Az Damiaan
Dicht bij het leven

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VALIDATION AND CLINICAL PERFORMANCE OF AN IN-HOUSE *JAK2* V617F MUTATION DETECTION ASSAY ON THE ROTOR GENE Q

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Background

- The presence of *JAK2* V617F mutation is part of the reference WHO criteria for the diagnosis of polycythemia vera, essential thrombocythemia and primary myelofibrosis in *BCR-ABL1* negative patients.

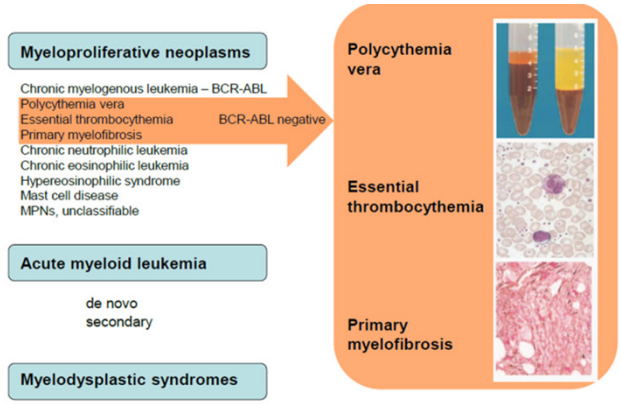

Fig. 1 Overview of myeloid malignancies

Methods (1)

- The mutation was detected by a TaqMan© based real-time PCR with a wild-type blocking LNA (Locked Nucleic Acid) probe, increasing the specificity and sensitivity. DNA quantity and possible inhibition were checked by a second control-qPCR.

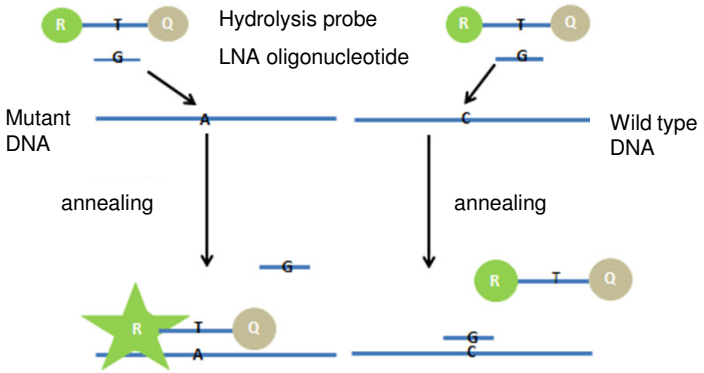

Fig. 2 Schematic overview of the mutation-specific LNA-based qPCR reaction (R: reporter; Q: quencher)

- Primers and probes were chosen from the literature (1).
- DNA was extracted using the MagNA Pure platform (Roche, DNA I High Performance protocol) from 117 patients (EDTA blood) and 4 external quality controls. The sensitivity panel of NIBSC and DNA-standards (HorizonDx) were also included in the validation process.
- 5 µl DNA was added to 20 µl mastermix. The mastermix used was LC480 Probes Master (Roche). The final concentration of the primers and hydrolysis probes was respectively 0,3 µM and 0,1 µM. The LNA-probe had a final concentration of 1 µM.

Methods (2)

- The assay was checked for accuracy, reproducibility, linearity, clinical sensitivity and specificity following the Belgian guidelines (2).

Results

- Patients and external quality controls were confirmed in a reference laboratory and were all concordant.
- 50 routine patient samples, clinically expected negative for *JAK2* V617F were analysed to determine the clinical specificity, one was weak positive and was later confirmed with 0,2 % allelic burden.
- The clinical sensitivity was 1% mutant allelic burden which is in line with the British guidelines (3).
- The variation coefficient for a high and low allelic burden sample was respectively 2,99 % and 1,46 %.
- The mutation and control PCR had an efficiency of 99 %.

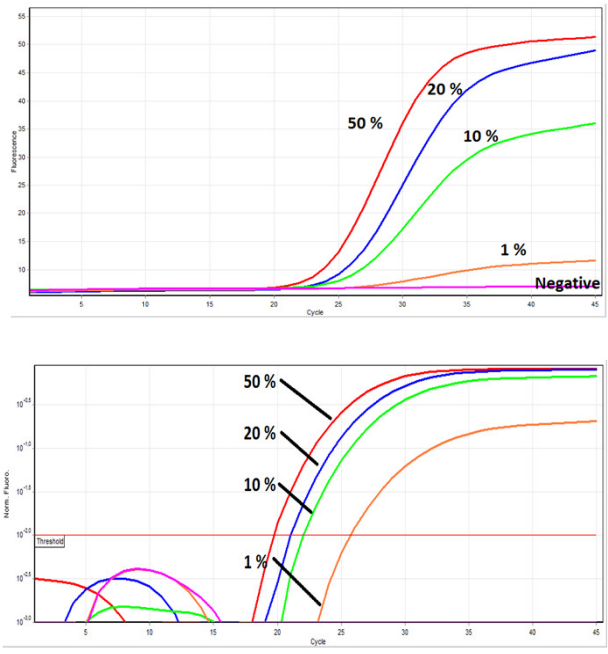

Fig. 3 Raw and analysed data of 4 samples with allelic burden from 50-1%

Conclusion

The cost-effective and rapid assay met all our analytical and clinical validation criteria and is therefore implemented in daily routine.

References:
(1) Denys et al., J of Molecular Diagnostics, 2010
(2) Raymaekers et al., Acta Clinica Belgica, 2011
(3) Bench et al., British J of Haematology, 2013

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