Evaluation of Signature® NPM1 Mutations (RUO)* for the Rapid Multiplex Detection of NPM1 Exon 12 Mutations

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SUMMARY POINTS
• The Signature® NPM1 Mutations (RUO)* assay has a streamlined workflow allowing simultaneous detection of wild-type and mutants A, B, D, and J NPM1 mutations in a liquid bead array using total RNA specimens.
• Sensitivity and specificity were 100% in this study with hematologic malignancies using capillary electrophoresis size fractionation as the reference method and sequencing to confirm positive specimens.

INTRODUCTION
NPM1 (NPM) gene mutations represent the most common genetic alteration in adult acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), but the significance of NPM1 mutations in the context of other hematologic malignancies is not yet fully understood. NPM1-positive AML and MDS patients show distinctive biological, clinical and prognostic features, molecular screening and monitoring of NPM1 mutations may be beneficial for improved risk-stratified treatment approaches. However, because the NPM1 mutation results in 4 nucleotide losses or insertions (Figure 1B) in a background of wild type sequences, sensitive and specific detection of NPM1 mutations by polymerase chain reaction (PCR) methods can be challenging. Signature® NPM1 Mutations RUO* is a research-use-only kit for the rapid detection of common NPM1 mutations using total RNA purified from cultured cells, bone marrow or peripheral blood. The assay utilizes multiplex reverse transcription-PCR (RT-PCR) in combination with fluorescent bead-based detection to simultaneously identify transcripts for NPM1 mutations and wild type targets (Figure 1A). The objective of this study was to evaluate the performance of the Signature® NPM1 assay.

RESULTS

Analytical Performance

Figure 2. Analytical Performance. (A) Sensitivity reached at best 0.01% in this study by dilution of mutant-positive cell line RNA in HL-60 RNA or the equivalent of 1,000 to 2,000 copies of mutant transcripts. The assay is compatible with other laboratory-developed assays using Signature reagents for the simultaneous detection and NPM1 assay and common AML-specific fusion transcripts. Sensitivity and specificity were 100% in this study with hematologic malignancies using capillary electrophoresis size fractionation as the reference method and sequencing to confirm positive specimens.}

MATERIALS AND METHODS
Peripheral blood or bone marrow from 63 patients with AML, ALL, CML, or MDS were collected as part of standard clinical care and following a protocol approved by the Johns Hopkins Medical Institutions IRB. Total RNA was isolated from peripheral blood or bone marrow using the QIAGEN® QIAamp® RNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions. The Signature® NPM1 Mutations RUO* kit was evaluated using Signature® General Purpose Reagents following the NPM1 Package Insert. Briefly, total RNA (5 μL) was reverse transcribed into cDNA and amplified by PCR using NPM1-specific primers (45 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec). NPM1 wild type transcripts were co-detected in the same reaction to serve as endogenous internal controls. The PCR products were then sorted on the liquid bead array containing oligonucleotide probes specific for NPM1 and detected using the Lumex® 2000 system. The mean fluorescence intensity (MFI) of NPM1 transcripts for each target was reported for co-amplification and co-detection of NPM1 mutations and AML fusion transcripts. 1 μL of Signature® NPM1 Mutations RUO* and Signature® AML1/ETO (Inv16) Primers (Inv16) was added to the NPM1 Amplification master mix and hybridization mix, respectively.

CONCLUSION
The Signature® NPM1 Mutations RUO* assay is highly compatible with the molecular laboratory workflow. It presents several advantages such as rapid time to result, 96-well plate format, broad range of RNA input, inclusion of positive and negative controls, and single-well multiplexing compatibility with other Signature reagents for simultaneous detection of leukemia-specific fusion transcripts. Evaluation with 63 newly diagnosed leukemia specimens resulted in 100% sensitivity and specificity in this study. The assay analytical sensitivity (0.01%) and dynamic range (4 Logs) warrant further evaluation to determine its potential clinical utility for monitoring residual disease during treatment of AML patients.

*For Research Use Only. Not for use in diagnostic procedures. The performance characteristics of this product have not been established.