Evaluation of an Automated Immunoassay for Quantification of Imatinib in Plasma

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Introduction

Therapeutic drug monitoring (TDM) of imatinib is an important tool for evaluating patient adherence to therapy and dose optimization, particularly in the setting of therapeutic failure or toxicity. The proposed therapeutic target for pre-dose (trough) plasma imatinib concentration (C_0) is 1000 ng/mL for chronic myeloid leukemia (CML) patients, and 1100 ng/mL for gastrointestinal stromal tumor (GIST) patients. However, TDM for imatinib is not widely available in the United States. Automated analysis of imatinib concentrations in plasma may improve accessibility of testing to support TDM for imatinib.

Methods

Immunoassay

Imatinib concentrations were determined using a laboratory developed competitive homogenous nanoparticle immunoassay, based on the method described by Beumer et al. The assay was performed with a Beckman Coulter AU5800 automated chemistry analyzer.

- Reagents
  - Imatinib mesylate
  - R1, Amino dextran conjugate
  - R2, Latex microparticle reagent bound to monoclonal antibody
  - Calibrators prepared in buffer: 0, 300, 600, 1000, 2000 and 3000 ng/mL
  - Quality control materials prepared in drug-free plasma
- Reaction includes 95 µL R1 and 4 µL sample, incubated for 3.4 min; then 95µL R2 is added
- Agglutination is monitored at 600 nm
- Concentration of imatinib is inversely proportional to the agglutination complex

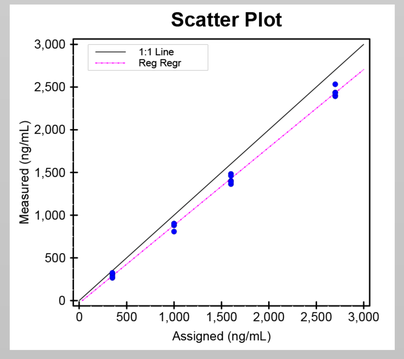
Evaluation Studies

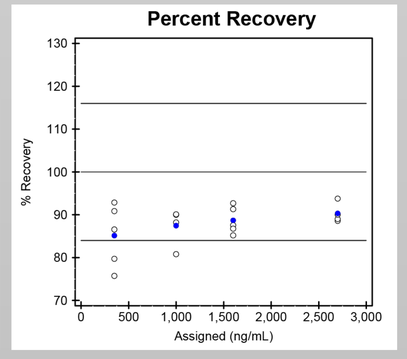
- **Accuracy** at four concentrations (350, 1000, 1600 and 2700 ng/mL) using fortified plasma samples. Each sample was tested five times in a single run.
- **Linearity** over eleven concentrations (251 - 3316 ng/mL) using fortified plasma samples. Each sample was tested twice in a single run.
- **Precision** at three concentrations (604, 1120, 1932 ng/mL) using fortified plasma samples. Each sample was tested four times per run, two runs per day, for four days (total n=32 results per sample)
- **Method comparison** with plasma collected from twenty CML patients that were prescribed imatinib for at least one month. The plasma samples were collected at the Huntsman Cancer Institute based on protocols approved by the University of Utah Institutional Review Board and were stored frozen in multiple aliquots, until tested. The samples were analyzed with the automated immunoassay described here and were also analyzed by the Oregon Health & Science University (OHSU) in Portland, OR, where imatinib quantification is performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). The lower reporting limits were 50 ng/mL for the LC-MS/MS assay and 300 ng/mL for the automated immunoassay.

Statistical analysis was performed by EP Evaluator

Results

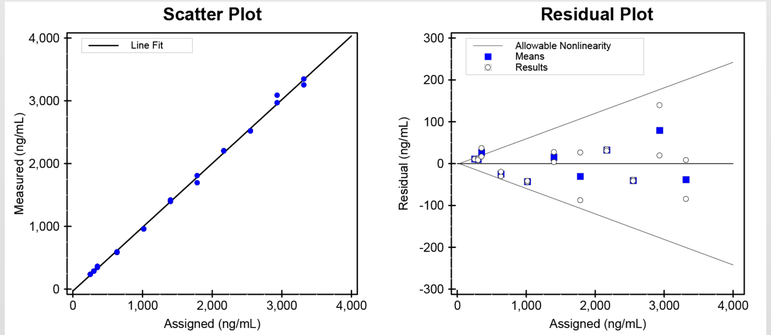
Accuracy





Results (cont.)

Linearity

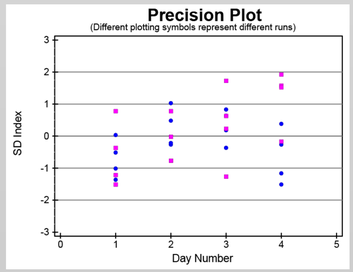


Data IS linear within allowable nonlinearity of 6%
Fit to data is acceptable (p=0.143)
Power of test to detect nonlinearity is acceptable (ratio=3.9)
All coefficients above order 1 are statistically equivalent to zero

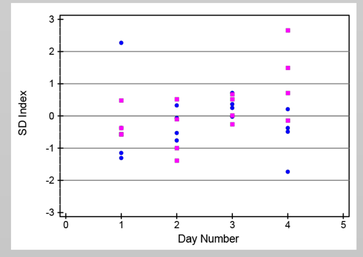
Precision

	%CV		
	604 ng/mL	1120 ng/mL	1932 ng/mL
Within Run	2.8	2.0	1.5
Between Run	1.7	1.0	1.6
Total	3.3	2.3	2.4

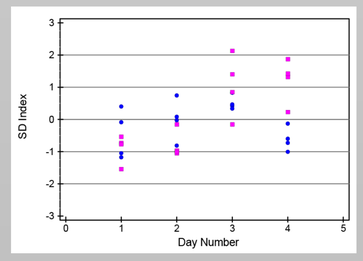
604 ng/mL
Total SD = 20.1



1120 ng/mL
Total SD = 25.7

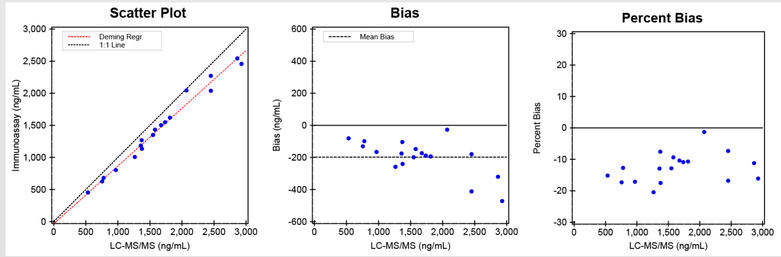


1932 ng/mL
Total SD = 46.9



Results (cont.)

Method Comparison with Authentic Patient Samples



	Imatinib (ng/mL)	
Patient ID	ARUP (immunoassay)	OHSU (LC-MS/MS)
1	<300	<50
2	<300	<50
3	<300	<50
4	626	757
5	680	779
6	803	969
7	1006	1265
8	1184	1360
9	1268	1372
10	1135	1376
11	1350	1549
12	1432	1580
13	1502	1676
14	1548	1737
15	1619	1813
16	2044	2071
17	2271	2451
18	2039	2451
19	2543	2864
20	2457	2929

Sub-therapeutic concentrations

Conclusions

The automated immunoassay described here for determination of plasma imatinib concentrations performed with excellent accuracy, linearity, and precision. There was an approximate 13% negative bias as compared to LC-MS/MS. Availability of testing could be used to support adherence testing and dose optimization for patients prescribed imatinib.

References

Beumer JH, Kozo D, Harney RL, Baldasano CN, Jarrah J, Christner SM, Parise R, Baburina I, Courtney JB, Salamone SJ. An Automated Homogeneous Immunoassay for Quantitating Imatinib Concentrations in Plasma. *Ther Drug Monit*. 2015 37(4):486-92

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