Significant advances in clinical genetics over the last decade have increased the demand for genetic tests that predict diagnosis and predisposition to disease (1). Moreover, many somatic diseases of genetic origin such as cancer require genetic studies during different phases for diagnosis, prognosis, control of disease progression, prediction of drug response, and monitoring of treatment response. Consequently, the use of genetic tests requires strict laboratory quality assessment (QA) procedures to fulfill clinical requirements.

A representative paradigm for higher-standard requirements is the quantification of BCR-ABL1 transcripts in chronic myeloid leukemia (CML) to assess the response to treatment with tyrosine-kinase inhibitors (TKIs) such as imatinib or second-generation inhibitors such as nilotinib and dasatinib. The relevance of transcript levels in determining disease prognosis and progression-free survival is clear (2, 3). The TKI treatment goal in CML is to achieve at least a thousand-fold reduction (3 logs) in transcript level from the baseline reference, based on BCR-ABL1 expression levels of the International Randomized Study of Interferon vs STI571 (IRIS) trial (4, 5). Such a reduction in transcript level is known as a major molecular response (MMR) and is associated with prolonged response to TKI and disease-free progression (2, 3). However, either IRIS trial samples or an international standard must be available to report the final results in international scale (IS) units, allowing harmonization in the reporting of BCR-ABL1 levels for uniform clinical interpretation.

To this effect, in 2009, the WHO approved the first WHO International Genetic Reference Panel for quantification of BCR-ABL1 mRNA by reverse-transcription quantitative PCR (RT-qPCR) (6, 7). However, the limited amount of primary WHO standards available requires the establishment of reference panels of BCR-ABL1 that are readily available in unlimited quantities to harmonize the expression of BCR-ABL1 and allow the reporting of BCR-ABL1 results in IS units. Accordingly, the report by White et al. (8) published in this issue of Clinical Chemistry represents an important effort that will undoubtedly make calibrators available and help to unify the reporting of results and ultimately improve CML treatment.

The major advantage of expressing transcript reduction results in IS units lies in the harmonized clinical meaning of results so reported and the ability to compare results among laboratories. However, even when a standardized protocol and common calibrator have been used and results have been expressed in IS units, as suggested by White et al. (8), significant variations in the results among laboratories (9) can hinder reliability of results. The reason for this variability among laboratories, despite their use of common calibrators and standardized protocol, is that the precision and sensitivity of their measurement methods is influenced by the whole procedure, reagents, and equipment used. No certified reference materials are currently available for molecular genetic testing, as the reagents and many currently used platforms do not meet the requirements for stability and traceability for diagnostic studies in vitro (10). Evidence suggests that to provide the highest standard of analysis, the use of calibrators for reporting BCR-ABL1 in IS units should be complemented with laboratory participation in an external QA (EQA) program to ensure consistent and acceptable test accuracy and sensitivity. Furthermore, scientific organizations such as the IFCC recognize participation in EQA schemes as a key tool for QA in clinical diagnostics (11).

Few EQA programs, such as the one from the European Molecular Genetics Quality Network (EMQN, http://www.emqn.org/emnqn), exist for common genetic tests. Even less common are EQA programs that include the quantification of BCR-ABL1 by RT-qPCR. Current programs in the latter category indicate wide variations in accuracy, precision, and sensitivity of results. For instance, Zhang et al. (12) conducted a study including 38 laboratories of North America using real-time RT-qPCR to measure BCR-ABL1 e14a2 transcript using K562 cells diluted from 10\(^{-1}\) to 10\(^{-5}\) in background U937 cells. The participating laboratories used...
different platforms, internal controls, reagents, and calculation methods. The results showed considerable variability, with log reduction calculations varying from 1.6 to 3 logs between laboratories at the same dilution. Moreover, 4 laboratories reported false-positive results in the samples of the cell line U937. Furthermore, 10 of 19 laboratories (56.2%) did not have reproducible results at the dilution level of $10^{-4}$, and 16 of 19 (84.2%) did not have reproducible results at the $10^{-2}$ level.

Likewise, Sher et al. (13) reported an EQA in which RNA samples were sent to 37 laboratories to evaluate their RT-qPCR assay methods for e19a2, e13a2, and e14a2 transcripts. RNA extracted from culture cells was diluted in RNA isolated from HL60 cells in a range from $10^{9}$ to $10^{-5}$. Laboratories performed 5 independent RT-qPCR reactions for each sample type at each dilution. In addition, 15 RT-qPCR reactions of the $10^{-3}$ e14a2 RNA dilution were run to assess reproducibility within and between laboratories. Participants were asked to run the samples according to their standard protocols to generate quantitative values for BCR/ABL, reference genes, and ratios of BCR/ABL for each sample RNA. Sensitivity varied widely among laboratories. The limit of detection was $\geq 10^{-3}$ in 14% of the laboratories for e13a2, 11% for e14a2, and 9% for e19a2. Furthermore, intralaboratory precision was low, with a mean CV of 19.2%, range 4.2%–82.6%.

Ramsdlen et al. (14) reported an EQA (Equal-quant) assessment of technical execution of RNA extraction, reverse transcription, and real-time PCR quantification of BCR/ABL copy number using hydrolysis probes. This multidisciplinary EQA scheme included 137 participating laboratories from 29 countries. The results showed important differences in performance among laboratories, with 20% of laboratories reporting at least 1 result lacking in precision and/or accuracy. For cDNA samples, the laboratories met the performance criteria, but for cellular samples requiring RNA extraction, approximately 33% of laboratories reported results outside the 95% CI.

These experiences in EQA confirm that there is still great variability of results in regard to accuracy and sensitivity. In addition, EQA assessment programs should include e19a2, since the patients harboring this transcript will also benefit from treatment with TKI.

Although the expression of results using IS units, as suggested by White et al. in this issue (8), represents an improvement in guiding treatment, BCR-ABL1 quantification must be complemented with an EQA program to ensure the reliability and homogeneity of results.

Finally, we must remember that the sensitivity and accuracy of PCR methods are limited. Therefore, TKI drugs that require higher sensitivity (>4 log reduction), may not be adequately assessed by conventional qPCR, and methods such as microfluidic digital PCR might be needed (15).

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