

BCR-ABL QUANTITATION SERVICES

TEST DESCRIPTION

Serial analysis of BCR-ABL mRNA levels by real-time quantitative polymerase chain reaction (QRT-PCR) during and/or after therapy (imatinib, dasatinib, nilotinib, or stem cell transplantation) accurately reflects the level of disease suppression and is an effective method for monitoring treatment efficacy. The importance of high quality, reproducible QRT-PCR for measuring BCR-ABL mRNA was recently emphasized by leaders in the CML field¹.

Major Molecular Response (MMR) is an easily interpreted and consequential value that reflects a patient's response to CML treatment. MMR is defined as greater than or equal to 3-log reduction in BCR-ABL/control gene ratio from a standardized median baseline value². In the International Randomized Interferon versus STI571 (IRIS) study, patients with a ratio at or below MMR within 18 months of starting treatment were 100% free from progressing to accelerated phase or blast crisis at 60 months³. MolecularMD employs a MMR reference value that has been validated with participants of the IRIS study.

MolecularMD BCR-ABL quantitation services allows accurate and reliable measurement of the BCR-ABL transcript and allows comparison of BCR-ABL levels to MMR. Quantitation of the transcript reflects the proportion of residual leukemia cells in the patient.

1. Hughes, T. et al., Molecular monitoring of BCR-ABL as a guide to clinical management in chronic myeloid leukaemia. *Blood Reviews* 2006; 20:29-41.
2. Hughes, T. et al., Frequency of Major Molecular Responses to imatinib or interferon alpha plus cytarabine in newly diagnosed chronic myeloid leukemia. *NEJM* 2003; 349:1423-32.
3. Druker, B. et al., Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *NEJM* 2006; 355:2408-17.

DISEASE RELEVANCE:

Chronic Myelogenous Leukemia (CML)

DRUG RELEVANCE:

ABL tyrosine kinase inhibitors such as imatinib (Gleevec®), dasatinib (Sprycel®) and nilotinib (Tasigna®).

TEST SPECIFICATIONS

METHODOLOGY:	Quantitative real-time PCR
CPT CODES:	Please call customer service or refer to MolecularMD website
INTERASSAY VARIABILITY:	Above MMR: less than 2-fold, Below MMR: less than 3-fold
SENSITIVITY:	One tumor cell in 1x10 ⁶ normal cells
LIMIT OF QUANTITATION:	3 BCR-ABL copies
REPORTING:	BCR-ABL/ABL ratio, MMR value, BCR-ABL copy number, ABL copy number, fold change from previous ratio
TURN AROUND TIME:	4-5 business days (MolecularMD accepts Saturday deliveries)

SPECIMEN REQUIREMENTS

SPECIMEN	PERIPHERAL BLOOD		BONE MARROW	
	EDTA	PAXgene	EDTA	PAXgene
SPECIMEN TUBE	EDTA	PAXgene	EDTA	PAXgene
VOLUME	10 mL	5-10 mL	2 mL	1-2 mL
STORAGE	R.T.*	R.T.**	R.T.	R.T.
STABILITY	48 hrs	72 hrs	48 hrs	72 hrs
SHIPPING	Ambient	Ambient	Ambient	Ambient
OTHER	DO NOT FREEZE	2 tubes	DO NOT FREEZE	2 tubes

* R.T. = Room Temperature

** If PAXgene tubes are expected to arrive at MolecularMD after 72 hours of blood draw, FREEZE at -20°C, then -70°C/80°C and ship on dry ice.



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PHYSICIAN / FACILITY / CLIENT INFORMATION
PHYSICIAN: Test physician
CONTACT PERSON: Test physician

SAMPLE REPORT

PATIENT INFORMATION
NAME/INITIALS: Test, Test
ID #: 001MMMD
DOB: 03/06/1947
AGE: 60 SEX: F

LOCATION: Test location
CONTACT PHONE #: Contact phone

SPECIMEN INFORMATION
SPECIMEN ID #: MMD2007-000001

RECEIVED DATE: 03/06/2007 TIME 9:30am
REPORT DATE: 03/12/2007 TIME 10:00am

TEST INFORMATION

BCR-ABL FOLD CHANGE: 10
From last analysis: 400
From lowest level: 400

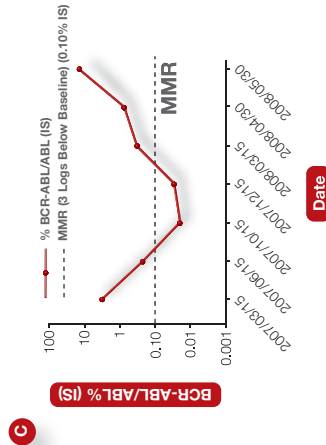
METHOD: ORT-PCR
SPECIMEN: Peripheral Blood
SAMPLE QUALITY: Good

A TEST 1 RESULTS: BCR-ABL (IS%): 16.19%
MUTATION 1: T3151

A TEST 2 RESULTS: BCR-ABL (raw): 4987
MUTATION 2: N/A

B INTERPRETATION

MMR: NO
BCR-ABL/ABL% >= 5 fold increase above MMR: YES
This greater than 5-fold increase in BCR-ABL/ABL ratio compared to the previous value could indicate resistance to therapy. Recommend repeat sample and/or mutation analysis if clinically indicated.



A TEST RESULTS: MolecularMD reports BCR-ABL RNA blood level as a ratio of BCR-ABL expression to an ABL gene based on international scale.

B INTERPRETATION: This figure indicates the patient's relationship to Major Molecular Response, a clear prognostic reference point. A greater than 5-fold increase is also indicated which according to the NCCN guidelines would require monitoring once a month.

C CUMULATIVE GRAPH: Percent BCR-ABL/ABL is plotted versus sample collection date on graph. The trend reflects the patient's response or lack thereof to therapy.

D HISTORY TABLE: MolecularMD also provides the raw data for up to seven previous tests

D CUMULATIVE PATIENT TESTING RESULTS: HISTORY TABLE003NOV

Specimen Number	Collection Date	MMR Status	BCR-ABL/ABL % Increase Above MMR	Fold Change From Last Analysis	Fold Change From Lowest Level
MMD2007-000003	2007-03-15				
MMD2007-000004	2007-06-15				
MMD2007-000005	2007-10-15				
MMD2007-000006	2007-12-15				
MMD2008-000001	2008-03-15				
MMD2008-000002	2008-04-30				
MMD2008-000003	2008-05-30	Loss (confirmed)			

Specimen Number	Collection Date	BCR-ABL/ABL (IS)	BCR-ABL/ABL (raw)	ABL Copies	BCR-ABL Copies	Mutation 1	Mutation 2
NOV2007-000003	2007-03-15	8.1%	10.00%	25357	2535		
MMD2007-000004	2007-06-15	0.405%	0.50%	35900	179		
MMD2007-000005	2007-10-15	0.04%	0.05%	28729	14		
MMD2007-000006	2007-12-15	0.07%	0.09%	18952	17		
MMD2008-000001	2008-03-15	0.81%	1.00%	3254	325		
MMD2008-000002	2008-04-30	1.62%	2.00%	19800	396		
MMD2008-000003	2008-05-30	16.19%	19.99%	4987	396	T3151	

TABLE KEY:

Specimen Number = Accession number for specimen set
Collection Date = Specimen collection date
MMR Status = report describing the maintenance or loss (confirmed or unconfirmed) of MMR
BCR/ABL (IS) International Scale = BCR-ABL/ABL value multiplied by the correction factor (0.81)
BCR-ABL/ABL (raw) = BCR-ABL Copies divided by ABL Copies
ABL = ABL Copies
BCR-ABL = BCR-ABL Copies
Mutation 1, 2, 3 = Identification of mutations found through Sequencing
ID = Unique Patient ID #

TEST DESCRIPTION:

BCR-ABL QRT-PCR Test:
The BCR-ABL QRT-PCR test quantitatively measures the RNA blood level of BCR-ABL, a marker for the presence and amount of transcriptionally active Philadelphia chromosome positive leukemia cells. For this test total RNA from whole leukocytes is reverse transcribed with random primers and the cDNA product is quantitated by fluorescent real-time QRT-PCR. The BCR-ABL QRT-PCR is a multiplex reaction containing primers from bcr exons 12 & 13 and abl exon 2, such that the major (p210) translocation breakpoint is detected. ABL cDNA copy numbers are used to control for the quality and quantity of sample RNA. Therefore, the result of this test is expressed in % as a fraction of BCR-ABL expression to that of the control gene ABL (% BCR-ABL/ABL).
Major Molecular Response (MMR) value at MolecularMD is designated 0.12%, which is equivalent to a 3-log reduction from a standardized baseline value of 1.0% IS. The MMR value at MolecularMD is determined versus 51071 (PIS) study or 0.1% International Scale (IS). A consecutive 4-log of IS is applied to all MolecularMD BCR-ABL/ABL ratios to obtain the International Scale value and MMR is reported when a patient shows a BCR-ABL/ABL % less than or equal to 0.1% IS.
Based on the limit of detection of this test and an ABL copy number greater than or equal to 25,614, a negative result indicates a greater than or equal to 4.5-log reduction of BCR-ABL copy number from a standardized baseline value on the international scale (defined as 3 logs above MMR).

BCR-ABL Mutational Test:

For this assay, the total RNA from whole blood leukocytes is reverse transcribed with random primers and the cDNA product is amplified with BCR-ABL-specific primer sets. The PCR product is then sequenced with ABL-specific primers. The assay detects mutations in the ABL kinase domain and additional ABL kinase domains of the BCR-ABL fusion protein, between amino-acids 30 and 510. The limit of detection of the assay is 25-40% depending on the mutation.

Electronically Signed By:

Courtney Fuller
Scientist
Chad Galders DO FCAP
Pathologist
(Case signed 03/12/2007)
(Case Reviewed 03/12/2007)

This test was developed and its performance characteristics determined by the laboratory. It has not been cleared or approved by the FDA. This test is used for clinical purposes. It should not be regarded as investigational or for research. The laboratory is registered under CLIA of 1988.

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