fant serum samples, only 1 of the 71 infants was misclassified at a 8-μg/L cutoff (NRA, 8.9 μg/L; epiRA, 7.36 μg/L). At a cutoff of 20 μg/L, 2 patient results were discordant, with NRA results of 21.5 μg/L and 21.5 μg/L and corresponding epiRA results of 18.2 μg/L and 19.6 μg/L.

In contrast to other studies, the results obtained with the NRA and epiRA methods agreed well, but no correlation was apparent between the difference between the 2 assays and the epiRA results ($r^2 = 0.016$). Other studies that used LC-MS/MS methods dedicated to quantifying 3-epi-25OHD$_3$ in adults reported that 3-epi-25OHD$_3$ is present at a mean proportion of 4% to 5% compared with 25OHD$_3$. Therefore, readers should be aware that LC-MS/MS methods that do not separate 3-epi-25OHD$_3$ have a bias on that order, compared with assays that distinguish 3-epi-25OHD$_3$ from 25OHD$_3$.

We conclude that separating 3-epi-25OHD$_3$ from 25OHD$_3$ is not important to provide clinically valid results for the majority of adult serum samples. The difference between the results we have presented can be explained by analytical imprecision rather than by 3-epi-25OHD$_3$ interference.

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Standardizing Next-Generation Sequencing Experiments and Analysis Methods

To the Editor:

The past few years have seen the emergence of new strategies for high-throughput DNA sequencing that have invigorated life science research. Because of these technological advances, the available sequencing data continue to expand. Additionally, cost reductions have made access to sequences of entire genomes easier for established laboratories and even easier for small research groups. A lack of consensus exists, however, on how best to design and analyze next-generation sequencing (NGS) studies and how to compare these data to previous and future work. Comparing different sequencing platforms and analysis methods, and thus their interpretations, is becoming more complicated. The main purpose of such studies is somehow getting lost in the process of experimental design as biologists confront new territory with NGS work, which requires handling more complex and powerful statistical methods and produces large data sets. Every new tool or method is being compared with existing methods, and investigators are using statistical arguments to propose that the new methods are better. Kiezun et al. *(1)* have recently highlighted current discrepancies and technical issues in the exome-sequencing approach and in whole-genome sequencing that could bias the interpretation and calling of variants. This situation calls for attention to establish a positive standard that can minimize discrepancies and increase the dependability of all sequencing studies. Some important questions include: Does the assembled and realigned genome represent the correct sequence of the sample?

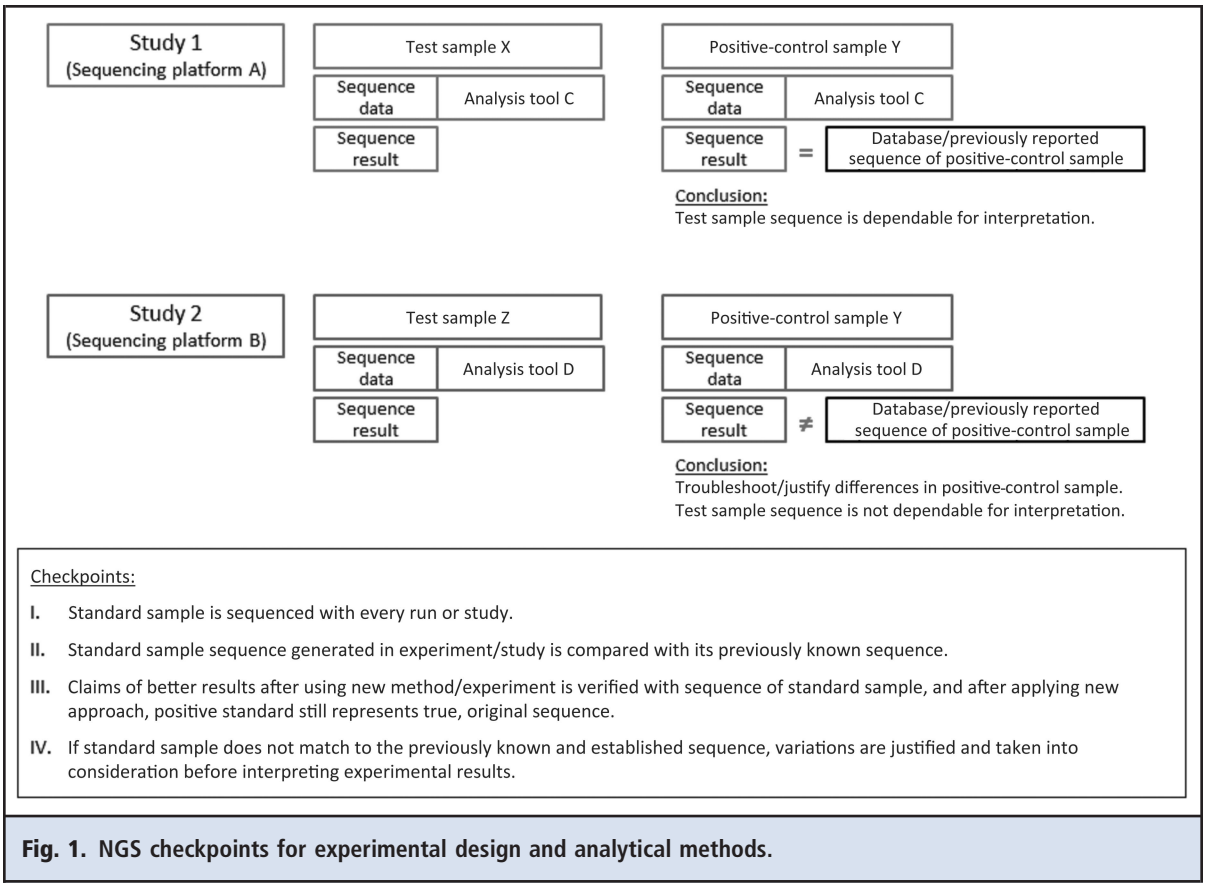

Fig. 1. NGS checkpoints for experimental design and analytical methods.

Are the observed effects real, or are they due to differences in analysis methods? Currently, there is no gold standard for high-throughput sequencing experiments and analysis techniques, but now would be an excellent time to develop one. The development of a sequencing gold standard would allow methods to be compared more easily and allow investigators to better understand the interpretations and nuances that derive from each method.

Three reports on autism spectrum disorder recently published in *Nature* have described exome-sequencing approaches *(2–4)*. The 3 reports concluded that genomic associations were weak and that individual candidate varying loci were incongruent. The 3 studies were different with respect to their approach to exome-enrichment methods, the sequencing platform used, the algorithm and parameters used in calling single-nucleotide polymorphisms, and pathway analysis. These studies confound the question of whether the differences are due to different sample populations or different methods of analysis. At present there is no right or wrong approach, but imagine having a positive standard. By using such a standard sample, we would be better able to compare these studies and determine whether the lack of agreement was simply due to differences in sample populations or to analysis techniques. We would then have the option to compare the 3 studies (and any future studies that use the standard) and then determine their consequences and interpretations to extract the most valuable information. If such standards existed, we could better compare 2 approaches or data from different groups and make appropriate decisions about sequences, genetic variation, or methodologic errors. With the introduction of less expensive DNA-sequencing methods, cost is no longer a justifiable reason as long as the costs support the integrity of the study. The availability of a large quantity of the biological sample that would serve as the standard is critical, and it will probably require commercial production, which might produce batch-to-batch variation in either cloned DNA or a fully characterized and stable cell line. NIST expects to publicly issue the samples as fully characterized standards by December 2014 *(5)*; therefore, it will be possible to mandate such characterized standards for all NGS studies. Manu-

facturers that supply a standard sequence for each batch of cloned standard DNA will make it an attractive choice over the standard cell line, which may be susceptible to genetic drift and not have a batch-to-batch standard sequence supplied by the US Food and Drug Administration or NIST.

Furthermore, most sequencers reserve a lane for a technical control sample. For example, PhiX is currently used in the control lane of the Illumina Genome Analyzer II sequencer. If PhiX were replaced with a biological standard sample (cloned characterized standard DNA or a characterized standard cell line) and those sequences were mapped and assembled with the method used for the rest of the samples, then new approaches could be better vetted, and a lack of agreement could be better understood. Simply replacing a sample of a technical standard sample with a more meaningful biological standard sample would allow us to better resolve many questions without appreciably increasing the cost.

Thus, we propose checkpoints and guidelines for standardizing NGS studies (Fig. 1) that we believe will help emphasize the importance of the positive standard in NGS studies and will help in establishing a consensus.

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Addressing Laboratory Workforce Issues in Australia

To the Editor:

The challenge of training future laboratory directors in a number of countries was discussed by Scott and his contributors in a previous issue *(1)*. In Australia the Pathology Associations Council, made up of representatives of the various national organizations and professions in Australian pathology service delivery, has been addressing similar issues through a number of different initiatives that involve better definition of the role of the scientist compared to the roles of other non–graduate-level professionals in pathology.

The first initiative was to update the competency based standards for medical scientists, given that that there is now a requirement for competency evaluations of staff under AS ISO 15189 (5.1.2) and such standards can be used in all laboratories to define minimum standards for practice *(2)*. These standards have been developed to reflect the contribution normally expected from a person with a degree in a relevant area of science or applied science from an Australian (or equivalent) university, together with 2 years of relevant professional experience in an accredited laboratory. These are the entry-level criteria of a scientist to this profession and reflect a combination of qualifications and skills and the assumption of personal responsibilities and accountability. Although the competencies described are for medical scientists, they are not all exclusive to this group; some (e.g., technical procedures, interpretation, clerical work) may be common to several groups but may be practiced in different contexts. Thus, these same competencies may be applied to all staff in the medical laboratory workforce on the basis of the appropriateness to their role.

The second initiative was to clarify by activity and level of authority the role definitions for the various grades of nonmedical technical and scientific staff employed in pathology laboratories *(3)*. In the scope-of-practice document