BCR/ABL1 Mbcr (P210) IS Quantitative Analysis

GOPATH® LABORATORIES
Global Pathology Services

"Patients with the Philadelphia chromosome who are treated with TKIs have a greater chance for survival."

BCR-ABL1 can be found in more than 95% of adult CML patients.[3]

BCR/ABL1 Fusions and Chronic Myelogenous Leukemia (CML)

Chronic myelogenous leukemia (CML) is a myeloproliferative neoplasm that accounts for 15%-20% of adult leukemia.[1] The hallmark of CML is a reciprocal translocation between chromosome 9 (ABL) and chromosome 22 (BCR), resulting in a fusion gene (BCR-ABL), otherwise known as the Philadelphia chromosome or Ph (Figure 1). This fusion leads to deregulated tyrosine kinase activity that plays a key role in CML pathogenesis.[2]

The Philadelphia chromosome is observed in more than 95% of adult CML patients, 15%-20% of adult acute lymphoblastic leukemia (ALL) patients, 3%-5% of pediatric ALL, and rarely in adult acute myelogenous leukemias (AML).[3] Although cytogenetically all Philadelphia chromosomes appear the same, molecular assessment could distinguish several clinically-important variant isoforms p190, p210, and p230, based on the different breakpoints. The transcript derived from major breakpoint (Mbcr), i.e, e13/a2 or e14/a2 forms protein p210, which is involved in 95% of CML but can also be associated with 15% of adult-onset ALL and 5% of adult-onset AML. The transcript derived from minor breakpoint (mbcr), i.e, e1/a2, forms protein p190, which is generally associated with 15% of adult-onset ALL and 5% of pediatric-onset ALL. P230 is usually associated with CML with neutrophilia and thrombocytosis.[4]

Treatment with tyrosine kinase inhibitors (TKIs) dramatically improves survival in CML patients and is thus the standard of care for CML patients. The goal of TKI treatment is to achieve a major molecular response (MMR), defined as a 3-log (1000 fold) reduction in BCR-ABL1 transcripts aligned to IRIS baseline.[5] Due to the demand of high sensitivity, reverse transcriptase quantitative PCR (RQ-PCR) is frequently used to assess BCR-ABL transcripts in CML patients in order to monitor disease course and treatment response.

The measured results can be aligned to international scale (IS) to determine if patients have achieved a particular milestone. As more potent TKI is being used in CML treatment, assessment of deep response is urgently needed, since it would be important to determine the safe point to discontinue treatment whereas remission can still be sustained.

Clinical Utility

Three levels of response are commonly used to evaluate CML status. First, hematologic response refers to the normalization of blood cell count, mainly needed to adjust the TKI dose for hematologic toxicity. Second, cytogenetic response (CyR) refers to chromosome G banding analysis (CBA) or fluorescence in situ hybridization (FISH), mainly needed to assist diagnosis of Ph in the pre-TKI era. Third, molecular response (MR), refers to real-time quantitative reverse-transcription PCR (RQ-PCR), mainly needed to measure the reduction of BCR-ABL1 fusion transcripts in order to monitor minimal residual disease (MRD) over time.

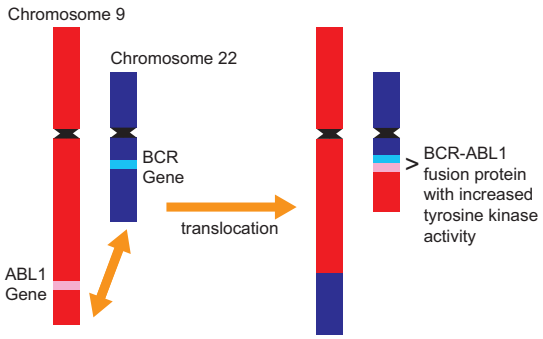


Figure 1. Example of chromosomal translocation/BCR-ABL1 fusion gene commonly called the Philadelphia chromosome

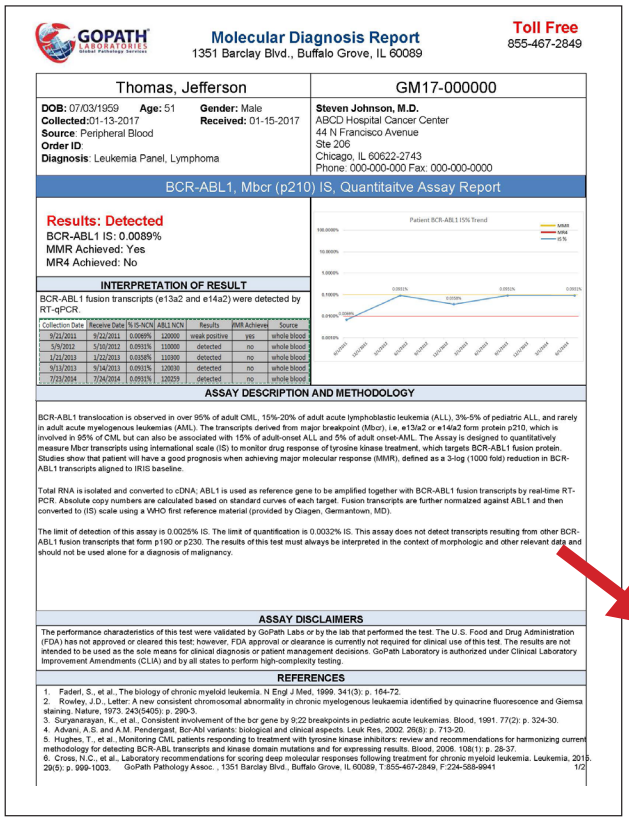
Assay Description

The assay is designed to quantitatively measure Mbcr transcripts using international scale (IS) to monitor drug response of tyrosine kinase inhibitor (TKI) treatment, which targets the BCR-ABL1 fusion protein. Studies show that a patient will have a good prognosis when achieving major molecular response (MMR), defined as a 3-log (1000 fold) reduction in BCR-ABL1 transcripts aligned to IRIS baseline.

Methodology

Total RNA is isolated from bone marrow or peripheral blood and converted to cDNA. ABL1 is used as a reference gene to be amplified together with BCR-ABL1 fusion transcripts by real-time RT-PCR. Absolute copy numbers are calculated based on standard curves of each target. Fusion transcripts are further normalized against ABL1 and then converted to (IS) scale using a WHO first reference material (provided by Qiagen, Germantown, MD). The limit of detection of this assay is 0.0025% IS. The limit of quantification is 0.0032% IS. This assay does not detect transcripts resulting from other BCR-ABL1 fusion transcripts that form p190 or p230.
The results of this test must always be interpreted in the context of morphologic and other relevant data and should not be used alone for a diagnosis of malignancy.

Sample Report & Requisition



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Molecular Diagnosis Report
1351 Barclay Blvd., Buffalo Grove, IL 60089

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855-467-2849

Thomas, Jefferson	GM17-000000
DOB: 07/03/1959 **Age:** 51 **Gender:** Male **Collected:**01-13-2017 **Received:** 01-15-2017 **Source**: Peripheral Blood **Order ID**: **Diagnosis**: Leukemia Panel, Lymphoma	**Steven Johnson, M.D.** ABCD Hospital Cancer Center 44 N Francisco Avenue Ste 206 Chicago, IL 60622-2743 Phone: 000-000-000 Fax: 000-000-0000

BCR-ABL1, Mbcr (p210) IS, Quantitaitve Assay Report

Results: Detected
BCR-ABL1 IS: 0.0089%
MMR Achieved: Yes
MR4 Achieved: No

Patient BCR-ABL1 IS% Trend

INTERPRETATION OF RESULT

BCR-ABL1 fusion transcripts (e13a2 and e14a2) were detected by RT-qPCR.

Collection Date	Receive Date	%IS-NCN	ABL1 NCN	Results	MMR Achieved	Source
9/21/2011	9/22/2011	0.0089%	120000	weak positive	yes	whole blood
5/9/2012	5/10/2012	0.0931%	110000	detected	no	whole blood
1/21/2013	1/22/2013	0.0358%	110300	detected	no	whole blood
9/13/2013	9/14/2013	0.0931%	120030	detected	no	whole blood
7/23/2014	7/24/2014	0.0931%	120259	detected	no	whole blood

ASSAY DESCRIPTION AND METHODOLOGY

BCR-ABL1 translocation is observed in over 95% of adult CML, 15%-20% of adult acute lymphoblastic leukemia (ALL), 3%-5% of pediatric ALL, and rarely in adult acute myelogenous leukemias (AML). The transcripts derived from major breakpoint (Mbcr), i.e, e13a2 or e14a2 form protein p210, which is involved in 95% of CML but can also be associated with 15% of adult-onset ALL and 5% of adult onset-AML. The Assay is designed to quantitatively measure Mbcr transcripts using international scale (IS) to monitor drug response of tyrosine kinase treatment, which targets BCR-ABL1 fusion protein. Studies show that patient will have a good prognosis when achieving major molecular response (MMR), defined as a 3-log (1000 fold) reduction in BCR-ABL1 transcripts aligned to IRIS baseline.

Total RNA is isolated and converted to cDNA; ABL1 is used as reference gene to be amplified together with BCR-ABL1 fusion transcripts by real-time RT-PCR. Absolute copy numbers are calculated based on standard curves of each target. Fusion transcripts are further normalized against ABL1 and then converted to (IS) scale using a WHO first reference material (provided by Qiagen, Germantown, MD).

The limit of detection of this assay is 0.0025% IS. The limit of quantification is 0.0032% IS. This assay does not detect transcripts resulting from other BCR-ABL1 fusion transcripts that form p190 or p230. The results of this test must always be interpreted in the context of morphologic and other relevant data and should not be used alone for a diagnosis of malignancy.

ASSAY DISCLAIMERS

The performance characteristics of this test were validated by GoPath Labs or by the lab that performed the test. The U.S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA approval or clearance is currently not required for clinical use of this test. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions. GoPath Laboratory is authorized under Clinical Laboratory Improvement Amendments (CLIA) and by all states to perform high-complexity testing.

REFERENCES

1. Faderl, S., et al., The biology of chronic myeloid leukemia. N Engl J Med, 1999. 341(3): p. 164-72.
2. Rowley, J.D., Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature, 1973. 243(5405): p. 290-3.
3. Suryanarayan, K., et al., Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. Blood, 1991. 77(2): p. 324-30.
4. Advani, A.S. and A.M. Pendergast, Bcr-Abl variants: biological and clinical aspects. Leuk Res, 2002. 26(8): p. 713-20.
5. Hughes, T., et al., Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood, 2006. 108(1): p. 28-37.
6. Cross, N.C., et al., Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia, 2015. 29(5): p. 999-1003.

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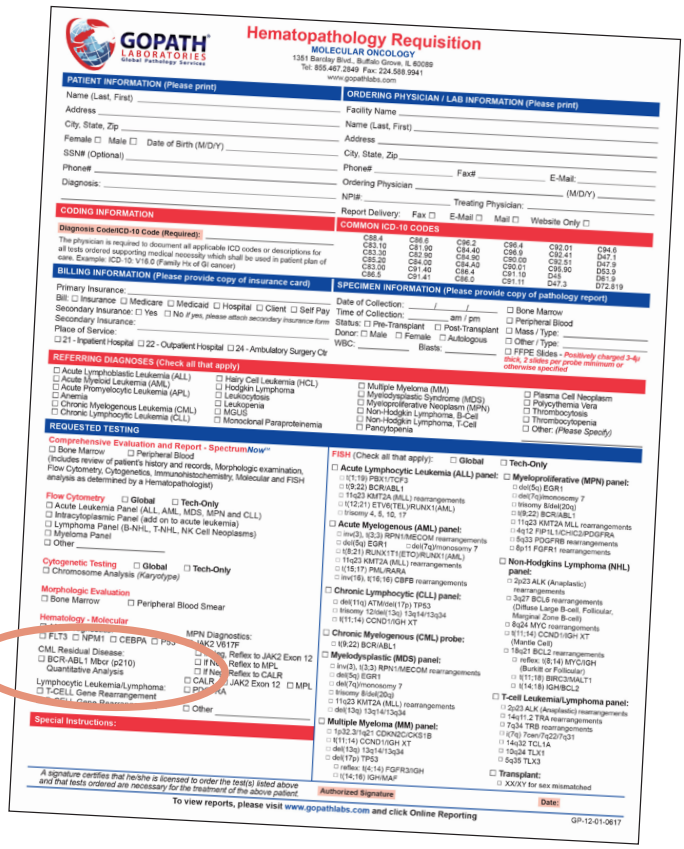

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www.gopathlabs.com

PATIENT INFORMATION (Please print)
Name (Last, First)
Address
City, State, Zip
Female ☐ Male ☐ Date of Birth (M/D/Y)
SSN# (Optional)
Phone#
Diagnosis:

ORDERING PHYSICIAN / LAB INFORMATION (Please print)
Facility Name
Name (Last, First)
Address
City, State, Zip
Phone# Fax# E-Mail:
Ordering Physician (M/D/Y)
NPI# Treating Physician:
Report Delivery: Fax ☐ E-Mail ☐ Mail ☐ Website Only ☐

CODING INFORMATION
Diagnosis Code/ICD-10 Code (Required):
The physician is required to document all applicable ICD codes or descriptions for all tests ordered supporting medical necessity which shall be used in patient plan of care. Example: ICD -10 V16.0 (Family Hx of GI cancer)

COMMON ICD-10 CODES
C88.4 C88.6 C96.2 C96.4 C92.01 C94.6
C83.10 C81.90 C84.40 C96.9 C92.41 D47.1
C83.30 C82.90 C84.90 C90.00 C92.51 D47.9
C85.20 C84.00 C84.00 C90.01 C95.90 D53.9
C83.00 C91.40 C86.4 C91.10 D45 D61.8
C86.5 C91.41 C86.0 C91.11 D47.3 D72.819

BILLING INFORMATION (Please provide copy of insurance card)
Primary Insurance:
Bill: ☐ Insurance ☐ Medicare ☐ Medicaid ☐ Hospital ☐ Client ☐ Self Pay
Secondary Insurance: ☐ Yes ☐ No If yes, please attach secondary insurance form
Secondary Insurance:
Place of Service:
☐ 21 - Inpatient Hospital ☐ 22 - Outpatient Hospital ☐ 24 - Ambulatory Surgery Ctr

SPECIMEN INFORMATION (Please provide copy of pathology report)
Date of Collection: / /
Time of Collection: am / pm
Status: ☐ Pre-Transplant ☐ Post-Transplant
Donor: ☐ Male ☐ Female ☐ Autologous
WBC: Blasts:
☐ Bone Marrow
☐ Peripheral Blood
☐ Mass / Type:
☐ Other / Type:
☐ FFPE Slides - Positively charged 3-4µ thick, 2 slides per probe minimum or otherwise specified

REFERRING DIAGNOSES (Check all that apply)
☐ Acute Lymphoblastic Leukemia (ALL)
☐ Acute Myeloid Leukemia (AML)
☐ Acute Promyelocytic Leukemia (APL)
☐ Anemia
☐ Chronic Myelogenous Leukemia (CML)
☐ Chronic Lymphocytic Leukemia (CLL)
☐ Hairy Cell Leukemia (HCL)
☐ Hodgkin Lymphoma
☐ Leukocytosis
☐ Leukopenia
☐ MGUS
☐ Monoclonal Paraproteinemia
☐ Multiple Myeloma (MM)
☐ Myelodysplastic Syndrome (MDS)
☐ Myeloproliferative Neoplasm (MPN)
☐ Non-Hodgkin Lymphoma, B-Cell
☐ Non-Hodgkin Lymphoma, T-Cell
☐ Pancytopenia
☐ Plasma Cell Neoplasm
☐ Polycythemia Vera
☐ Thrombocytosis
☐ Thrombocytopenia
☐ Other: (Please Specify)

REQUESTED TESTING
Comprehensive Evaluation and Report - SpectrumNow™
☐ Bone Marrow ☐ Peripheral Blood
(Includes review of patient's history and records, Morphologic examination, Flow Cytometry, Cytogenetics, Immunohistochemistry, Molecular and FISH analysis as determined by a Hematopathologist)

Flow Cytometry ☐ Global ☐ Tech-Only
☐ Acute Leukemia Panel (ALL, AML, MDS, MPN and CLL)
☐ Intracytoplasmic Panel (add on to acute leukemia)
☐ Lymphoma Panel (B-NHL, T-NHL, NK Cell Neoplasms)
☐ Myeloma Panel
☐ Other

Cytogenetic Testing ☐ Global ☐ Tech-Only
☐ Chromosome Analysis (Karyotype)

Morphologic Evaluation
☐ Bone Marrow ☐ Peripheral Blood Smear

Hematology - Molecular
☐ FLT3 ☐ NPM1 ☐ CEBPA ☐ P53 MPN Diagnostics: JAK2 V617F
CML Residual Disease:
☐ BCR-ABL1 Mbcr (p210) Quantitative Analysis ☐ If No, Reflex to JAK2 Exon 12 ☐ If No, Reflex to MPL ☐ If No, Reflex to CALR
Lymphocytic Leukemia/Lymphoma: ☐ CALR ☐ JAK2 Exon 12 ☐ MPL ☐ PDGFRA
☐ T-CELL Gene Rearrangement
☐ Other

FISH (Check all that apply): ☐ Global ☐ Tech-Only
☐ Acute Lymphocytic Leukemia (ALL) panel:
☐ t(1;19) PBX1/TCF3
☐ t(9;22) BCR/ABL1
☐ 11q23 KMT2A (MLL) rearrangements
☐ t(12;21) ETV6(TEL)/RUNX1(AML)
☐ t(9;22) BCR/ABL1
☐ Acute Myelogenous (AML) panel:
☐ inv(3), t(3;3) RPN1/MECOM rearrangements
☐ del(5q) EGR1 ☐ del(7q)/monosomy 7
☐ t(8;21) RUNX1T1/(ETO/RUNX1/AML)
☐ 11q23 KMT2A (MLL) rearrangement
☐ t(15;17) PML/RARA
☐ inv(16), t(16;16) CBFB rearrangements
☐ Chronic Lymphocytic (CLL) panel:
☐ del(11q) ATM/del(17p) TP53
☐ trisomy 12/del(13q) 13q14/13q34
☐ t(11;14) CCND1/IGH XT
☐ Chronic Myelogenous (CML) probe:
☐ t(9;22) BCR/ABL1
☐ Myelodysplastic (MDS) panel:
☐ inv(3), t(3;3) RPN1/MECOM rearrangements
☐ del(5q) EGR1
☐ del(7q)/monosomy 7
☐ trisomy 8/del(20q)
☐ 11q23 KMT2A (MLL) rearrangements
☐ del(13q) 13q14/13q34
☐ Multiple Myeloma (MM) panel:
☐ 1p32.3/1q21 CDKN2C/CKS1B
☐ t(11;14) CCND1/IGH XT
☐ del(13q) 13q14/13q34
☐ del(17p) TP53
☐ reflex: t(4;14) FGFR3/IGH
☐ t(14;16) IGH/MAF
☐ Myeloproliferative (MPN) panel:
☐ del(5q) EGR1
☐ del(7q)/monosomy 7
☐ trisomy 8/del(20q)
☐ t(9;22) BCR/ABL1
☐ 11q23 KMT2A MLL rearrangements
☐ 4q12 FIP1L1/CHIC2/PDGFRA
☐ 8p33 PDGFRB rearrangements
☐ 8p11 FGFR1 rearrangements
☐ Non-Hodgkins Lymphoma (NHL) panel:
☐ 2p23 ALK (Anaplastic) rearrangements
☐ 3q27 BCL6 rearrangements (Diffuse Large B-cell, Follicular, Marginal Zone B-cell)
☐ 8q24 MYC rearrangements
☐ t(11;14) CCND1/IGH XT (Mantle Cell)
☐ 18q21 BCL2 rearrangements
☐ reflex: t(8;14) MYC/IGH (Burkitt or Follicular)
☐ t(11;18) BIRC3/MALT1
☐ t(14;18) IGH/BCL2
☐ T-cell Leukemia/Lymphoma panel:
☐ 2p23 ALK (Anaplastic) rearrangements
☐ 14q11.2 TRA rearrangements
☐ 7q34 TRB rearrangements
☐ t(7q) 7cen/7q22/7q31
☐ 14q32 TCL1A
☐ 10q24 TLX1
☐ 5q35 TLX3
☐ Transplant:
☐ XX/XY for sex mismatched

Special Instructions:

A signature certifies that he/she is licensed to order the test(s) listed above and that tests ordered are necessary for the treatment of the above patient

Authorized Signature Date:

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GP-12-01-0617

Samples for Submission

Collect 3-5 mL of whole blood or 1-3 mL of bone marrow and place in EDTA or citrate tubes supplied by GoPath Laboratories. Samples MUST BE RECEIVED WITHIN 48 HOURS of collection due to lability of RNA. Transport specimen using a cool pad to maintain a refrigerated temperature around 4ºC. Do not use frozen blood. All specimens must be labeled with the patient name, which must match the name listed on the requisition form.

References

1. Faderl, S., et al., The biology of chronic myeloid leukemia. N Engl J Med, 1999. 341(3): p. 164-72.
2. Rowley, J.D., Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature, 1973. 243(5405): p. 290-3.
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