to describe the utilization of real-time PCR with a specific MGB probe to detect the V617F mutation in the JAK-2 gene. We also present the retrospective results obtained from peripheral blood samples of 250 patients who were assessed between July 2022 and July 2023 due to suspected myeloproliferative disorders (MPD). **Objectives:** The primary objectives of this study are to validate a rapid, cost-effective, and sensitive MGB TaqMan probe-based real-time PCR method, coupled with automatic magnetic DNA extraction (EXTRACT MPTA, Loccus) compared with column manual extraction (Promega), for detecting the JAK-2 V617F mutation in an onco-hematology remote laboratory (ROLF). Additionally, we aim to evaluate the incidence of the V617F mutation in the 250 cases submitted to ROLF. **Methods:** For the real-time PCR reaction, we utilized a specific MGB probe (Thermo) targeting the V617F mutation (rs77375493) along with the SNP detection master mix reagent. The PCR was performed using a Rotor-Gene Q instrument (Qiagen), and the raw fluorescence data were analyzed at cycle 45. To assess sensitivity, we conducted tests using a homozygous cell line for the mutation (HEL 92). **Results:** Among the 250 cases (126 females and 124 males) originating from 28 different hospitals in Brazil, 63 (25.2%) tested positive for the V617F mutation, with a relatively equal distribution between genders ($p = 0.1459$). The mean age of V617F positive samples was 66 years compared to 55 years for negative samples ($p < 0.0001$). Analysis of fluorescence intensity at cycle 45 of real-time PCR in positive and negative samples for the V617F mutation revealed remarkable sensitivity, detecting the presence of the mutated allele down to 1% (limit of detection - LOD). The average fluorescence intensity was 0.52 for mutated samples and 0.04 for non-mutated samples ($p < 0.0001$, Mann-Whitney test). DNA from 32 samples and diluted controls were extract manually and automatically (magnetic) and 100% agreement was obtained. **Conclusion:** Our study demonstrates the clinical relevance and high sensitivity of real-time PCR with the MGB probe for detecting the V617F mutation in the JAK-2 gene. It accurately identifies V617F mutation even at low levels (1% LOD). The automatic magnetic DNA extraction setting offers a rapid and cost-effective alternative to manual column extraction. Notably, we observed a significant correlation between the V617F mutation and older age, with positive samples having a mean age of 66 years compared to 55 years for negative samples ($p < 0.0001$). These findings have important implications for molecular diagnostics in onco-hematology, enabling early and precise diagnosis of myeloproliferative disorders (MPD).

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FAST AND COST-EFFECTIVE RNA EXTRACTION FOR BCR-ABL QUANTIFICATION IN ONCO-HEMATOLOGY LABORATORIES

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of a *BCR-ABL1* rearrangement; its detection and quantification by real-time quantitative PCR (RQ-PCR) plays a central role in CML diagnosis, therapy monitoring, and sequencing to identify resistance mutations in case of therapy failure. To evaluate the molecular response of CML, *BCR-ABL1* quantification must be calculated on the international scale (IS%) for which RQ-PCR has the necessary sensitivity (up to 10−5). Currently, *BCR-ABL1* quantifications are ubiquitous in onco-hematology laboratories (OHLs; ~70% of tests performed in our OHL), whereby quality, cost, and turn-around-time are of critical importance. Today, the majority of OHLs in Brazil rely on laborious manual RNA-extraction methods (e.g., Trizol) that usually require 8 mL of peripheral blood (PB) to monitor CML. Here, we report a strategy that requires only 2 mL of PB in combination with the automated magnetic DNA/RNA-extraction kit Extracta-MPTA (Loccus do Brasil) for CML diagnosis and monitoring. **Methods:** *BCR:ABL1* was quantified and its IS% determined using our developed kit, which was calibrated using ERM-AD263 and UK-NEQAS (external quality assessment). Total RNA was extracted from 40 PB samples, after erythrocyte lysis using two different methods: 1) Leukocytes from 8 mL of PB were extracted manually using Trizol; 2) leukocytes from 2 mL of PB were automatically extracted using Extracta-MPTA (Loccus do Brasil). *BCR-ABL1* quantification, *BCR-ABL1* IS%, and BCR quantification (internal control) were compared statistically (paired t-test). The reproducibility was evaluated by analyzing 20 replicates; the low limit of the leucocyte number to *BCR:ABL1* quantification using Extracta-MPTA were also evaluated. Median and moving means of 135 unpaired samples were used to monitor *BCR-ABL1* IS% between Trizol, Extracta-MPTA, while EQA from UK-NEQAS was also evaluated using both methods. In parallel, RNA was extracted by both methods from 20 samples for the diagnosis of acute lymphoblastic leukemia (ALL) (BCR-ABL p190 transcript) and acute promyelocytic leukemia (APL) (PML-RARA transcript). **Results:** A comparison of *BRC-ABL1*, BCR, and %IS in 40 paired samples showed that the results obtained from Trizol or Extracta-MPTA are comparable (paired t-test: $p = 0{,}4222$, $p = 0{,}9263$, and $p = 0{,}071$, respectively). The reproducibility was evaluated by BCR quantification (24%).. A comparison of the medians from 135 unpaired samples extracted using Trizol and Extracta-MPTA revealed that *BCR-ABL1* IS% distribution among our patients is stable over time ($p = 0{,}7704$; Mann Whitney: $r^2 = 0{,}0012$). EQA was extracted using both methods and the results were identical. A comparison between Trizol and EXTRACTA_MPTA for ALL and APL was 100% identical (Fischer; $p = 1{,}000$). **Conclusions:** Both RNA extraction methods are suitable for OHLs for PML-RARA and BCR-ABL (p190 and p210) detection and CML diagnosis/monitoring by BCR-ABL1 detection/quantification. By using Extracta-MPTA the amount of PB used to obtain RNA can be reduced by 75%, test results can be obtained within 4 h, and direct reagent costs are reduced by 25% with a minimum of hands-on time.

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