Standardizing BCR-ABL MBr Monitoring Without External Standards

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Abstract: BCR-ABL blood transcript abundance is a key marker for monitoring tyrosine kinase inhibitor treatment of chronic myelogenous leukemia (CML). Standardization of BCR-ABL qPCR measurements suffers from variability introduced by sample purity, preparation, reagents and instrument. International standardization efforts have made modest improvements but rely on cumbersome external reference material and lab calibration. This poster describes initial testing of a novel qPCR method that controls for the majority of real-time qPCR variation.

Currently, the quantitative definition of a significant “rise” in BCR-ABL RNA (to warrant mutation analysis) is controversial and varies among laboratories. However, because PCR negativity is poorly standardized among laboratories and varies depending on the sensitivity of PCR assays, the definition of CNR to evolve, and calling an inconsistent diagnostic value for CNR has been problematic.

RE: International Standardization... This process has generally worked well, it is apparent that the establishment of conversion factors is time-consuming, complex, expensive, and open to only a limited number of laboratories at any given time. Furthermore, it is unclear how frequently any individual CF will need to be reevaluated.

Blood is collected and shipped to laboratory for RNA extraction and cDNA synthesis. cDNA is divided into PCR & real-time qPCR variation. The goal of this experiment was to demonstrate that interlaboratory imprecision would support claims of COPY NUMBER

METHOD CONCORDANCE

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Leukemia

blood

2010 116: e111-e117

SNAG Software Calculates

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BCR-ABL/GUSB QA METRICS

Purpose: Observe SNAG BCR-ABL MBr in cDNA quantification linearity and interday reproducibility. Method: Cell line cDNA mixtures derived from K522 (a b2a2 source, black) and K562 (b2a2, red) and 1:4000 dilution of different BCR-ABL Western-negative were quantified for BCR-ABL and GUSB using a CE based BCR-ABL SNAQ Soft.

Results: The measured vs. expected responses for both reagents were well measured by SNAG BCR-ABL MBr method (y-axis, std dev. error bars) and plotted against expected answer (x-axis). Tables indicates interday replicate CV.

Conclusions: Both p210 transcripts were accurately measured over a 4-log reduction of BCR-ABL with interday variation <25%.

SNAQ Quantification by Ratio Analysis. The cDNA sample contains native template (blue) which is spiked with a known quantity of internal standard (red) to which a fluorescent probe (green) may hybridize. The two templates are kinetically identical PCR, so their ratio is maintained during amplification and melting curve analysis. During melting curve analysis, the probe fluorescence decreases as it dissociates from the template. The difference between internal standard and native template arises from the base pair mismatch between internal standard and probe. Intermediate melting profile (orange circles) indicate presence of both templates, the extension of each derived by curve fitting (red dashed line, internal standard) blue dashed line, native template). Native transcript abundance is calculated from the known internal standard transcript quantity spiked into the PCR and the resulting ratio.

A single internal standard supports a dynamic range of about 1000-fold. SNAG BCR-ABL MBr achieves seven log dynamic range by mixing specimens with three different Mixtures of Internal Standard (MS). MS A & B each have GUSB internal standard at 4x10⁶ copies (purple box), and BCR-ABL internal standard at 4x10⁷ copies (orange box), the dynamic range of each MS is indicated on the above graph). GUSB transcript abundance measurements in every PCR provides a QA to ensure sufficient sample loading. Using a mixture of reference and target genets) in a competitive PCR ensures that the BCR-ABL/GUSB results are consistent despite PCR efficiency and volumes variation.

Purpose: Compare SNAG BCR-ABL MBr and Cepheid method concordance using clinical specimen. Method: B20 and ABL transcripts abundance of twenty-three CML patient whole blood samples were measured using the BCR-ABL assay in the Cepheid GeneXpert® system (x-axis). Frozen cell pellets were made from the remaining blood specimen. RNA was extracted from the frozen cell pellets and provided blinded for p210 fusion (b2a2 or b3a2) identification and interday reproducibility.

Results: The laboratory CV ranges for all six samples spanned 8% to 25%, better than the 15% CV requirements necessary for half-log accuracy.

SNAG BCR-ABL MBr reagent beta test demonstrated an excellent interday, interlaboratory reproducibility sufficient for 2-fold interlaboratory accuracy claims without reliance on external reference material. Follow up studies will gauge impact of sample collection & RNA isolation. Further, the SNAG method should be applicable for other qPCR applications.