MAYO CLINIC LABORATORIES

Test Definition: PM3CX

Lymph3Cx Assay, Primary Mediastinal Large B-cell Lymphoma and Diffuse Large B-cell Lymphoma, mRNA Gene Expression, NanoString, Tissue

Overview

Useful For

Only indicated for formalin-fixed paraffin-embedded specimens from patients diagnosed with large B-cell lymphoma

Genetics Test Information

This assay is only indicated for patients diagnosed with large B-cell lymphoma for the purposes of determining a final diagnosis of primary mediastinal large B-cell lymphoma (PMBCL) vs diffuse large B-cell lymphoma (DLBCL) and to provide the cell-of-origin information for cases determined to be DLBCL. This assay is only intended to be run using RNA derived from formalin-fixed paraffin-embedded tissue specimens.

PMBCL is recognized by the World Health Organization's (WHO) publication "Classification of Tumours of the Haematopoietic and Lymphoid Systems" as a distinct lymphoid neoplasm,(1) but correct diagnosis can be difficult due to dependence upon correlation of clinical variables, presentation, and immunohistochemistry assays. This is further complicated by the occasional presentation of PMBCL outside the mediastinum.(2)

WHO also recognizes 2 distinct molecular subtypes of diffuse large B-cell lymphoma (DLBCL): Germinal center B-cell (GCB) type and activated B-cell (ABC) type, as well as a third "Unclassifiable" group.(1)

Molecular gene expression offers robust and accurate determination of both the diagnosis of PMBCL and sub-classification of DLBCL.(3,4)

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
AZSLR	Slide Review	No, (Bill Only)	Yes

Testing Algorithm

When this test is ordered, slide review will always be performed at an additional charge.

Highlights

The NanoString nCounter system analyzes gene expression without a complementary DNA synthesis step, greatly increasing tolerance of degraded RNA, inhibitors associated with the formalin fixation process, bias, and other artifacts that can often adversely affect enzymatic reactions. Following RNA isolation and overnight hybridization, all washing, immobilization, and scanning steps are automated. This improves precision of the assay and reduces hands-on processing time, providing a rapid turnaround for results, highly desirable attributes for testing in a clinical setting.(4)

Method Name

Gene Expression by Digital Counting of Fluorescent Bar-Coded Probes

NY State Available
No

Specimen

Specimen Type
Tissue, Paraffin

Ordering Guidance
This test does not include a pathology consultation. If a pathology consultation is requested, PATHC / Pathology Consultation will be ordered and performed at an additional charge.

Necessary Information
Pathology report (final or preliminary) **must accompany specimen in order for testing to be performed.** At minimum, it should contain the following information:
1. Patient name
2. Block number-must be on all blocks, slides, and paperwork (can be handwritten on the paperwork)
3. Tissue collection date
4. Source of the tissue

Specimen Required
Submit only 1 of the following specimens:
Preferred:
Specimen Type: Tissue slides
Slides: 1 Hematoxylin and eosin stained and 6 to 10 unstained
Collection Instructions:
1. Submit 1 slide stained with hematoxylin and eosin.
2. If the formalin-fixed paraffin-embedded (FFPE) biopsy specimen is a core needle biopsy or fine needle aspirate, submit 10 unstained slides.
3. If the FFPE biopsy specimen is a resection, submit 6 unstained slides.
4. Submitted slides should be 4- to 5-micron thick sections placed on positively charged slides.
Additional Information: Paraffin embedded specimens can be from any anatomic location (skin, soft tissue, lymph node, etc).

Acceptable:
Specimen Type: Tissue block
Collection Instructions: Submit an FFPE tumor tissue block.
Additional Information: Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

Specimen Minimum Volume

Minimum 60% tumor with or without macrodissection. Minimum required unstained tissue section input: 0.12 mm(3); Slides: See Specimen Required

Reject Due To

Exhausted tissue block	Reject
Tissue with non-formalin fixation	Reject
Broken or cracked unstained slides	Reject
Broken or cracked stained slides that cannot be repaired	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Tissue, Paraffin	Ambient (preferred)		
	Refrigerated		

Clinical & Interpretive

Clinical Information

Primary mediastinal large B-cell lymphoma (PMBCL) is recognized as a distinct entity in the World Health Organization (WHO) classification and accounts for approximately 2% to 3% of all non-Hodgkin lymphomas (NHL).(1) Currently, diagnosis relies on consensus of histopathology, clinical variables, and presentation (as it can present outside the mediastinum), giving rise to diagnostic inaccuracy in routine practice. This is complicated by recent studies that identified lymphomas sharing molecular and morphological features with PMBCL, yet without involvement in the mediastinum.(2) Since classification can impact therapeutic approach, the need has arisen for a more robust method to identify PMBCL vs diffuse large B-cell lymphoma (DLBCL).

Gene expression profiling studies have demonstrated that PMBCL can be distinguished from subtypes of DLBCL based on

gene expression signatures in fresh/frozen tissues,(3,4) which are often difficult to obtain in conventional clinic settings. Members of the Lymphoma/Leukemia Molecular Profiling Project have developed a robust and accurate molecular classification assay (Lymph3Cx) for the distinction of PMBCL from DLBCL subtypes based on gene expression measurements in clinically-available FFPE tissue,(5) which has been subsequently validated against this published data in the Molecular Diagnostics Arizona Lab.

Research suggests that novel therapeutic approaches might have preferential benefit in PMBCL as compared to DLBCL.(6,7,8,9)

The Lymph3Cx assay builds upon the previously described and validated Lymph2Cx assay.(4,10) The Lymph3Cx assay is a qualitative assay utilizing a 58-gene signature (45 endogenous targets and 13 housekeeping genes), reporting calculated scores to distinguish PMBCL from DLBCL.(5) After determination of PMBCL vs DLBCL status, the built-in Lymph2Cx assay then provides the cell-of-origin (COO) for samples determined to be DLBCL as previously described.

DLBCL is the most common form of NHL, accounting for up to 30% of newly diagnosed cases in the United States. DLBCL is an aggressive (fast-growing) lymphoma caused by the uncontrollable growth and proliferation of B lymphocytes. It can arise in lymph nodes or outside of the lymphatic system in the gastrointestinal tract, testes, thyroid, skin, breast, bone, or brain. Although DLBCL is the most common form of NHL, there are distinct subtypes that may affect prognosis (how well patients will do with standard treatment) and treatment options. Since 2008, WHO classification of lymphoid neoplasms has recognized 2 distinct molecular subgroups of DLBCL: a germinal center B-cell (GCB) type, an activated B-cell (ABC) type, as well as a third group of cases that do not belong to either (unclassifiable). These were all originally grouped together based on shared morphology but can be distinctly separated based on their biology, particularly their gene expression pattern. According to a review of the 2016 revision of the WHO guidelines,(1) a better understanding of the molecular pathogenesis of these 2 subgroups has led to the investigation of more specific therapeutic strategies based on COO classification. Current data suggests that the ABC subtype of DLBCL has a poorer prognosis compared to the GCB subtype or unclassifiable cases; and that the ABC subtype may differentially respond to specific therapies.(11,12,13)

Reference Values

Not applicable

Interpretation

Results of the consultation are reported in a formal pathology report that includes a description of ancillary test results and an interpretive comment.

Cautions

Test results should be interpreted in the context of clinical findings, tumor sampling, and other laboratory data.

Paraffin-embedded tissues that have been decalcified are sometimes unsuccessful for analysis. The pathologist reviewing the hematoxylin and eosin-stained slide may find it necessary to cancel testing.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and

will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

Clinical Reference

1. Swerdlow SH, Campo E, Jaffe ES, et al. The 2016 Revision to the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375-2390
2. Yuan J, Wright G, Rosenwald A, et al. Identification of primary mediastinal large B-cell lymphoma at nonmediastinal sites by gene expression profiling. Am J Surg Pathol. 2015;39(10):1322-1330
3. Savage KJ, Monti S, Kutok JL, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. Blood. 2003;102(12):3871-3879
4. Rosenwald A, Wright G, Leroy K, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med. 2003;198(6):851-862
5. Mottok A, Wright G, Rimsza L, et al. Molecular classification of primary mediastinal large B-cell lymphoma using routinely available tissue specimens. Blood. 2018;132(22):2401-2405
6. Dunleavy K, Pittaluga S, Maeda LS, et al. Dose-adjusted EPOCH-rituximab therapy in primary mediastinal B-cell lymphoma. N Engl J Med. 2013;368(15):1408-1416
7. Xu-Monette ZY, Zhou J, Young KH. PD-1 expression and clinical PD-1 blockade in B-cell lymphomas. Blood. 2018;131(1):68-83
8. Zinzani PL, Ribrag V, Moskowitz CH, et al. Safety and tolerability of pembrolizumab in patients with relapsed/refractory primary mediastinal large B-cell lymphoma. Blood. 2017;130(3):267-270
9. Scott DW, Wright GW, Williams PM, et al. Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. Blood. 2014;123(8):1214-1217
10. Robetorye RS, Ramsower CA, Rimsza LM, et al. Incorporation of digital gene expression profiling for cell-of-origin determination (Lymph2Cx testing) into the routine work-up of diffuse large B cell lymphoma. J Hematop. 2019;12(1):3-10. doi:10.1007/s12308-019-00344-0
11. Nowakowski GS, Chiappella A, Witzig TE, et al. Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non-germinal center B-cell phenotype in newly diagnosed diffuse large B-cell lymphoma: a phase II study. J Clin Oncol. 2015;33(3):251-257
12. Dunleavy K, Pittaluga S, Czuczman MS, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. Blood. 2009;113(24):6069-6076
13. Thieblemont C, Briere J, Mounier N, et al. The germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse large B-cell lymphoma: a bio-CORAL study. J Clin Oncol. 2011;29(31):4079-4087

Performance

Method Description

The Lymph3Cx assay is performed on RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue of suspected large B-cell lymphoma (BCL) specimens. A pathologist examines a hematoxylin and eosin stained slide and marks an area containing at least 60% tumor suitable for the test. A technologist measures the area, determines the appropriate number of unstained sections to utilize, and performs macrodissection if necessary. RNA is extracted from FFPE tissue sections using a column-based RNA isolation kit. Purified RNA is hybridized to target-specific oligonucleotide probe pairs (A and B), which also contain universal tag sequences. Probe A hybridizes to a specific Reporter Tag and the 5' region of the target nucleic acid sequence, and probe B hybridizes to the universal Capture Tag and the 3' region of the target nucleic acid sequence, creating a "tag complex." Sequential wash steps are performed to remove excess tags, probes, and unhybridized nucleic acids. After washing, the purified Tag Complexes are eluted off the beads and immobilized for data collection. The nCounter Digital Analyzer digitally resolves and counts each fluorescent barcode molecule representing each gene in the RNA. A positive control (Ultramer RNA targets for each of the 58 genes) and a negative control (Molecular Biology Grade water) are included with each cartridge containing patient specimens to qualify the run. The patented Lymph3Cx algorithm performs quality control using the 14 housekeeping genes and reports the model score (to differentiate primary mediastinal large BCL from diffuse large [DL] BCL); if DLBCL, the cell-of-origin is calculated and reported.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

7 to 12 days

Specimen Retention Time

Extracted RNA, H and E-stained slides, and unused unstained slides: Indefinitely; Client provided paraffin blocks: Returned after testing is complete

Performing Laboratory Location

Mayo Clinic Molecular Diagnostics - Scottsdale

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

Test Definition: PM3CX

Lymph3Cx Assay, Primary Mediastinal Large B-cell Lymphoma and Diffuse Large B-cell Lymphoma, mRNA Gene Expression, NanoString, Tissue

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

0120U
88381

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
PM3CX	Lymph3Cx, Lg Bcell Lymphoma,mRNA,Ts	93785-4

Result ID	Test Result Name	Result LOINC® Value
606164	Lymph3Cx, Large B-cell Lymphoma, mRNA, Tissue	93779-7
606165	PMBCL Probability	93780-5
606166	DLBCL Probability	93781-3
606185	PMBCL Call	93782-1
606186	DLBCL COO	93783-9