HIGH-RESOLUTION MELTING ASSAY FOR RAPID SIMULTANEOUS DETECTION OF *JAK2*, *MPL* AND *CALR* VARIANTS IN PATIENTS WITH CLINICAL SUSPICION FOR MYELOPROLIFERATIVE NEOPLASMS

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Introduction

- Genetic testing for *JAK2*, *MPL*, and *CALR* mutations remains crucial in the diagnosis of Philadelphia-negative myeloproliferative neoplasms (MPN).
  - Emphasized in current NCCN guidelines as well as recent ICC and WHO diagnostic criteria
- Common shortcomings in current testing algorithms:
  - Depend on batching and/or sequential testing
  - Involve multiple testing modalities
  - May involve send-out testing
- The HemeScreen® MPN Assay addresses this need and entails
  - High-resolution melting (HRM) analysis
  - Multiplex mutation testing:
    - *JAK2* exons 12-14, *MPL* exon 10, *CALR* exon 9

Materials and Methods

Indications

- Clinical suspicion for MPN

Samples

- Peripheral blood or bone marrow aspirates

Study Design

- Initial DNA extraction was performed centrally with two aliquots created for testing
- Each sample was tested with the HemeScreen® MPN Assay and Sanger sequencing
- Each assay was performed in an independent, CLIA-certified laboratory.
- Teams were blinded to patient clinical information and to the other assay's results

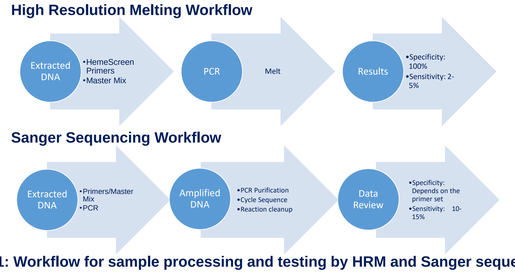


Figure 1: Workflow for sample processing and testing by HRM and Sanger sequencing.

HemeScreen® MPN Assay

- Quantstudio™ 3 Real Time PCR System
- Analyzed with HRM v3.1 software (Thermo Fisher)

Sanger Sequencing

- ABI 3730XL DNA Analyzer (Life Technologies)
- Analyzed with Sequencher™ 5.4.6 software (Gene Codes Corp)

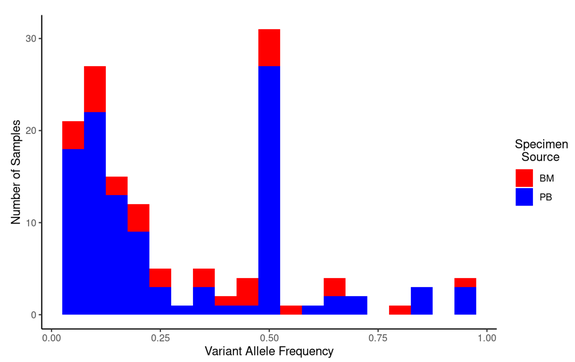
Statistical Analysis

- Analytical and clinical performance calculated
- Sanger sequencing served as the gold standard
- Disease-associated (DA) and variants of uncertain significance (VUS) considered clinically relevant

Results

Specimens consisted of a wide range of variant allele frequencies of mixed sources, and testing was completed at a high success rate.

A.



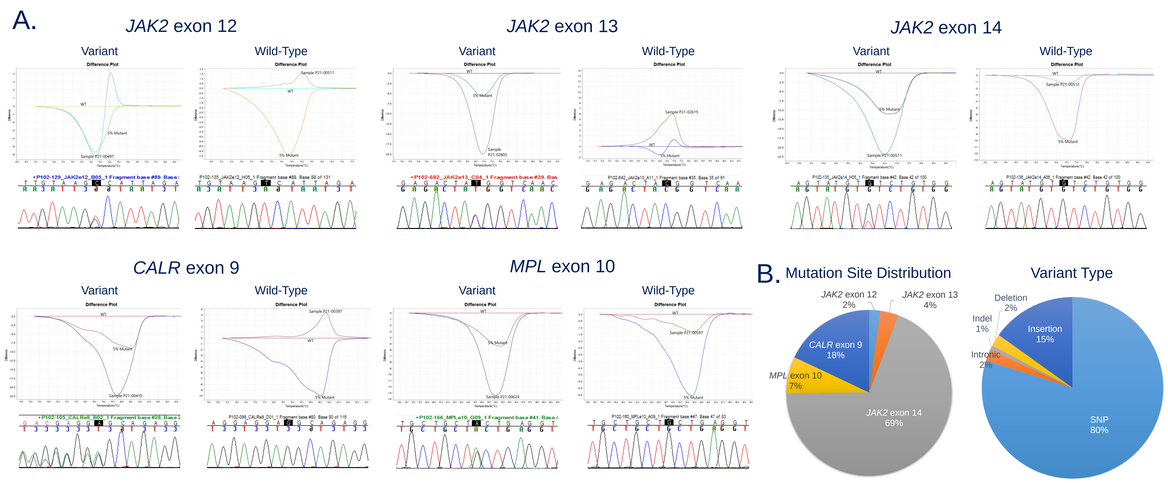
B.

1043 Specimens Received → 982 (94%) Suitable for Testing → 955 (92%) Complete at All Targets

Figure 2: A. Tested specimens consisted of 90% peripheral blood (PB) and 10% bone marrow (BM) samples with variant allele frequencies (VAF) ranging from <5-95%. B. 1043 specimens were initially received for testing on the basis of clinical concern for MPN, 982 of which were suitable for testing. All 982 samples underwent successful HRM testing, of which 955 specimens had successful Sanger sequencing at all targets.

HRM accurately identified both variant and wild-type sequences at all targets with variants consisting of point mutations, insertions, and deletions.

A.



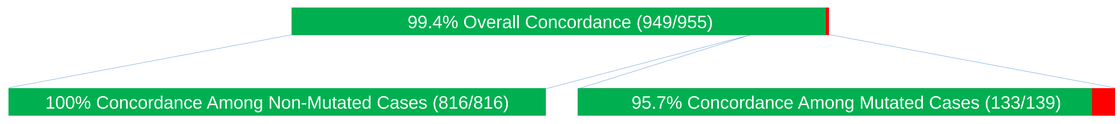
B. Mutation Site Distribution

Variant Type

Figure 3: A. For each target, variant (left) and wild-type (right) patterns on HRM, represented by difference plots (top row), and Sanger sequencing (bottom row) were identified among the submitted patient samples. For each test, a 5% mutant sample was run as a point of comparison, allowing for accurate identification of variants, occasionally even at levels below 5% variant allele fractions (VAF). Overall, mutations were identified in each of the 5 targeted domains: *JAK2* exons 12, 13, and 14; *CALR* exon 9, and *MPL* exon 10. B. Among samples with mutations, the distribution was consistent with those reported in non-CML MPNs with the majority consisting of *JAK2* exon 14 (V617F) mutations and fewer *MPL* and *CALR* mutations. Variants included 114 single nucleotide variants, 3 insertions (5 bp), 21 deletions (3-52 bp), and 1 deletion-insertion.

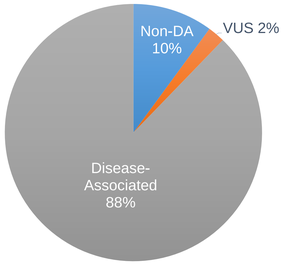
HRM showed a high degree of analytical consistency with Sanger sequencing and high sensitivity and specificity in patient samples.

A. Analytical Performance



B. Clinical Performance

Variant Categorization



Overall Clinical Performance

Sensitivity	96.7%
Specificity	98.7%
PPV	92.3%
NPV	99.5%

HRM Performance by Variant

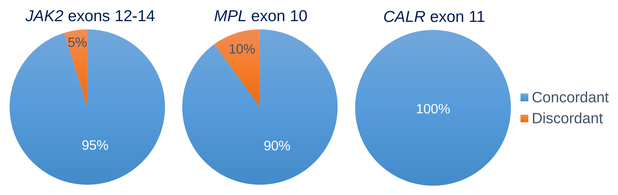
Variant	Detection Rate
JAK2 V617F	94/96 (98%)
MPL W515L/S	8/9 (89%)
CALR in/del	19/19 (100%)

Characteristics of variants not detected by HRM

Target	Variant	VAF	Type	Effect
MPL exon 10	c.1544G>T; p.W515L	0.05	SNV	Disease Associated
JAK2 exon 14	c.1849G>T; p.V617F	0.5	SNV	Disease Associated
JAK2 exon 12	c.1641+6T>C; p.?	0.5	Intronic	Non-Disease Associated
JAK2 exon 12	c.1641+6T>C; p.?	0.5	Intronic	Non-Disease Associated
JAK2 exon 13	c.1711G>A; p.G571S	0.5	SNV	VUS
JAK2 exon 14	c.1849G>T; p.V617F	0.1	SNV	Disease Associated

Figure 4: A. Overall, HRM and Sanger sequencing had an analytical concordance of 99.4% with HRM detecting 133/139 (96%) variants confirmed by Sanger sequencing (9/10 *MPL*, 25/25 *CALR*, 99/104 *JAK2*). B. The HRM-based assay identified known disease-associated (DA) variants (89%), variants of undetermined significance (2%), and non-DA variants (9%) with a positive predictive value (PPV) of 92.3% and negative predictive value (NPV) of 99.5%. Non-DA variants were predominantly benign 3 or 9 bp *CALR* deletions. Among *JAK2* V617F cases, 94/96 (98%) were detected by the HRM assay.

HRM-Sanger Concordance by Target



Conclusions

- Our studies demonstrate the accuracy, exquisite sensitivity, and specificity of the HemeScreen® MPN Assay in detecting genetic variants observed in MPN.
- The multiplex design with HRM technology offers an opportunity for a low-cost approach with a simplified workflow for laboratories while providing a quick turnaround time and accurate results.
- Overall, these data demonstrate the capability of HRM-based multiplex assays to serve as powerful, clinically-applicable platforms for rapid, simultaneous molecular detection of clinically-relevant, somatic disease variants.