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617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Streamlining the Detection of FLT3 Internal Tandem Duplication (FLT3-ITD) Using the Cepheid Genexpert® Automated System

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Objectives: The development of targeted therapies for Acute Myeloid leukemia (AML) demands fast and reliable diagnostic tests. Specifically, FMS-like tyrosine kinase 3 (FLT3-ITD), comprising nearly 25% of all AML cases, is the most common targetable mutation in AML. Current diagnosis of FLT3-ITD by PCR involves numerous laborious steps, with bench-top steps involving time-consuming whole blood processing, DNA isolation, plate-based PCR and subsequent fragment analysis by capillary electrophoresis. Consequently this adds significant overhead, time-consuming personnel training, and numerous steps which could lead to errors, all of which increase the resource burden and time to result. The objective of this study was to create an automated FLT3-ITD diagnostic prototype that is faster, requires less hands-on time that can detect all clinically relevant ITD lengths.
Methods: We have developed a simplified FLT3-ITD prototype assay using the Cepheid GeneXpert® automated platform for FLT3-ITD detection. The prototype substantially streamlines the process, using a less than 30 minutes off-board time to prepare a universal blood lysate that is compatible with all Cepheid Hematology Oncology products. Once the sample is loaded into the cartridge and inserted into the instrument, the cartridge performs automated DNA isolation, purification, and qPCR. A Taqman-based probe for ABL is utilized in the qPCR step as a PCR control to ensure that the blood processing and qPCR was successful and avoid wasted downstream steps. Quantitative fragment analysis can easily be performed on the PCR product using capillary electrophoresis (CE) for precise allelic ratio (AR) callout. Additionally, the PCR product is compatible be run on a bioanalyzer or standard Agarose Gel-Electrophoresis if available.
Results: The developed automated FLT3-ITD diagnostic prototype demonstrated significant improvements over the existing methodologies with substantially reduced time to result and simplicity in use. To assess the sensitivity of this prototype, we used MV4-11 cancer cells harboring a 30 bp ITD and CRISPR-CAS engineered human cell lines with 3 bp, 21 bp, and 75 bp FLT3 ITD insertions, comprising the most commonly detected ITD lengths spiked into EDTA blood as a test material. For these ITD's, we observed sensitivity and linearity down to 0.01-0.02 by CE for the ITDs tested. Importantly, the prototype detected all lengths of ITD mutations with inserts from 3bp to 300 bp using plasmid test materials.
Conclusion: We have successfully developed a novel FLT3-ITD diagnostic prototype that has several major advantages over existing methodologies. The prototype significantly reduces the time and hands-on effort required for analysis, streamlining the diagnostic process for same day results. The larger sample volume used allows improved sensitivity to ensure accurate detection, even at low allelic frequencies. Additionally, the prototype requires fewer reagents in that sample prep and qPCR are performed in a single cartridge and run hands-free. Downstream analysis of amplified products can be easily performed and validated using capillary electrophoresis, bioanalyzer, or gel electrophoresis. Finally, because this prototype uses the same universal blood lysate as other AML tests, it has the potential to improve future diagnostic therapeutic interventions in the clinic.

Disclosures Sirajuddin: *Cepheid:* Current Employment. **Gregory:** *Cepheid:* Current Employment. **Kushal:** *Cepheid:* Current Employment.

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