Editors of journals were strongly recommended to require that submitted manuscripts report Hb A1c in both SI (IFCC) and NGSP/DCCT units.

The most recent meeting was held in December 2011 in Dubai. The objective of the deliberation was to evaluate the progress of implementation of the prior recommendations. Unfortunately, the goal of reporting the same units worldwide has not been realized. Dual reporting has been adopted by very few countries and has been transitory for the vast majority of these countries. Some countries have elected to report only SI units, while other countries have decided to maintain NGSP/DCCT units. Notwithstanding these developments, the meeting attendees recommended that both SI (IFCC) and NGSP/DCCT units be used for reporting Hb A1c in journals and other printed materials. To facilitate this goal, calculators to convert between millimoles per mole and percentage have been made available (http://www.hba1c.nu/eng2.html and http://www.ngsp.org/convert1.asp). Several journals have adopted this policy and all journals are encouraged to do so.

Meeting Participants


Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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Quality Guidelines for Next-Generation Sequencing

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Next-generation sequencing (NGS) technology has been embraced for its ability to revolutionize genetic testing, owing to its massively high-throughput nature. Sanger sequencing, the current gold standard for sequencing, is being replaced in many instances by NGS because of the latter’s capacity to sequence large gene panels, including entire exomes and genomes, at a lower cost. Although both Sanger and NGS are methods for sequencing, the technologies are vastly different, and therefore the quality definitions and metrics for the Sanger method do not necessarily apply to those of NGS. Given that so many laboratories are offering or gearing up to offer NGS-based tests, it has become imperative to establish consistent quality guidelines to help ensure that NGS results can be used for clinical decision-making.

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In light of this consideration, the Next-Generation Sequencing: Standardization of Clinical Testing workgroup, consisting of 41 participants and convened by the CDC, recently published a 64-page supplementary document providing principles and guidelines for “assuring the quality of next-generation sequencing in clinical laboratory practice” (1). The aim of this document was to address elements of a quality-management system related to the analytical NGS processes: test validation, quality control, proficiency testing (or alternative approaches for assessing test performance), and reference materials. The workgroup focused on processes related to the analytical validity of sequence results but did not address annotation or clinical interpretation of test findings. Additionally, the workgroup covered NGS validation as it pertains to inherited disorders but did not tackle additional complex topics, such as infectious disease, oncology, and structural variation.

The validation processes of NGS were separated by the workgroup into 3 distinct yet interdependent phases: platform validation, test-specific validation, and informatics pipeline validation. These 3 phases cover the essential bases that (a) the sequence data are reliable (i.e., platform validation), (b) the assay can detect test-specific clinically important sequence variations (i.e., test-specific validation), and (c) the bioinformatics analyses provide reliable accuracy, precision, sensitivity, and specificity (i.e., informatics pipeline validation).

Because of the numerous distinctions between Sanger and NGS technology, as well as the increased complexity of NGS compared with the Sanger technology, the measurement of performance characteristics (accuracy, precision, reportable interval of test results, and reference interval) was discussed in depth. Whereas Sanger sequencing provides long, reliable reads with very low error rates, most current NGS platforms have numerous short reads requiring alignment to the genome, with high error rates for individual reads. Therefore, the workgroup recommended establishing cutoff values and determining limitations of NGS quality metrics (e.g., depth and uniformity of coverage, GC and strand bias, allelic read percentages, and base-call quality scores) for each individual test during development and validation, as well as ongoing monitoring of these metrics once the test is established. They also recommended using another validated method, such as Sanger sequencing or single-nucleotide polymorphism array, to provide concordance and confirmation before the reporting of results.

Determining the precision of NGS for each test requires the assessment of reproducibility (interassay) and repeatability (intraassay); however, the high cost of NGS is prohibitive to validate precision across a large number of samples. The workgroup suggested a minimum practice of using 3 reference samples and sequencing them 3 times in the same and different runs, to establish platform precision. For test-specific validation of precision, the participants recommended that additional metrics, including depth and uniformity of sequencing coverage, be included in the evaluative processes, thereby limiting the number of samples required (and the associated costs).

In addition to ongoing monitoring of performance metrics, proficiency testing and external quality-assessment programs are important aspects of NGS quality management. Because a formal proficiency-testing program for NGS is currently not available, the workgroup advised that laboratories establish alternative assessment processes, which might include interlaboratory exchange of previously characterized samples and the use of reference materials, which could be in the format of DNA and/or electronic data files.

NGS technology as a whole is a moving target owing to its rapid, ongoing evolution, as well as the availability of so many different technologies. The guidelines put forth by the Next-Generation Sequencing: Standardization of Clinical Testing workgroup therefore did not address quality as it pertains to all of the intricacies of each different NGS technology, but rather approached the guidelines from a more general standpoint. The participants conceded that the guidelines will need updating and expanding as the NGS technologies change. In the meantime, however, these guidelines are a welcome addition to laboratories that have adopted or are in the process of adopting NGS technology for clinical-test offerings.

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