New tool for monitoring molecular response in chronic myeloid leukemia

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Methods

- **Background:** Chronic myeloid leukemia (CML) treatment monitoring using PCR based peripheral blood testing provides improved test sensitivity over cytology but suffers from inadequate standardization in most labs due to variations, inherent in the existing PCR methodologies.

- **Variable results in most labs are probably due to different techniques, internal controls, reagents, and methods of calculation.**

- We present the initial analytic performance evaluation of a novel competitive template-based peripheral blood b2a2/b3a2 transcript abundance method.

- **Standardized Nucleic Acid Quantification BCR-ABL (SNAQ-BCRABL)** uses mixtures of b2a2 or b3a2 and GusB competitive templates and melting curve analysis to provide clinically needed quality controls to correct for operator, instrument, sample, and reagent variation when measuring transcript abundance.

- We hypothesized that the SNAQ-BCRABL method produced more consistent results with less frequent outliers compared to the LDT methods.

- Blood is collected and shipped to laboratory for RNA extraction and cDNA synthesis.

- Copy DNA is divided into three mixtures of internal standard (GusB A, B & C), GusB & BCR-ABL primer/probes & master mix (100 ng PCR).

- Samples are amplified and melting curve data collected and exported to a spreadsheet where melt curves are fit to a hybridization model to generate a native template: internal standard ratio and GusA metrics (sample load, probe specificity).

- This ratio is used to calculate transcript abundance, from which BCR-ABL/GusB ratio is calculated.

- **SNAQ-BCRABL work flow:**

  - Collected blood
  - Extract RNA
  - QIAamp RNA Blood Mini Kit
  - Synthesize cDNA (20 μl)
  - PCR & melting curve
  - SuperScript VILO cDNA Synthesis Kit
  - PCR & melting curve
  - HotStarTaq Plus®
  - Import melting curve data into automatic data analysis tool
  - Snak Software calculates
  - Copy number BCR-ABL/GusB QA METRICS

- **Background:** Non-molecular methods for monitoring molecular response in chronic myeloid leukemia (CML) are less sensitive than molecular methods. The ability to perform more accurate monitoring of CML patients is limited by the lack of standardized methodologies and variability in results among laboratories.

- **Objective:** To establish a standardized method for monitoring molecular response in chronic myeloid leukemia (CML) that is accurate and consistent among laboratories.

- **Methods:** Standardized Nucleic Acid Quantification BCR-ABL (SNAQ-BCRABL) uses mixtures of b2a2 or b3a2 and GusB competitive templates and melting curve analysis to provide clinically needed quality controls to correct for operator, instrument, sample, and reagent variation when measuring transcript abundance.

- **Assessment:** The primary study goal required a >0.85 correlation between SNAQ and LDT was met with correlations of 0.96, 0.97 and 0.94 with 1Lx2S, 2Lx2S, 1Sx1L and 2Sx2L respectively.

- **Conclusions:** The SNAQ methods correlate well with the LDT methods with a 95% limit of agreement of ±3-fold between laboratories.

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