younger than 14 years of age. Testing will be canceled if a specimen is received from a patient in this age group.

Necessary Information

Age and sex are required.

Specimen Required

Both serum and plasma are required for this test.

Patient Preparation:
Fasting:12 hours, required

Specimen Type: Serum
Supplies: Amber Frosted Tube, 5 mL (T915)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Amber vial
Specimen Volume: 4 mL
Collection Instructions:
1. Centrifuge and aliquot serum into an amber vial within 2 hours of collection.

2. Centrifuged serum must be light protected within 4 hours of collection. It is acceptable to draw the blood and then protect it from light after centrifugation as long as it's within 4 hours of collection.
3. Label specimen as serum.

Specimen Type: Plasma
Collection Container/Tube: Gray top (potassium oxalate/sodium fluoride)
Submission Container/Tube: Plastic vial
Specimen Volume: 1 mL
Collection Instructions:
1. Centrifuge and aliquot plasma into plastic vial.
2. Label specimen as plasma.

Forms
If not ordering electronically, complete, print, and send Gastroenterology and Hepatology Test Request (T728) with the specimen

Specimen Minimum Volume
Serum: 2 mL
Plasma: 0.25 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject
Patients <14 years of age	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Plasma NaFl-KOx	Refrigerated (preferred)	7 days	
	Ambient	7 days	
	Frozen	14 days	
Serum	Refrigerated (preferred)	7 days	LIGHT PROTECTED
	Ambient	24 hours	LIGHT PROTECTED
	Frozen	14 days	LIGHT PROTECTED

Clinical & Interpretive

Clinical Information
This test estimates the 3 elementary features of metabolic liver disease: steatosis, activity, and fibrosis. The estimation is

made by measuring 10 standard serum biomarkers (gamma-glutamyl transferase, total bilirubin, alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides, and fasting plasma glucose). Results from these tests are combined with the patient's age and sex to estimate hepatic fibrosis (FibroTest), steatosis (SteatoTest 2), and activity (NashTest 2) scores.

Reference Values

NASHTEST 2 INTERPRETATION

NashTest 2 score	Grade	Interpretation
0.00-0.25*	N0	No NASH
0.25-0.50*	N1	Mild NASH
0.50-0.75*	N2	Moderate NASH
0.75-1.00*	N3	Severe NASH

*Boundary values can apply to 2 stages based on rounding. For example, a NashTest 2 score of 0.245 will round up to 0.25 and be staged N0. A NashTest 2 score of 0.254 will round down to 0.25 and be staged N2.

STEATOTEST 2 INTERPRETATION

SteatoTest 2 score	Grade	Interpretation
0.00-0.40*	S0	No steatosis (<5%)
0.40-0.55*	S1	Mild steatosis (5-33%)
0.55-1.00*	S2	Moderate/severe steatosis (34-100%)

*Boundary values can apply to 2 stages based on rounding. For example, a SteatoTest 2 score of 0.395 will round up to 0.40 and be staged S0. A SteatoTest 2 score of 0.404 will round down to 0.40 and be staged S1.

FIBROTEST INTERPRETATION

FibroTest score	Stage	Interpretation
0.00-0.21*	F0	No fibrosis
0.21-0.27*	F0-F1	No fibrosis
0.27-0.31*	F1	Minimal fibrosis
0.31-0.48*	F1-F2	Minimal fibrosis
0.48-0.58*	F2	Moderate fibrosis
0.58-0.72*	F3	Advanced fibrosis
0.72-0.74*	F3-F4	Advanced fibrosis
0.74-1.00	F4	Severe fibrosis (Cirrhosis)

*Boundary values can apply to 2 stages based on rounding. For example, a FibroTest score of 0.305 will round up to 0.31 and be staged F1. A FibroTest score of 0.314 will round down to 0.31 and be staged F1-F2.

ALPHA-2-MACROGLOBULIN
< or =18 years: 178-495 mg/dL
>18 years: 100-280 mg/dL

ALANINE AMINOTRANSFERASE (ALT)
Males
<12 months: Not established

> or =1 year: 7-55 U/L

Females
<12 months: Not established
> or =1 year: 7-45 U/L

APOLIPOPROTEIN A1
Males
<24 months: Not established
2-17 years
Low: <115 mg/dL
Borderline low: 115-120 mg/dL
Acceptable: >120 mg/dL
> or =18 years: > or =120 mg/dL

Females
<24 months: Not established
2-17 years
Low: <115 mg/dL
Borderline low: 115-120 mg/dL
Acceptable: >120 mg/dL
> or =18 years: > or =140 mg/dL

GAMMA-GLUTAMYLTRANSFERASE (GGT)
Males
0-11 months: <178 U/L
12 months-6 years: <21 U/L
7-12 years: <24 U/L
13-17 years: <43 U/L
> or =18 years: 8-61 U/L

Females
0-11 months: <178 U/L
12 months-6 years: <21 U/L
7-12 years: <24 U/L
13-17 years: <26 U/L
> or =18 years: 5-36 U/L

HAPTOGLOBIN:
30-200 mg/dL

BILIRUBIN, TOTAL
0-6 days: Refer to www.bilitool.org for information on age-specific (postnatal hour of life) serum bilirubin values.
7-14 days: 0.0-14.9 mg/dL

15 days to 17 years: 0.0-1.0 mg/dL
> or =18 years: 0.0-1.2 mg/dL

ASPARTATE AMINOTRANSFERASE (AST)
Males
0-11 months: Not established
1-13 years: 8-60 U/L
> or =14 years: 8-48 U/L

Females
0-11 months: Not established
1-13 years: 8-50 U/L
> or =14 years: 8-43 U/L

CHOLESTEROL, TOTAL
The National Lipid Association and the National Cholesterol Education Program (NCEP) have set the following guidelines for lipids in adults 18 years old and older:

TOTAL CHOLESTEROL
Desirable: <200 mg/dL
Borderline High: 200-239 mg/dL
High: > or =240 mg/dL

TRIGLYCERIDES
Normal: <150 mg/dL
Borderline High: 150-199 mg/dL
High: 200-499 mg/dL
Very High: > or =500 mg/dL

The Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents has set the following guidelines for lipids in children 2 to 17 years of age:

TOTAL CHOLESTEROL
Acceptable: <170 mg/dL
Borderline High: 170-199 mg/dL
High: > or =200 mg/dL

TRIGLYCERIDES
2-9 years
Acceptable: <75 mg/dL
Borderline High: 75-99 mg/dL
High: > or =100 mg/dL

10-17 years

Acceptable: <90 mg/dL
Borderline High: 90-129 mg/dL
High: > or =130 mg/dL

GLUCOSE FASTING
0-11 months: Not established
> or =1 year: 70-100 mg/dL

Interpretation

This test provides numeric scores that assess hepatic fibrosis (FibroTest), hepatic inflammation (NashTest 2), and steatosis (SteatoTest 2). Interpretation of the scores is provided in the report. Individual results from the 10 component tests are also provided with institution-specific reference intervals.

FibroTest is reported relative to a scale ranging from F0-F4 (F0=no fibrosis, F1=minimal fibrosis, F2=moderate fibrosis, F3=advanced fibrosis, F4=severe fibrosis [cirrhosis]). Fibrosis scores may overlap (eg, F0/F1, F1/F2).

NashTest 2 is reported relative to a scale ranging from N0-N3 (N0=no nonalcoholic steatohepatitis [NASH], N1=mild NASH, N2=moderate NASH, N3=severe NASH).

Steatosis is reported relative to a scale ranging from S0-S2S3 (S0=no steatosis [<5%], S1=mild steatosis [5-33%], S2/S3=moderate/severe steatosis [34-100%]). A stage of S1 or S2S3 is considered clinically significant.

Cautions

The tests will be deferred for patients with acute hemolysis, acute hepatitis, acute inflammation, and extra hepatic cholestasis.

The advice of a specialist should be sought for interpretation in chronic hemolysis or Gilbert syndrome.

The test interpretation has not been validated for liver transplant patients.

Isolated extreme values of one of the components should lead to caution in interpreting the results.

In case of discordance between a biopsy result and a test, it is recommended to seek the advice of a specialist. The causes of these discordances could be due to a flaw of the test or to a flaw in the biopsy: ie, a liver biopsy has a 33% variability rate for one fibrosis stage.

NashTest 2 is set to N0 is the absence of steatosis based on the definition of nonalcoholic steatohepatitis (NASH). SteatoTest 2 scores of less than 0.40 (S0) will default NashTest 2 to N0.

Clinical Reference

1. BioPredictive. Technical Recommendations for FibroTest, FibroMax, and NASH-FibroTest assays. Version 3.5.3. Bio Predictive; 2022
2. Poynard T, Peta V, Munteanu M, et al. The diagnostic performance of a simplified blood test (SteatoTest-2) for the prediction of liver steatosis. Eur J Gastroenterol Hepatol. 2019;31(3)393-402. doi:10.1097/MEG.0000000000001304
3. Munteanu M, Pais R, Peta V, et al. Long-term prognostic value of the FibroTest in patients with non-alcoholic fatty

liver disease, compared to chronic hepatitis C, B, and alcoholic liver disease. Aliment Pharmacol Ther. 2018:48(10):1117-1127. doi:10.1111/apt.14990
4. Poynard T, Munteanu M, Charlotte F, et al. Diagnostic performance of a new noninvasive test for nonalcoholic steatohepatitis using a simplified histological reference. Eur J Gastroenterol Hepatol. 2018;30(5):569-577. doi:10.1097/MEG.0000000000001064
5. Poynard T, Munteanu M, Charlotte F, et al. Impact of steatosis and inflammation definitions on the performance of NASH tests. Eur J Gastroenterol Hepatol. 2018;30(4):384-391. doi:10.1097/MEG.0000000000001033
6. Munteanu M, Tiniakos D, Anstee Q, et al. Diagnostic performance of FibroTest, SteatoTest and ActiTest in patients with NAFLD using the SAF score as histological reference. Aliment Pharmacol Ther. 2016;44(8):877-889. doi:10.1111/apt.13770
7. Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. J Hepatol. 2018;68(2):305-315. doi:10.1016/j.jhep.2017.11.013
8. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128(7):1898-1906. doi:10.1053/j.gastro.2005.03.084

Performance

Method Description

NASH-FibroTest Interpretation:
Proprietary algorithm owned by BioPredictive

Alpha-2-Macroglobulin and Haptoglobin:
In this method, the light scattered onto the antigen-antibody complexes is measured. The intensity of the measured scattered light is proportional to the amount of antigen-antibody complexes in the sample under certain conditions. If the antibody volume is kept constant, the signal behaves proportionally to the antigen volume.

A reference curve is generated by a standard with a known antigen content on which the scattered light signals of the samples can be evaluated and calculated as an antigen concentration. Antigen-antibody complexes are formed when a sample containing antigen and the corresponding antiserum are put into a cuvette. A light beam is generated with a light-emitting diode, which is transmitted through the cuvette. The light is scattered onto the immuno-complexes that are present. Antigen and antibody are mixed in the initial measurement, but no complex is formed yet. An antigen-antibody complex is formed in the final measurement.

The result is calculated by subtracting value of the final measurement from the initial measurement. The distribution of intensity of the scattered light depends on the ratio of the particle size of the antigen-antibody complexes to the radiated wavelength.(Unpublished Mayo method; instruction manual: Siemens Nephelometer II, Siemens, Inc; Version 2.4. 07/2019)

Alanine Aminotransferase:
Alanine aminotransferase (ALT) catalyzes the transfer of an amino group between L-alanine and 2-oxoglutarate to form pyruvate and L-glutamate. The pyruvate then reacts with reduced nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase to form L-lactate and NAD[+]. Pyridoxal phosphate serves as a coenzyme in the

amino transfer reaction. It ensures full enzyme activation. The rate of NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.(Package insert: ALT reagent. Roche Diagnostics; 04/2024)

Apolipoprotein A1:
Anti-apolipoprotein A-1 antibodies react with the antigen in the sample to form antigen/antibody complexes which, following agglutination, can be measured turbidimetrically.(Package insert: Tina-quant Apolipoprotein A-1. Roche Diagnostics; 02/2022)

Gamma-Glutamyltransferase:
This is an enzymatic colorimetric assay where gamma-glutamyltransferase (GGT) transfers the gamma-glutamyl group of L-gamma-glutamyl-3-carboxy-44-nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity. It is determined by measuring the increase in absorbance photometrically.(Package insert: GGT-2 reagent, Roche Diagnostics; 09/2022)

Total Bilirubin:
Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium. The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.(Package insert: Bilirubin Total Gen. 3. Roche Diagnostics; 01/2024)

Aspartate Aminotransferase:
Aspartate aminotransferase (AST) catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH) to form L-malate and NAD+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation. The rate of NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance. (Aspartate Aminotransferase reagent. Roche Diagnostics, 01/2024)

Total Cholesterol:
Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye. The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.(Package insert: Cholesterol Gen2 Reagent. Roche Diagnostics; 09/2023)

Triglycerides:
This test uses a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.(Package insert: Triglycerides. Roche Diagnostics; 05/2024)

Glucose:
Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP. Glucose-6-phosphate

dehydrogenase oxidizes glucose-6-phosphate in the presence of nicotinamide adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.(Package insert: Glucose HK Gen.3. Roche Diagnostics; 07/2024)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
2 to 4 days

Specimen Retention Time
7 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

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 was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
0003M

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NSFIB	NASH-FibroTest	93691-4

Result ID	Test Result Name	Result LOINC® Value
TBILF	Bilirubin, Total, S	1975-2
ALTF	Alanine Aminotransferase (ALT), S	1743-4
A2MF	Alpha-2-Macroglobulin, S	1835-8

APOAF	Apolipoprotein A1, S	1869-7
GGTF	Gamma Glutamyltransferase (GGT), S	2324-2
HAPTF	Haptoglobin, S	46127-7
GLURF	Glucose, Fasting, P	1558-6
TRIGF	Triglycerides, S	2571-8
CHOLF	Cholesterol, Total, S	2093-3
ASTF	Aspartate Aminotransferase (AST), S	30239-8
SCRF	FibroTest Score	48795-9
STGF	FibroTest Stage	48794-2
INTEF	FibroTest Interpretation	88447-8
NUM	BioPredictive Serial Number	74715-4
ERROR	BioPredictive Analyte Error	No LOINC Needed
SCRN2	NashTest 2 Score	93692-2
GRDN	NashTest 2 Grade	93693-0
INTEN	NashTest 2 Interpretation	93694-8
SCRS2	SteatoTest 2 Score	93695-5
GRDS	SteatoTest 2 Grade	93696-3
INTEI	SteatoTest 2 Interpretation	93697-1
CMMS	NASH-FibroTest Comment	48767-8