

IMPROVING DIAGNOSTIC STANDARDIZATION IN MYELOPROLIFERATIVE NEOPLASMS: RESULTS FROM THE ITALIAN JAKNET PROGRAM

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Introduction: The *JAK2V617F* mutation is the most common driver event in Myeloproliferative Neoplasms (MPNs), leading to cytokine-independent JAK/STAT activation and contributing to both disease onset and progression. Accurate quantification of the *JAK2V617F* variant allele frequency (VAF) is essential for diagnosis and monitoring. In 2018, GIMEMA established JakNet, a network of Italian hematology laboratories (labs) supported by Novartis, to promote methodological harmonization and standardize *JAK2V617F* testing through shared recommendations and annual quality controls (QC).

Methods: Since 2018, labs have undergone yearly QC rounds using the NIBSC 1st Panel *JAK2V617F* reference standards, comprising seven DNA samples with defined VAF (0-100%). Labs were defined as "compliant" when all the values were included in the acceptable intervals, "operationally non-compliant" when the discrepancies detected did not impact on the negative/positive classification, "non-compliant" if the discrepancies were in the crucial interval 0 to 1% interfering with the negative/positive definition. Analytical methods (HRMA, RT-qPCR, ddPCR) were compared over time.

Results: The network expanded from 35 lab in 2018-2019 to 50 in 2025, across Italy. Overall, a total of 54 labs participated: 22 in Northern Italy, 13 in Central Italy, and 19 in Southern Italy; 35 were university- and 19 hospital-based. Analytical performance improved: compliant labs rose from 74% in

2018 to 78% in 2024, peaking at 82% in 2023. Operationally non-compliant labs decreased from 20-31% to 5-11% in 2023-2024, while the proportion of non-compliant remained consistently low over years, usually below 15%, reflecting the overall robustness of laboratory performance. In 2025, compliance slightly dropped to 70% due to an increase in operationally non-compliant labs (22%), while non-compliant labs remained at 8%. Method adoption evolved: in 2018, 71% of labs used RT-qPCR, 26% ddPCR, and 3% HRMA; by 2020, HRMA was nearly phased out. Between 2022 and 2025, RT-qPCR remained predominant (70-76%) and ddPCR slightly increased (23-30%). The use of ddPCR and standardized CE-IVD kits ensured QC compliance, while semi-quantitative approaches showed variable accuracy (figure 1). Between 2018 and 2025, of 13 labs initially considered as operationally non-compliant, 8 became fully compliant, 4 remained operationally non-compliant, and 1 became non-compliant. Of 5 labs initially non-compliant, 2 improved to full compliance, demonstrating the favorable impact of continuous QC and proficiency programs in fostering methodological standardization.

Conclusions: JakNet established a national framework for standardizing *JAK2V617F* testing in MPNs. Quantitative molecular methods and systematic QC improved assay accuracy and inter-laboratory consistency. Continued participation and data sharing strengthen diagnostic harmonization and support integration of innovative technologies in molecular hematology.

MYELOPROLIFERATIVE DISORDERS

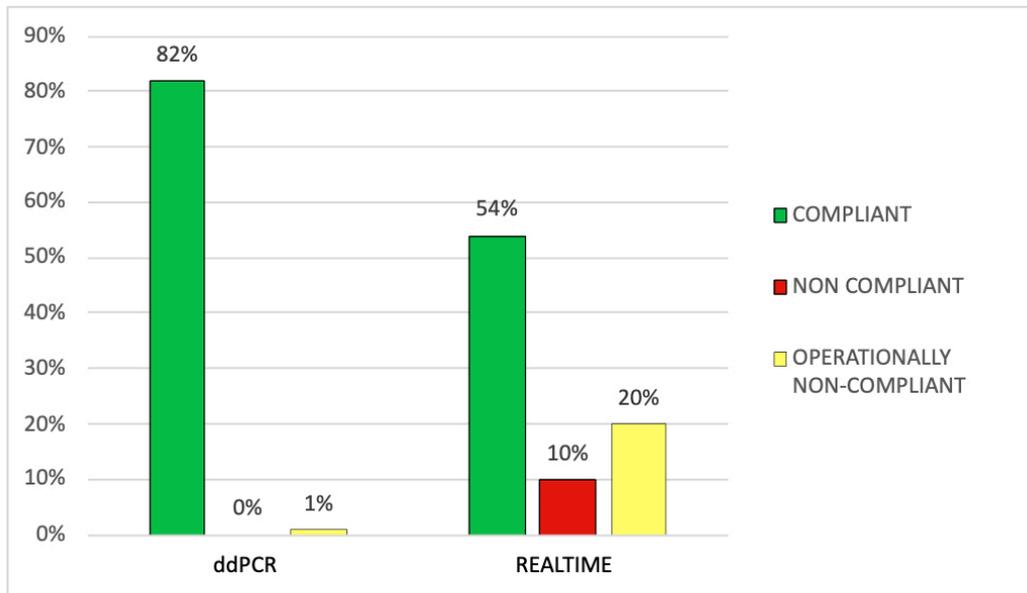


Figure 1: ddPCR vs Real time performance trend (QC rounds from 2018 to 2025)