MAYO CLINIC LABORATORIES

Test Definition: HCCGS

Hepatocellular Carcinoma Risk Panel with GALAD Score, Serum

Overview

Useful For

Risk assessment for development of hepatocellular carcinoma in patients with chronic liver disease

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
L3AFP	AFP-L3% and Total AFP, S	Yes	Yes
DCP	Des-Gamma-Carboxy Prothrombin, S	Yes	Yes
GAL1	GALAD Model Score	No	Yes

Highlights

This test includes a GALAD (gender, age, alpha-fetoprotein [AFP]-L3, AFP, des-gamma-carboxy prothrombin [DCP]) model score calculation. The GALAD model has been demonstrated to have higher diagnostic accuracy for the detection of hepatocellular carcinoma (HCC) when compared to the use AFP, AFP-L3, and DCP markers alone or in combination. The performance of the GALAD score has also been reported to be superior to ultrasound for HCC detection.

Method Name

L3AFP and DCP: Isotachophoresis with Laser-Induced Fluorescence
GAL1: Calculation

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

GALAD (gender, age, alpha-fetoprotein [AFP]-L3, AFP, des-gamma-carboxy prothrombin [DCP]) score testing (this test) **should not** be performed for patients who are pregnant, as alpha-fetoprotein results are elevated during pregnancy.

Necessary Information

Sex and age are required.

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
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:
-Gastroenterology and Hepatology Test Request (T728)
-Oncology Test Request (T729)

Specimen Minimum Volume

0.25 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Frozen (preferred)	90 days	
	Refrigerated	5 days	

Clinical & Interpretive

Clinical Information

Worldwide, hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death.(1) While HCC can be treated effectively in its early stages, most patients are not diagnosed until they are symptomatic and at higher grades and stages, which are less responsive to therapies. Alpha-fetoprotein (AFP) is the standard serum tumor marker utilized in the evaluation of suspected HCC. However, increased serum concentrations of AFP might be found in chronic hepatitis and liver cirrhosis, as well as in other tumor types (eg, germ cell tumors),(2) decreasing the specificity of AFP testing for HCC. Furthermore, AFP is not expressed at high levels in all HCC patients, resulting in decreased sensitivity, especially in potentially curable small tumors.

AFP-L3:
AFP is differentially glycosylated in several hepatic diseases. For example, alpha-(1,6)-fucosyltransferase is differentially expressed in hepatocytes following malignant transformation.(3) This enzyme incorporates fucose residues on the carbohydrate chains of AFP. Different glycosylated forms of AFP can be recognized following electrophoresis by reaction

with different carbohydrate-binding plant lectins. The fucosylated form of serum AFP that is most closely associated with HCC is recognized by a lectin from the common lentil (*Lens culinaris*). This is designated as AFP-L3 (third electrophoretic form of lentil lectin-reactive AFP). AFP-L3 is most useful in the differential diagnosis of individuals with total serum AFP of 200 ng/mL or less, which may result from a variety of benign pathologies, such as chronic liver diseases.

Des-gamma-carboxy prothrombin:
Des-gamma-carboxy prothrombin (DCP), also known as the protein induced by vitamin K absence or antagonist II (PIVKA-II), is an abnormal form of the coagulation protein, prothrombin. DCP is a nonfunctional prothrombin resulting from a lack of carboxylation of 10 glutamic acid residues in the N-terminal portion of the molecule. In normal liver, prothrombin undergoes post-translational carboxylation before release into the peripheral blood. The carboxylation converts specific amino-terminal glutamic acid residues to gamma-carboxyglutamic acid. The vitamin K-dependent carboxylase responsible for the carboxylation is absent in many HCC cells, and an abnormal prothrombin with all or some unconverted glutamic acid is secreted. Therefore, this non-carboxylated form (DCP) has been used as an HCC biomarker.

DCP is considered a complementary biomarker to AFP and AFP-L3 for assessing the risk of developing HCC. Elevations of both AFP-L3 and DCP indicate progression of HCC, albeit they reflect different features of the progression. In a prospective study of patients in the United States with an established diagnosis of HCC, sensitivities for AFP, AFP-L3, and DCP were 68%, 62%, and 73%, respectively. When the 3 markers were combined, the sensitivity was 86%. In another study, DCP levels were shown to correlate with tumor size and metastatic HCC. In this study, compared to AFP and AFP-L3, DCP had the highest sensitivity (87%) and the highest positive predictive value (87%) in patients with HCC due to chronic hepatitis B and C infections. A number of studies have shown that elevated serum DCP is significantly related to portal vein invasion or intrahepatic metastasis, which significantly affect prognosis for patients with HCC.

DCP can be elevated in other conditions besides HCC. Conditions such as obstructive jaundice, intrahepatic cholestasis causing chronic decrease in vitamin K, and ingestion of drugs such as warfarin or wide-spectrum antibiotics can result in high concentrations of DCP. In addition, 25% to 50% of patients with HCC will have a DCP value within the reference range. Because of this, a normal DCP value does not rule out HCC.

Gender, Age, AFP-L3, AFP, DCP (GALAD) Score:
Biomarkers of HCC include AFP, AFP-L3, and DCP. The GALAD model combines these three biomarkers with the patient's gender and age to estimate the risk of HCC in patients with chronic liver disease based on the following equation Z = -10.08 + 0.09 x age + 1.67 x sex + 2.34 log(10) (AFP) + 0.04 x AFP - L3 + 1.33 x log(10) (DCP), where sex = 1 for males, 0 for females.

The GALAD score is calculated using the lower limit of quantitation (LLOQ) when one or more of the following values are below the lower limit of quantitation: %L3, Total AFP, or DCP. In the event this occurs, the GALAD score is resulted as (<)GALAD score.

The GALAD model has been demonstrated to have higher diagnostic accuracy for the detection of HCC when compared to the use AFP, AFP-L3, and DCP markers alone or in combination. The performance of the GALAD score has also been reported to be superior to ultrasound for HCC detection.

Reference Values

TOTAL ALPHA-FETOPROTEIN (AFP):
<4.7 ng/mL

AFP L3-PERCENT:
<10%

DES-GAMMA-CARBOXY PROTHROMBIN:
<7.5 ng/mL

GAL1:
Not applicable

Interpretation

Alpha-fetoprotein (AFP)-L3 results of 10% or more are associated with a 7-fold increased risk of developing hepatocellular carcinoma (HCC). Patients with AFP-L3 levels of 10% or more should be monitored more intensely for evidence of hepatocellular carcinoma according to current practice guidelines.

Total serum AFP results above 200 ng/mL are highly suggestive of a diagnosis of HCC. In patients with liver disease, a total serum AFP level above 200 ng/mL is near 100% predictive of HCC. With lower total AFP levels, there is an increased likelihood that chronic liver disease, rather than HCC, is responsible for the AFP elevation.

Based on a retrospective study at Mayo Clinic, for patients with total AFP levels 200 ng/mL or less, AFP-L3 specificity approaches 100% for HCC when its percentage exceeds 35% of the total AFP.(4)

AFP concentrations over 100,000 ng/mL have been reported in normal newborns, and the values rapidly decline in the first 6 years of life.

Des-gamma-carboxy prothrombin:
In patients with an elevated des-gamma-carboxy prothrombin (DCP) result (> or =7.5 ng/mL), the risk of developing HCC is 36.5% (95% CI 23.5%-49.6%). The risk of developing HCC with a negative DCP result (<7.5 ng/mL) is 7.6% (95% CI 4.4%-10.8%).

Gender, Age, AFP-L3, AFP, DCP (GALAD) Score:
Higher GALAD model scores correlate with increased risk of HCC. The area under the curve (AUC) of a receiver operating characteristic curve of the GALAD score was 0.95 for all HCC detection, and 0.92 for the detection of early-stage HCC. Additionally, the AUC of the GALAD score (0.95) was higher than that of ultrasound alone for all HCC detection (AUC of 0.82, *P* <0.01).

The sensitivity and specificity performance characteristics of the GALAD score for HCC will be influenced by the selected GALAD score cut-off. For example, at an optimal AUC cutoff of -0.76, the GALAD score had 91% sensitivity and 85% specificity for HCC detection. At a more specific GALAD score cutoff of 0.88, the observed sensitivity was 80% for HCC detection with an observed specificity of 97%.

The GALAD model was developed and validated in patient cohorts with a prevalence of HCC ranging from 35% to 49%.

The performance of the model may be altered in populations with different HCC prevalence. In addition, the clinical performance of the GALAD score varies by etiology of HCC and therefore may be different in different regions of the world.

Cautions

Serum markers are not specific for malignancy, and values may vary by method. Do not interpret alpha-fetoprotein (AFP), AFP-L3, and des-gamma-carboxy prothrombin (DCP) levels as absolute evidence of the presence or absence of malignant disease. Results should be used in conjunction with information from the clinical evaluation of the patient, cytology, and imaging procedures.

Values obtained with different assay methods or kits cannot be used interchangeably.

In rare cases, some individuals can develop antibodies to mouse or other animal antibodies (often referred to as human anti-mouse antibodies [HAMA] or heterophile antibodies), which may cause interference in some immunoassays. Caution should be used in interpretation of results, and the laboratory should be alerted if the result does not correlate with the clinical presentation.

Test results for AFP are not interpretable if the patient is pregnant.

DCP-producing tumors other than hepatocellular carcinoma can show elevated DCP values. Liver disease caused by other etiologies, such as alcohol-induced liver disease, hemochromatosis, Wilson disease, autoimmune hepatitis, and steatohepatitis have not been studied with the DCP assay.

Medications containing vitamin K preparations may cause a negative bias with DCP values. Medications containing vitamin K antagonist or antibiotic may cause a positive bias with DCP values.

The total AFP and AFP-L3 test values must be obtained using the uTASWako i30 in the GALAD (gender, age, AFP-L3, AFP, des-gamma-carboxy prothrombin) score calculation.

Clinical Reference

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Performance

Method Description

Testing is performed using the uTASWako i30 instrument and the test system reagents.

Alpha-Fetoprotein L3:
Total alpha-fetoprotein (AFP) is measured by laser-induced fluorescence with separation of the lentil lectin-reactive AFP-L3 and lectin nonreactive forms of AFP by isotachophoresis of their immune-complexes. Results are expressed as the percent ratio of AFP-L3 to total AFP.(Package insert: uTASWako i30 AFP-L3. Wako Diagnostics; 21.11.18K17, 12/2021)

Des-Gamma-Carboxy Prothrombin:
Sample is added to the reagent well with the fluorescent dye labeled antihuman prothrombin antibody (mouse monoclonal) to form the primary immunocomplex. The second labeled antibody solution, anion-conjugated antihuman des-gamma-carboxy prothrombin (DCP) antibody (mouse monoclonal), is concentrated by isotachophoresis when voltage is applied. The concentrated anion-conjugated antibody then reacts with the primary immunocomplex to form the secondary immunocomplex. This secondary complex is further concentrated during isotachophoresis and is separated from unbound fluorescent dye-labeled antibody by capillary gel electrophoresis. The remaining dye labeled DCP is measured by laser-induced fluorescence. The concentration of DCP in the specimen is directly proportional to the amount of fluorescence.(Package insert: uTASWako i30 DCP. Wako Diagnostics; V 21.11.18K15, 12//2021)

Gender, Age, AFP-L3, AFP, DCP, and (GALAD) Model Score:
The GALAD model is a statistical model for estimating the likelihood of hepatocellular carcinoma in patients with chronic liver disease. The GALAD score is calculated based on gender, age, and measured concentrations of AFP-L3, AFP and DCP.

PDF Report

No

Day(s) Performed
Monday through Friday

Report Available
1 to 4 days

Specimen Retention Time
2 weeks

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
82107
83951

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
HCCGS	HCC Risk Panel with GALAD Score, S	96452-8

Result ID	Test Result Name	Result LOINC® Value
TAFP	Total AFP, S	1834-1
L3	%L3	42332-7
INT67	Interpretation	69048-7
DCP	Des-Gamma-Carboxy Prothrombin, S	34444-0
GAL1	GALAD Model Score	96450-2