MAYO CLINIC LABORATORIES

Test Definition: P53CA

TP53 gene somatic mutation analysis

Overview

Useful For

Evaluating chronic lymphocytic leukemia patients at diagnosis or during disease course for the presence of *TP53* gene variants indicating high risk of disease progression and adverse outcomes

This test is **not intended for** the evaluation of patients suspected of having an inherited or germline *TP53* cancer syndrome (eg, Li Fraumeni syndrome)

Testing Algorithm

Flow cytometry will be performed on peripheral blood samples to verify diagnosis of chronic lymphocytic leukemia (CLL) and to selectively enrich for B-cells in samples with a clonal population.

See *TP53* Sequencing Testing Algorithm in Special Instructions.

Special Instructions

- TP53 Mutation Testing Algorithm
- Molecular Hematopathology Patient Information

Highlights

This test is complementary to fluorescence in situ hybridization (FISH) analysis for the 17p- abnormality but more appropriately identifies the presence of variant alteration and gene inactivation in tumor cells.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CSP53	TP53 Pre-Analysis Cell Sorting, V	No	No

Method Name

Polymerase Chain Reaction (PCR) and Sanger Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

For the evaluation of patients suspected of having an inherited or germline *TP53* cancer syndrome (eg, Li Fraumeni syndrome), order TP53Z / *TP53* Gene, Li Fraumeni Syndrome, Full Gene Analysis, Varies.

Shipping Instructions

Blood and bone marrow specimens must arrive within 10 days of collection.

Necessary Information

The following information is required:

1. Pertinent clinical history

2. Clinical or morphologic suspicion

3. Date of collection

4. Specimen source

Specimen Required

Submit only 1 of the following specimens:

Specimen Type: Blood (preferred)

Container/Tube: Lavender top (EDTA) or yellow top (ACD solution B)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send specimen in original tube.

3. Label specimen as blood.

Specimen Stability Information: Ambient/Refrigerate <10 days

Specimen Type: Bone marrow

Container/Tube: Lavender top (EDTA), yellow top (ACD solution B), or green top (heparin)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix bone marrow.

2. Send specimen in original tube.

3. Label specimen as bone marrow.

Specimen Stability Information: Ambient/Refrigerate <10 days

Specimen Type: Tissue

Container/Tube: Plastic container

Specimen Volume: 100 mg

Collection Instructions: Stabilize fresh tissue in tissue culture medium or freeze immediately after collection.

Specimen Stability Information: Refrigerate 24 hours/ Frozen

Forms

1. Molecular Hematopathology Patient Information: B-Cell Chronic Lymphocytic Leukemia (CLL) for *IGVH* and/or *TP53* Somatic Mutation Testing in Special Instructions

2. If not ordering electronically, complete, print, and send a Hematopathology/Cytogenetics Test Request (T726) with the specimen.

Reject Due To

Gross hemolysis	Reject
Extracted DNA	Reject
Moderately to severely clotted	Reject

Specimen Minimum Volume

Blood, bone marrow: 1 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container

Varies	Varies (preferred)	10 days	

Clinical & Interpretive

Clinical Information

Patients with chronic lymphocytic leukemia (CLL) have variable disease course influenced by a series of tumor biologic factors. The presence of chromosomal 17p- or a *TP53* gene variant confers a very poor prognosis to a subset of CLL patients, both at time of initial diagnosis, as well as at disease progression, or in the setting of therapeutic resistance. *TP53* gene variant status in CLL has emerged as the single most predictive tumor genetic abnormality associated with adverse outcome and poor response to standard immunochemotherapy; however, patients can be managed with alternative therapeutic options.

Although the prognostic relevance of an acquired *TP53* gene variant is best studied for CLL, similar findings are also reported for other hematologic malignancies including low-grade B-cell lymphoma, diffuse large B-cell lymphoma, and some types of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Therefore, while this test has been developed to be primarily focused on high-risk CLL patients, *TP53* gene sequencing analysis can also be performed in additional neoplasms, as clinically indicated.

Reference Values

Genetic variants present or absent as compared to a reference sequence of the normal *TP53* gene

Interpretation

Results are reported in standard nomenclature according to the most recent Human Genome Variation Society (HGVS) recommendations and an interpretive comment regarding the nature of the sequence variant (eg, known deleterious, suspected deleterious, synonymous change) will be included to complete the clinical report.

Cautions

This test will not detect all possible acquired variants in the *TP53* gene because it is restricted to analyzing exons 4 to 9. However, this region encompasses more than 90% of described pathologic variants and covers the coding exons of the critical DNA binding regions.

The analytical sensitivity of the assay can be affected by the absolute B-cell number in the peripheral blood or tissue sample, as well as the often subclonal nature of this tumor genetic abnormality. The assay attempts to compensate in part for this by performing an initial screening flow cytometry to assess B-cell quantity and by performing the cell enrichment step (for the peripheral blood specimens only) to isolate relatively pure CD19+ B-cells for analysis. Nevertheless, the nature of the Sanger sequencing method is such that typical reproducible analytic sensitivity will be in the order of 25% variant allele burden.

Because optimal cell enrichment is dependent on the absolute B-cell quantity, samples with a very low WBC or initial percentage of B cells (determined from flow cytometry or WBC automated cell count) will likely result in poor assay performance and inability to detect possible *TP53* gene variants in the tumor population.

Clinical Reference

1. Zenz T, Krober A, Scherer K, et al: Monoallelic TP53 inactivation is associated with poor prognosis in chronic lymphocytic leukemia: results from a detailed genetic characterization with long-term follow-up. Blood. 2008;112:3322-3329

2. Lehmann S, Oqawa S, Raynaud SD, et al: Molecular allelokaryotyping of early-stage, untreated chronic lymphocytic leukemia. Cancer. 2008;112:1296-1305

3. Rossi D, Cerri M, Deambrogi C, et al: The prognostic value of TP53 mutations in chronic lymphocytic leukemia is independent of Del17p13: implications for overall survival and chemorefractoriness. Clin Cancer Res. 2009;15(3):995-1004

4. Zent CS, Call TG, Hogan WJ, et al: Update on risk-stratified management for chronic lymphocytic leukemia. Leuk Lymphoma. 2006;47(9):1738-1746

5. Trbusek M, Smardova J, Malcikova J, et al: Missense mutations located in structural p53 DNA-binding motifs are associated with extremely poor survival in chronic lymphocytic leukemia. J Clin Oncol. 2011;29:2703-2708

6. Halldorsdottir AM, Lundin A, Murray F, et al: Impact of TP53 mutation and 17p deletion in mantle cell lymphoma. Leukemia. 2011;25:1904-1908

7. Young KH, Leroy K, Moller MB, et al: Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. Blood. 2008;112:3088-3098

Performance

Method Description

Peripheral blood specimens from chronic lymphocytic leukemia (CLL) patients only will be analyzed by a screening flow cytometry method to determine B-cell content and confirm the presence of a clonal B-cell population. Blood samples (but not bone marrows) from patients with CLL are enriched for B-lymphocytes by cell sorting and DNA is extracted from the B-cell fraction. For other sample types (bone marrow, fresh or frozen tissues) DNA is extracted directly without prior enrichment. Polymerase chain reaction (PCR) and Sanger sequencing of *TP53* exons 4 to 9 is performed. Sequence analysis is performed using Mutation Surveyor and Alamut software. The presence of a detected variant is then assessed using curated public databases of known *TP53* gene mutations.(The TP53 Web Site entry UMD TP53 Mutation Database. Accessed 12/3/2013; den Dunnen JT, Antonarakis SE: Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat. 2000;15:7-12)

PDF Report

No

Specimen Retention Time

DNA 3 months

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

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

CPT Code Information

81352-TP53 (tumor protein 53) (eg, tumor samples), full gene sequence or targeted sequence analysis of >5 exons

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
P53CA	TP53 gene somatic mutation analysis	21739-8

Result ID	Reporting Name	LOINC®
MP018	Specimen Type:	31208-2
35759	Final Diagnosis:	34574-4
607075	Signing Pathologist	19139-5