Saladax BIOMEDICAL
DIAGNOSTICS THAT MAKE A DIFFERENCE

REF PI IM-RGT

MyImatinib™

MyImatinib™ Assay

This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service:

Telephone: +1-610 419-6731
Fax: +1-484-547-0590

Key to Symbols Used

IVD	*in vitro* Diagnostic Device		Consult Instructions for Use
LOT	Batch Code (Lot)		Temperature Limitation
REF	Catalog Number		Use By
	Caution: Consult accompanying documents	EC REP	Authorized Representative in the European Community
R1	Reagent 1		Manufacturer
R2	Reagent 2		

EC REP
EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Saladax Biomedical, Inc.
116 Research Drive
Bethlehem, PA 18015
USA

CE

English 1
Printed in USA
PI IM-RGT, Rev 03

Intended Use

The Saladax MyImatinib assay is an *in vitro* diagnostic medical device intended for the quantitative determination of imatinib in human plasma using automated clinical chemistry analyzers as an aid in the management of imatinib therapy.

Summary and Explanation of the Test

Imatinib mesylate (Gleevec®, Glivec®) is a potent inhibitor of BCR-ABL tyrosine kinase and is widely used to treat chronic myelogenous leukemia (CML) and gastro-intestinal stromal tumors (GIST)[1,2]. The drug, available in 100 and 400 mg tablets, is taken daily or twice daily by mouth at standard doses of 400 or 600 mg/day. This drug has demonstrated significant clinical efficacy in both CML and GIST, producing durable responses and prolonged survival and has become the standard of care in the treatment of these diseases. Despite the impressive efficacy, suboptimal response and treatment failure have been reported.

Factors affecting efficacy include PK-related factors effecting exposure, biological factors such as the presence or later emergence of BCR-ABL mutations, other genetic mutations, and clinical features such as the disease state[3,4].

A number of studies[5-7] have demonstrated that imatinib exhibits a wide range of variation among patients, and that this variability has been correlated with lack of efficacy and resistance to the drug. This interpatient variability of trough levels can be as high as 16 fold and the coefficient of variation (CV) is greater than 50%[6-8]. There are a number of factors that affect PK exposure of imatinib. These include patient adherence, demographic factors, absorption from the gastrointestinal tract, variability in CYP enzyme activity, genetic polymorphisms and drug-drug interactions[8-17].

The PK parameter that is most associated with the biological effect of imatinib is trough concentration, where a sample is taken just before the next dose of the drug, after the drug has achieved steady state concentration[8]. Steady state is achieved when patients have had uninterrupted imatinib therapy for at least 29 days and the imatinib dose or treatment regimen has remained the same for at least 8 days[6,8,18].

The major toxicities observed during imatinib treatment are fluid retention, rash, myalgia, and anemia. These are more common in patients with higher imatinib trough concentrations, but have not been statistically correlated[3]. Less frequent adverse events include fatigue, abdominal and joint pain, and neutropenia.

Data from several studies[4,6,7,19,20] in CML and one study from GIST[5] have shown a correlation between trough concentrations of imatinib and clinical response, leading to guidelines with recommended therapeutic target concentrations of greater than 1,000 ng/mL for CML and greater than 1,100 ng/mL for GIST[21]. In a randomized clinical trial, CML patients who received personalized dose adjustment to obtain plasma concentratons of at least 1,000 ng/mL had a higher rate of major molecular response at 12 months than patients on standard therapy[22].

Principles of the Procedure

The MyImatinib assay (US patent No. 8,273,860) is a homogeneous two-reagent nanoparticle agglutination immunoassay used to measure the concentration of imatinib in human plasma. It is based on the principle of measuring changes in scattered light, or apparent absorbance, which result when antibody coated nanoparticles aggregate. This aggregation is measured at wavelengths between 400 and 650 nm using automated clinical chemistry analyzers. In this technology, multivalent drug-conjugates serve as binding partners to antibodies selective for imatinib, which are covalently attached to the surface of nanoparticles. In the absence of free imatinib, this reaction creates large aggregates, resulting in a solution that scatters incident light and leads to an increase in the observed absorption of the solution. When a sample containing imatinib is introduced, the aggregation reaction is partially inhibited. Antibody bound to drug in the sample is no longer available to promote nanoparticle aggregation, resulting in less scattering of incident light and lower observed absorption of the solution. This results in an inhibition curve with increasing imatinib concentration in that no drug in the sample results in maximum absorbance and high drug levels in the sample result in a minimal amount of absorbance. Monitoring the changes in scattered light or absorbance as a function of drug levels results in a concentration-dependent curve.

Reagents

MyImatinib Assay (REF IM-RGT) includes:

1. **Reaction Buffer** R1 **:** Contains agglutination enhancers, protein, and buffer.
2. **Nanoparticle Reagent** R2 **:** Contains monoclonal antibody bound to nanoparticles in a buffered solution.

Additional Materials Required But Not Provided:

REF IM-CAL – MyImatinib Calibrator Kit

REF IM-CON – MyImatinib Control Kit

Precautions and Warnings

For *In Vitro* Diagnostic Use Only.

Exercise the normal precautions required for handling all laboratory reagents.

All components of the MyImatinib Assay contain less than 0.1% sodium azide. For specific listing, refer to the reagent section of this package insert. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Seek immediate medical attention if reagents are ingested or come into contact with eyes. When disposing of such reagents, always flush with large amounts of water to prevent accumulation of azide.

Handling and Storage Instructions

Store reagents, calibrators and controls refrigerated at 2-8°C (35-46°F). Do not freeze.

Before use, mix the Nanoparticle Reagent (R2) by gently inverting the R2 reagent vial three to five times, avoiding the formation of bubbles.

Indications of Stability

Reagents are stable until the expiration date when stored and handled as directed.

Sample Collection and Handling

Use K_2EDTA plasma specimens when measuring imatinib concentrations with the MyImatinib Assay. Only draw specimens from patients who have had uninterrupted imatinib therapy for **at least** 29 days and have not had a change in imatinib dose or treatment regimen for **at least** 8 days. On the day of collection, draw the specimen **before** the next dose is taken. For once-daily dosing: draw the specimen at least 21 hours from the last dose, but before the next dose. For twice-daily dosing: draw the specimen at least 9 hours from the last dose, but before the next dose. When collecting a specimen, fill the tube until the vacuum stops drawing blood to ensure that testing is not performed using a "short draw" specimen. Do not collect specimens into tubes containing a plasma separator gel.

Once a blood specimen is drawn, process the specimen to plasma within 48 hours of collection. The whole blood may be stored at 2-8°C or room temperature before centrifugation. To process the specimen into plasma, centrifuge the whole blood for a minimum of 10 minutes to separate the plasma from cells. Carefully draw off the plasma starting from the top of the plasma layer, avoiding the cell layer

(contamination of plasma with blood cells may interfere with results), transfer the plasma to a secondary tube, and cap. Test plasma within 15 days of collection or store at ≤ -20°C. Imatinib in plasma is stable for up to 15 days at 2-8°C and room temperature.

Assay Procedure

Refer to the instrument specific Application Sheet for instructions and parameters before performing the assay.

Specimen Dilution Procedure

Specimens containing imatinib concentrations greater than 3,000 ng/mL imatinib can be diluted up to 1:2 to yield an assay clinical reportable range up to 9,000 ng/mL. Specimens containing greater than 3,000 ng/mL imatinib can be manually diluted up to 1:2 with deionized H_2O prior to testing. Specimens containing greater than 3,000 ng/mL imatinib may also be diluted using the auto-dilution function on the analyzer.

Calibration

The MyImatinib Assay produces a calibration curve spanning 0 to 3,000 ng/mL using the MyImatinib Calibrator Kit. The minimum detectable concentration of imatinib in plasma for the MyImatinib Assay is 154 ng/mL.

Verify the assay calibration by testing MyImatinib Controls.

Calibration Frequency

Calibration is recommended:

- After a calibrator or reagent lot change,
- After performing major instrument maintenance or repairs,
- As required by each laboratory's quality control procedures.

Quality Control

The MyImatinib Control Kit contains three levels of controls with imatinib concentrations at 750, 1,500 and 2,500 ng/mL.

Each laboratory should establish its own control ranges and frequency. Good laboratory practice suggests that at least two control levels be tested each day patient

samples are assayed and each time a calibration is performed. Reassess control targets and ranges following a change of reagent, calibrator, or control lot.

Results and Expected Values

The analyzer software calculates a best fit non-linear curve equation that is used to generate a calibration curve that ranges from 0 to 3,000 ng/mL of imatinib. Concentrations of imatinib in the unknown samples are calculated from this calibration curve using absorbance values generated for each sample.

Limitations of the Procedure

As with all analyte determinations, the MyImatinib Assay should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Performance characteristics for the MyImatinib Assay have not been established for body fluids other than K_2EDTA human plasma.

No significant interferences were observed from samples with the following conditions:

	Level
Rheumatoid Factor	500 IU/mL
Human Serum Albumin	12 g/dL
Human IgG	12 g/dL
Icteric Interference	30 mg/dL
Lipemic Interference	593 mg/dL
Hemolysate	1,000 mg/dL

Imatinib is stable in whole blood for 4 hours at ambient room temperature or 24 hours at 2-8°C / on wet ice.

As with any assay utilizing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Samples containing such antibodies can potentially produce erroneous results, which are inconsistent with the patient's clinical profile. If HAMA interference is suspected, please contact Saladax Technical Service for assistance.

Expected Values

The target minimum trough concentration of imatinib in plasma is 1,000 ng/mL for CML patients and 1,100 ng/mL for GIST patients. Specimens are to be taken at a timepoint near, but prior to ingesting the next dose in order to ensure that the

imatinib plasma trough concentration is measured. Specimens must be collected **at least** 29 days after imatinib treatment begins and **at least** 8 days after a change in imatinib dose or treatment regimen for steady state to be reached[6,8,18].

The patient's current and past medical condition, the complexity of the clinical state, individual differences in sensitivity to the compound, co-administration with other treatment drugs, time of administration, and a number of other factors may cause different requirements for optimal blood concentrations of imatinib. Individual imatinib concentrations should not be used as the sole criteria for changing the treatment regimen. Each patient should undergo comprehensive clinical assessment prior to modification of the treatment plan. Given the heterogeneity of the patient's clinical state, clinicians should establish a desired therapeutic management range based upon their own experience as well as each patient's clinical requirements.

Specific Performance Characteristics

Typical performance data for the MyImatinib Assay obtained on a Beckman Coulter AU480™ are shown below. Results obtained in individual laboratories may differ from these data.

Precision

Precision was determined as described in CLSI Guideline EP5-A2.

A normal plasma pool spiked at 4 imatinib concentrations, and three levels of MyImatinib controls, were tested up to 23 days to determine the repeatability and within-laboratory precision of the assay. The complete results for the condition that resulted in the maximum within-laboratory CV (for all the reagent/calibrator lots and analyzers evaluated) are found in the table below.

Sample Type	Assigned Value (ng/mL)	N	Mean (ng/mL)	Repeatability (%CV)	Within-Laboratory Precision (%CV)
Controls	750	92	762	3	4
	1,500	92	1,468	3	4
	2,500	92	2,548	2	3
Human Plasma	350	92	363	6	7
	900	92	946	3	4
	1,600	92	1,665	3	4
	2,700	88	2,649	3	3

Lower Limit of Quantitation (LLoQ)

LLoQ was determined as described in CLSI Guideline EP17-A.

The LLoQ of an assay is the lowest analyte concentration that can be measured with acceptable accuracy and precision. For the MyImatinib assay, the LLoQ was defined as the lowest imatinib concentration at which the total analytical error was ≤ 35%. The LLoQ was determined to be 296 ng/mL.

Limit of Detection (LoD)

LoD was determined as described in CLSI Guideline EP17-A.

The LoD of an assay is the lowest analyte concentration that can be detected with a stated confidence, but not neccesarily measured quantitatively. For the MyImatinib assay, the LoD was defined as the lowest imatinib concentration at which 95% of the results exceeded the assay Limit Of Blank (LoB), where the assay LoB was the 95th percentile of the results generated from the analysis of specimens that did not contain imatinib. The LoD was determined to be 154 ng/mL.

Specificity

The specificity of an assay is the degree to which the assay detects only a specific substance and does not detect other substances. Percent cross-reactivity was determined for an imatinib metabolite, a related tyrosine kinase inhibitor, and each of a series of common co-administered drugs.

<u>Imatinib metabolites and Related Compounds</u>

N-desmethyl imatinib (an imatinib metabolite) and nilotinib (a related tyrosine kinase inhibitor) were individually added to a normal plasma pool spiked with imatinib at 1,100 ng/mL and tested using the MyImatinib assay. The percent cross-reactivities determined for each compound are given below.

Compound	Spike concentration (ng/mL)	% Cross-Reactivity
N-desmethyl imatinib	100,000	0.2
Nilotinib	100,000	0.1

Common Co-Administered Drugs

Unless otherwise indicated, 100,000 ng/mL of each compound was spiked into imatinib free plasma or plasma spiked with 1,100 ng/mL of imatinib. The cross-reactivities of all compounds evaluated were ≤ 0.1%.

(+)-γ-Tocopherol	Ethacrynic acid	Oxazepam
5-fluorouracil	Fentanyl	Paclitaxel
8-chlorotheophylline	Fexofenadine HCl	Pantothenic acid
Acetaminophen*	Fluconazole	Pimozide
Acetazolamide	Fluticasone propionate	Prednisolone
Acetylsalicylic acid*	Folic acid	Prednisone
Alprazolam	Furosemide	Pyridoxine HCl
Amikacin sulfate salt	Gemcitabine HCl*	Quinidine
Amiloride HCl	Gentamycin sulfate	R,R(-)-pseudoephedrine
Amlodipine besylate	Heparin Sodium salt	Retinol
Atorvastatin calcium	Hydrochlorothiazide	Riboflavin
Betamethasone	Ibuprofen*	Risperidone
Biotin	Indinavir sulfate	Rosuvastatin calcium
Budesonide	Irinotecan HCl	S,S(+)-pseudoephedrine
Caffeine	Itraconazole	Salicylic acid*
Calcium carbonate*	Kanamycin sulfate	Simvastatin
Ceftriaxone sodium*	L-ascorbic acid	Sirolimus
Cetirizine dihydrochloride	Lisinopril dihydrate	Sodium fluoride
Chlorpromazine HCl	Lorazepam	Spironolactone
Clarithromycin	Lovastatin	Streptomycin sulfate
Clonidine HCl	Meclizine dihydrochloride monohydrate	Tacrolimus
Clotrimazole	Metformin	Theophylline
Codeine	Metoclopramide HCl	Thiamine HCl
Cortisol	Metoprolol tartrate	Triamcinolone acetonide
(-)-Cotinine	Mirtazapine	Triamterene
Cyclophosphamide*	Mometasone furoate	Triazolam
Cyclosporin A	Naproxen sodium*	Valproic acid, sodium salt*
Desloratadine	Nateglinide	Vancomycin HCl
Diazepam	Nefazodone HCl	Vitamin B12
Diphenhydramine HCl	(-)-Nicotine Tartrate	Vitamin D2
Docetaxel	Nicotinic acid	Vitamin K1
Doxorubicin HCl	Nordiazepam	Zolpidem hemitartrate
Doxycycline HCl	Omeprazole	Zopiclon
K_2EDTA		

*1,000,000 ng/mL

Recovery

Assay recovery is the degree to which an analyte measurement yielded by an assay agrees with the true analyte concentration in the specimen. For the MyImatinib assay, recovery was defined as the percent recovery of imatinib in normal plasma specimens spiked with imatinib relative to the theoretical imatinib concentration.

To assess recovery, imatinib was spiked into 5 normal plasmas at 4 concentrations: 350, 1,000, 1,600, and 2,700 ng/mL. The greatest absolute mean percent deviations at each spike level among the reagent/calibrator lots and analyzers evaluated ranged from 11 to 18%.

Linearity

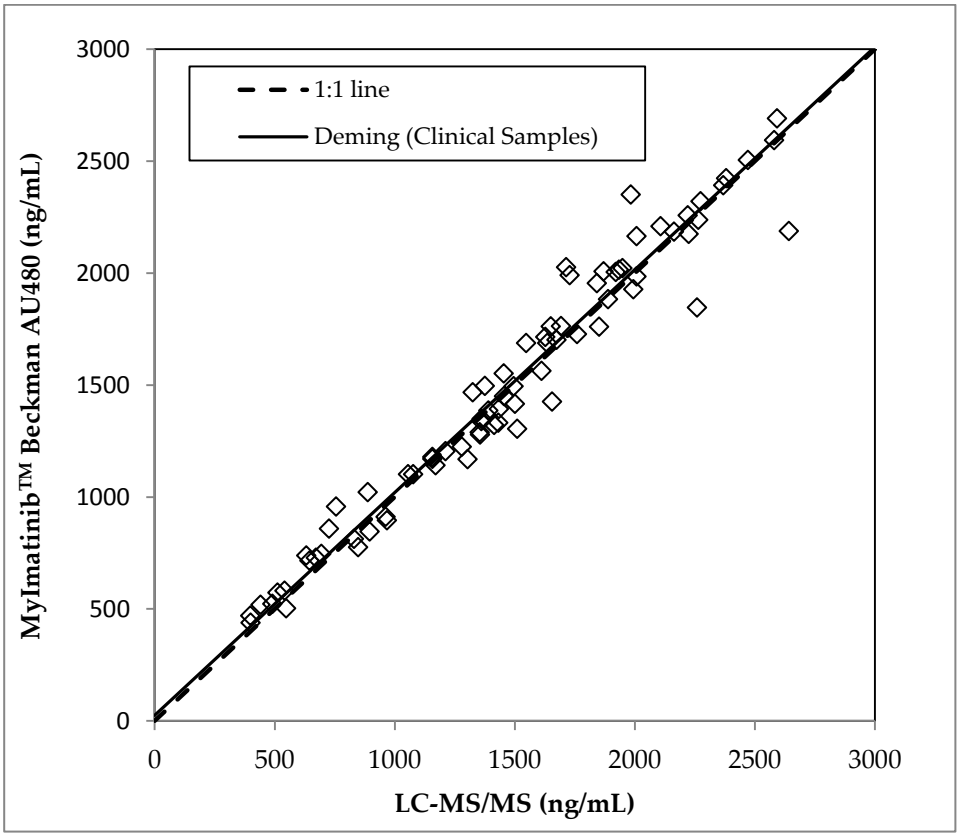
Assay linearity was determined as described in CLSI Guideline EP6-A.

Assay linearity is the degree to which a plot of assay results vs. the known concentrations of analyte conforms to a straight line. According to CLSI EP6-A, an appropriate measure of linearity is the deviation of a polynomial fit of the data from a linear fit. For the MyImatinib assay, the linear range was defined as the range of imatinib concentrations that yielded mean percent recoveries (vs. theoretical) ranging from 80 to 120% and percent deviations of the polynomial fit from the linear fit that were ≤ 15%.

To determine assay linearity, 11 samples with theoretical imatinib concentrations ranging from 251 to 3,316 ng/mL were prepared according to CLSI EP6-A. The theoretical imatinib concentrations that yielded acceptable mean percent recoveries and deviations from the linear fit among the reagent/calibrator lots and analyzers evaluated ranged from 301 to 3,316 ng/mL.

Method Comparison

A comparison between the MyImatinib assay and a validated LC-MS/MS[23] reference method was performed using 77 human plasma samples obtained from patients receiving imatinib therapy. The range of imatinib concentrations measured by the MyImatinib assay was 438 – 2,691 ng/mL with a mean of 1,492 ng/mL. The range of concentrations measured by LC-MS/MS method was 399 – 2,643 ng/mL with a mean of 1,475 ng/mL. Results of the Deming regression analysis are below.



Package Insert References:

1. Druker BJ, Sawyers CL, Kantarjian H, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001;344(14):1031-37.
2. Savage DG, Antman KH. Imatinib mesylate--a new oral targeted therapy. *N Engl J Med*, 2002;346(9):683-93.
3. Cortes JE, Egorin MJ, Guilhot F, Molimard M, et al. Pharmacokinetic/pharmaco-dynamic correlation and blood-level testing in imatinib therapy for chronic myeloid leukemia. *Leukemia*. 2009;23(9):1537-44.
4. Takahashi N, Miura M. Therapeutic Drug Monitoring of Imatinib for Chronic Myeloid Leukemia Patients in the Chronic Phase. *Pharmacology*. 2011;87(5-6): 241-48.
5. Demetri GD, Wang Y, Wehrle E, et al. Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors. *J Clin Oncol*. 2009;27(19):3141-47.
6. Larson RA, Druker BJ, Guilhot F, et al. Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. *Blood*. 2008;111(8):4022-28.
7. Picard S, Titier K, Etienne G, et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood*. 2007;109(8):3496-99.
8. Peng B, Hayes M, Resta D, et al. Pharmacokinetics and pharmacodynamics of imatinib in a phase I trial with chronic myeloid leukemia patients. *J Clin Oncol*. 2004;22(5):935-42.
9. Beumer JH, Natale JJ, Lagattuta TF, et al. Disposition of imatinib and its metabolite CGP74588 in a patient with chronic myelogenous leukemia and short-bowel syndrome. *Pharmacotherapy*. 2006;26(7):903-7.
10. Levine AM, Richardson JL, Marks G, et al. Compliance with oral drug therapy in patients with hematologic malignancy. *J Clin Oncol*. 1987;5(9):1469-76.
11. Marin D, Bazeos A, Mahon FX, et al. Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. *J Clin Oncol*. 2010;28(14):2381-8.
12. Wilkinson GR. Cytochrome P4503A (CYP3A) metabolism: prediction of in vivo activity in humans. *J Pharmacokinet Biopharm*. 1996;24(5):475-90.
13. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. *Clin Pharmacokinet*. 2005;44(9):879-94.
14. Tsang J, Rudychev I, Pescatore SL. Prescription compliance and persistency in chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor (GIST) patients (pts) on imatinib (IM) [abstract]. *J Clin Oncol*. 2006;24(18)(supp l):6119.
15. Ibrahim AR, Eliasson L, Apperley JF, et al. Poor adherence is the main reason for loss of CCyR and imatinib failure for chronic myeloid leukemia patients on long-term therapy *Blood*. 2011;117:3733-36.
16. Yamakawa Y, Hamada A, Nakashima R, et al. Association of genetic polymorphisms in the influx transporter SLCO1B3 and the efflux transporter ABCB1 with imatinib

pharmacokinetics in patients with chronic myeloid leukemia *Ther Drug Monit*. 2011;33:244-50
17. Jabbour E, Branford S, Saglio G, et al. Practical advice for determining the role of BCR-ABL mutations in guiding tyrosine kinase inhibitor therapy in patients with chronic myeloid leukemia *Cancer.* 2011;117:1800-11.
18. le Coutre P, Kreuzer KA, Pursche S, et al. Pharmacokinetics and cellular uptake of imatinib and its main metabolite CGP74588. *Cancer Chemother Pharmacol*. 2004;53(4):313-23.
19. Bouchet S, Titier K, Moore N, et al. Therapeutic drug monitoring of imatinib in chronic myeloid leukemia: experience from 1216 patients at a centralized laboratory Fundam Clin Pharmacol. 2013;27:690-97.
20. Guilhot F, Hughes TP, Cortes J, et al. Plasma exposure of imatinib and its correlation with clinical response in the Tyrosine Kinase Inhibitor Optimization and Selectivity Trial *Haematologica*. 2012;97:731-738.
21. Yu H, Steeghs N, Nijenhuis CM, et al. Practical Guidelines for Therapeutic Drug Monitoring of Anticancer Tyrosine Kinase Inhibitors: Focus on the Pharmacokinetic Targets. *Clin Pharmacokinet*. 2014;53:305-25.
22. Rousselot P, Johnson-Ansah H, Huguet F, et al. Personalized Daliy Doses of Imatinib By Therapeutic Drug Monitoring Increase teh Rates of Molecular Responses in Patients with Chronic Myeloid Leukemia. Final Results of teh Randomized OPTIM Imatinib Study *Blood*. 2015;126.
23. Beumer JH, Kozo D, Harney RL, et al. An Automated Homogeneous Immunoassay for Quantitating Imatinib Concentrations in Plasma. *Ther Drug Monit.* 2014;37(4)486-92.