

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 3-fold, Below MMR: less than 5-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.

PHYSICIAN / FACILITY / CLIENT INFORMATION

 PHYSICIAN: Test MD, MD
 CONTACT PERSON: Test MD, MD

STUDY: Test

 LOCATION: test location
 CONTACT PHONE

CASE: MMD2010-631825
PATIENT INFORMATION

 NAME/INITIALS: Test, 011510
 ID #: 011510
 DOB: 03/15/1978
 AGE: 31 SEX: F

SPECIMEN INFORMATION

 SPECIMEN ID #: 631825
 COLLECTION DATE: 10/15/2009 TIME 10:00
 RECEIVED DATE: 01/15/2010 TIME 9:30
 REPORT DATE: 01/27/2010

TEST INFORMATION

FFS BCR-ABL PCR Results

TEST:	METHOD:	SPECIMEN:	SAMPLE QUALITY:
BCR-ABL QUANT.	QRT-PCR	Peripheral Blood	Good

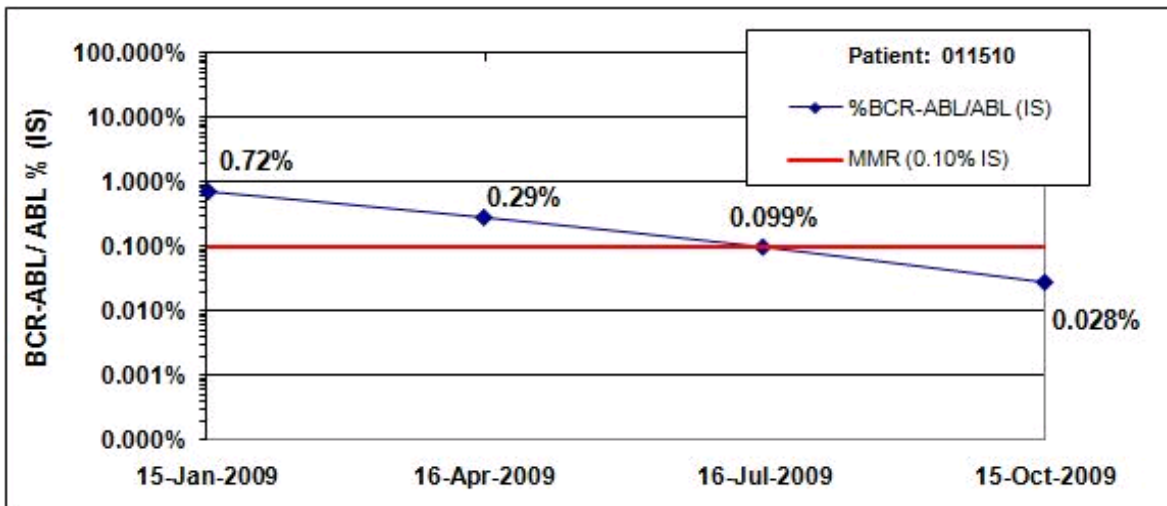
TEST RESULTS:	BCR-ABL/ABL (raw%):	ABL Copies	BCR-ABL Copies
	0.00034 (0.034%)	34785	12

INTERPRETATION:

The ratio of BCR-ABL/ABL is 0.00028 (0.028 %) IS.

MMR: YES

The ABL copy number indicates that the quality of the RNA was adequate to accurately report this result.

CUMULATIVE RESULTS: QRT-PCR TREND


011510

Specimen #	Collection Date	MMR	BCR-ABL/ABL % ≥ 5 fold increase from lowest	Fold Change from last analysis	Fold Change from lowest level
MMD2010-631822	15-Jan-2009	No	No	NA	25.61
MMD2010-631823	15-Apr-2009	No	No	-2.51	10.20
MMD2010-631824	15-Jul-2009	Yes	No	-2.89	3.529
MMD2010-631825	15-Oct-2009	Yes	No	-3.52	0

Specimen #	Collection Date	%BCR-ABL/ABL (IS)	%BCR-ABL/ABL (raw)	ABL Copies	BCR-ABL Copies
MMD2010-631822	15-Jan-2009	0.716%	0.884%	28975	256
MMD2010-631823	15-Apr-2009	0.285%	0.352%	34098	120
MMD2010-631824	15-Jul-2009	0.099%	0.122%	45987	56
MMD2010-631825	15-Oct-2009	0.028%	0.034%	34785	12

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

TEST DESCRIPTION(S):

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 b2 & b3 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 from the International Randomized Interferon versus STI571 (IRIS) study or 0.1% per International Scale (IS). A correction factor of 0.81 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. This conversion factor is validated for BCR-ABL/ABL ratios of 10% and below.

Electronically Signed By:

Test PQTester3
Pathologist
(Case Reviewed 01/27/2010)

Courtney Fuller
Director, Clinical Operations
(Case signed 01/27/2010)

This test was developed and its performance characteristics determined by this 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 regulated under CLIA of 1988. *False positive or negative results may occur for reasons that include genetic variants, blood transfusions, or somatic heterogeneity of the tissue sample. 1341 SW Custer Drive, Portland, OR 97219 T: 503-459-4974 F: 503-459-4976 www.molecularmd.com