

BCR-ABL KINASE DOMAIN SEQUENCING SERVICES

TEST DESCRIPTION

MolecularMD has developed a sensitive and specific direct sequencing assay for screening of mutations in BCR-ABL. Our assay detects mutations between amino acids 30 and 510 including the kinase domain SH3, SH2 and regions 3' to the ABL kinase domain.

Over 40 amino acid substitutions in the tyrosine kinase domain of BCR-ABL can be detected by MolecularMD's sequencing assay. These substitutions have been identified in CML patients who develop resistance to tyrosine kinase inhibitors and related treatments.

Direct sequencing is a non-biased approach that has a sensitivity of 20-30% in the identification of BCR-ABL mutant clones.

DISEASE RELEVANCE:

Chronic Myelogenous Leukemia (CML)

DRUG RELEVANCE:

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

TEST SPECIFICATIONS

METHODOLOGY:	Direct Sequencing
CPT CODES:	Please call Customer Service or refer to the MolecularMD website.
LIMIT OF DETECTION:	20-30% of the mutant allele among non-mutated alleles
CONTROLS:	BCR-ABL WT and various mutants covering the entire kinase domain
REPORTING:	Amino acid change in ABL kinase domain
TURN AROUND TIME:	8 business days, 4 business days if RNA is available (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.

SAMPLE REPORT

PHYSICIAN / FACILITY / CLIENT INFORMATION

PHYSICIAN: Test physician

LOCATION: Test location

CONTACT PERSON: Test physician

CONTACT PHONE #: Contact phone

PATIENT INFORMATION

NAME/INITIALS: Test, Test

ID #: 001MMD

DOB: 03/06/1947

AGE: 60 SEX: F

SPECIMEN INFORMATION

SPECIMEN ID #: MMD2007-000001

SPECIMEN DATE: 03/06/2007 TIME 9:30am

RECEIVED DATE: 03/07/2007 TIME 10:00am

REPORT DATE: 03/12/2007

TEST INFORMATION

BCR-ABL FOLD CHANGE: From last analysis: From lowest level:
 N/A N/A

TEST: METHOD: SPECIMEN: SAMPLE QUALITY:
 1. Mutation Analysis Sequencing Peripheral Blood Good

TEST 1 RESULTS: MUTATION 1: MUTATION 2:
 E255V N/A

INTERPRETATION

The E255V mutation was detected.

CUMULATIVE PATIENT TESTING RESULTS: HISTORY TABLE003NOV

Specimen Number	Collection Date	BCR-ABL/ABL (IS)	BCR-ABL/ABL (raw)	ABL Copies	BCR-ABL Copies	Mutation 1	Mutation 2
MMD2007-000003	2007-03-15	8.1%	10.00%	25357	2535		
MMD2007-000004	2007-06-15	0.405%	0.50%	35800	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	N/A	N/A	N/A	N/A	E255V	

TABLE KEY:

Specimen Number = Accession number for specimen set
 Collection Date = Specimen collection date
 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 or FRET
 ID = Unique Patient ID #

TEST DESCRIPTION:

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 Galderisi DO FCAP
 Pathologist

(Case signed 03/12/2007)

(Case Reviewed 03/12/2007)

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.