



ASSOCIATION FOR MOLECULAR PATHOLOGY

Education. Innovation & Improved Patient Care. Advocacy.

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Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852.

Comments re: Docket No. FDA-2016-D-1270: Draft Guidance on “Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases”

Submitted electronically at www.regulations.gov

To Whom It May Concern:

Thank you for the opportunity to submit written comments to Docket NO. FDA-2016-D-1270 on the draft guidance on “Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases.” The Association for Molecular Pathology (AMP) is an international medical and professional association representing approximately 2,300 physicians, doctoral scientists, and medical technologists who perform or are involved with laboratory testing based on knowledge derived from molecular biology, genetics, and genomics. Membership includes professionals from the government, academic medicine, clinical testing laboratories, and the in vitro diagnostics (IVD) industry.

Our members are among the early adopters and users of next generation sequencing (NGS) in a clinical setting, and have accumulated substantial knowledge and expertise as it relates to this novel and powerful technology. All laboratories, including those directed by AMP members, share the same goal of providing high quality clinical services to patients and their treating physicians. This is our main mission as laboratorians and the manner in which we achieve this is the same for both NGS and non-NGS test procedures.

AMP believes there is a need to modernize the Food and Drug Administration (FDA)’s approach to the regulation of IVD test kits that are manufactured and sold to laboratories. This includes development of more consistent and predictable regulatory pathways, with reasonable requirements that are appropriate to the context in which a test is generally used.¹

¹ In filing comments on this proposed guidance document, AMP does not waive any legal claim that the FDA lacks the statutory authority to regulate laboratory developed testing services. Furthermore, AMP strongly maintains that, to the extent that it is established that the FDA does have such authority, the overwhelming weight of legal authority dictates that the proposed new requirements for laboratories outlined in the draft guidance must be issued through notice and comment rulemaking. Nothing in these comments is intended to impact adversely in any way AMP’s right, alone or in

General Comments:

AMP submitted detailed comments on November 25, 2015 to Docket No. FDA-2015-N-2881 on the “Standards-Based Approach to Analytical Performance Evaluation of Next Generation Sequencing In Vitro Diagnostic Tests,” which can easily be accessed on the AMP website at http://amp.org/publications_resources/position_statements_letters/documents/AMPCommentsOnAnalyticalStandardsforNGS-FDA-2015-N-2881-FINAL.pdf. AMP asks the FDA to refer back to these comments as it considers regulatory policy related to NGS-based IVDs. In those comments, AMP described its efforts in collaboration with other professional societies to develop accreditation requirements and professional guidelines to ensure high quality performance of NGS-based test procedures, its working groups focused on clinical NGS testing, and the requirements in the College of American Pathologists’ (CAP) accreditation requirements and proficiency testing for NGS-based tests.

Molecular pathology professionals are highly trained and well qualified to design and validate NGS-based test procedures using established standards. FDA would be a welcome partner in helping us craft rigorous yet flexible guidelines and standards that laboratories, professional societies, and established oversight and accreditation programs for clinical laboratories could adopt to achieve high quality performance of NGS assays for the benefit of patients. However, AMP believes that these standards are best established through the professional associations’ development of practice guidelines.

NGS is a rapidly advancing technology that requires complex lab processes and bioinformatics to bring usable results to treating physicians. AMP believes that FDA can best contribute to patient care and public health by helping to ensure the performance characteristics of NGS instruments, software, and analyte-specific reagents sold to customer laboratories. However, an approach is needed that is sufficiently flexible to accommodate rapid technological developments and exponentially increasing medical and scientific knowledge in a timely manner. To accomplish this, AMP recommends that FDA partner with private sector organizations and experts to set standards for FDA-cleared or approved instruments, analyte-specific reagents, and software.

Questions Asked in the Federal Register Notice:

1. Does the draft guidance content adequately address the analytical performance of targeted and whole exome human DNA sequencing (WES) NGS-based tests intended to aid in the diagnosis of individuals with suspected germline diseases or other conditions (referred to as “NGS-based tests for germline diseases” or “NGS-based tests” in the guidance)? For example, do the recommendations outlined in the draft guidance adequately address the analytical performance of NGS-based tests used as an aid in diagnosis of patients with signs and symptoms of developmental delay or intellectual disability, undiagnosed diseases, or hereditary cancer syndromes? If not, what additional test design, development, or validation activities are necessary for analytical validation of such tests? Are there specific indications within this broad intended use that require different or additional test design, development, or validation activities from those described in the draft guidance?

No, the draft guidance uses standards and metrics for assays that might not be applicable to all germline NGS-based tests. It’s inappropriate to use a one size fits all approach and as sequencing technologies evolve, it is also imperative that the standards evolve simultaneously. This flexibility to evolve along with the technology and avoid stifling innovation in diagnostics is best achieved through the development of practice guidelines. Furthermore, these recommendations are not appropriate for NGS application used for somatic testing and it would be inappropriate to apply the same analytical standards for all of the potential uses and it would be

combination with other stakeholders, to pursue separate comments, litigation, or other remedies with respect to the proposed regulatory framework or related issues.

dangerous to do so. If the agency plans to use these criteria for another indication of NGS-based IVDs, we request that a subsequent draft guidance be released and stakeholders be provided the opportunity to comment.

The performance standards outlined in the draft guidance are too restrictive and only applicable to limited technology platforms. Laboratories purchase and select sequencing platforms based on their needs, budgets, and services they provide. It's unreasonable to include metrics like those in the draft guidance as not all sequencing platforms can achieve them and further, accurate and precise results are obtainable even at different coverage and confidence interval levels. Instead, a more nuanced approach, such as specifying the ideal but allowing laboratories to make a case for why an alternate performance is acceptable. There are some metrics where this may be possible but in general there are far too many factors influencing the needs of a particular clinical test.

It is important to establish a boundary for the analytical validation of NGS assays that delineates it from the portion that relies on professional interpretation by the molecular professional. There is typically a complicated analysis of sequence variants that occurs. The variant call file (VCF) is an initial file that needs to be further processed and refined in order to determine the analytic validity of the variant call. No automated algorithm can currently identify and accurately characterize all sequence variants. However, a molecular professional can review the data and determine if an orthogonal assay is required to confirm the presence and/or validity of a sequence variant. It's possible to write a SOP for this practice, but it's not possible to write an algorithm or develop a standard that describes this professional aspect. Review of the sequence variants present in a VCF file is a professional activity and not amenable to a standard at the present time. At the current state of technology that is also continually evolving, it would be impractical or impossible to apply a standard to this activity and as such, should remain within the practice of medicine.

Additionally, language in the draft guidance to "validate individual steps" is very confusing, because the only situation where one would need to validate one or more steps of the entire assay separately is if and when they are offered as a standalone test and the draft guidance states that it does not apply to standalone tests. Rather, more appropriate language would be calling for the optimization of the individual steps to the point that they reach target performance characteristics.

2. Do the recommendations in the draft guidance adequately address the analytical validation of NGS-based tests that use targeted panels or WES? Targeted sequencing panels? Are there differences between the use of targeted panels and WES that were not adequately distinguished in the recommendations described in the draft guidance?

Next generation sequencing is a continuum and the laboratory director needs to establish the coverage, technical needs, and parameters of the assay to answer the clinical questions. WES is not different from targeted panels for many steps in the testing process as far as the wet lab process is concerned. The front and back end, however, are extremely different and any guidance on test development and validation must also include upfront test design as well as bioinformatics processing of data at the back end. These steps are mentioned in the draft guidance, but they lack sufficient depth.

At this time, AMP does not believe there is a difference between the analytical validation of a targeted panel and WES. The use of either a targeted panel or WES are heavily influenced by clinical judgement, which places it clearly within the practice of medicine and therefore not within the purview of the FDA. Such clinical judgement activities include but are not limited to incidental findings, filtering WES to gene panels, selection of genes for analysis, interpretation of variants, and the highly specialized clinical judgement that is required to interpret a patient result to aid in determination of a clinical condition.

3. The recommendations in this document focus on WES and targeted NGS-based tests for germline diseases. Are the recommendations outlined in the guidance sufficient to address analytical validation for whole genome sequencing (WGS) NGS-based tests for germline diseases? If not, what additional test design, development, and validation activities are needed to address the analytical validation of such tests?

AMP believes that there is not enough clinical experience yet with WGS to create appropriately informed guidelines or standards. Creation of guidelines or standards for WGS is premature as they would be out of date by the time WGS was more extensively entering clinical practice.

4. Accuracy is generally described using an agreement, typically positive and negative percent agreement (PPA and NPA), between a new test and an accepted reference method. For NGS-based tests, positive predictive value (PPV) may be a more meaningful metric than NPA when calculating the likelihood that a variant call detected by the test is a true positive. If PPV is calculated using only analytical results without taking into account prevalence in a population, it is sometimes called “technical” PPV (TPPV) to distinguish it from prevalence-based PPV. What are the benefits and weaknesses to assessing NGS-based test accuracy using TPPV in addition to PPA and NPA, or instead of NPA?

AMP agrees with the metrics proposed by the FDA and believes that CLIA should be modernized to reflect this new metric. When CLIA was established, NGS technology did not exist and hence, this metric was not considered to be the gold standard like it is today. AMP recommends that the FDA include definitions and harmonize technology for statistical terms applied to NGS including technical positive predictive value.

5. Are the minimum performance thresholds presented in this draft guidance appropriate, or are alternative thresholds more appropriate? Are there “best ways” to determine acceptable thresholds for each metric? Are there performance metrics that do not require minimum thresholds? Are there test scenarios where minimum thresholds are not useful or relevant?

Performance requirements and stated metrics are important to establish, but they should be stated with levels of confidence based on requirements by CAP, ACMG, AMP and other professional organizations. Performance requirements need to be based on scientific evidence, which does not yet exist for most, and the field would greatly benefit from large meta-analyses of the NGS data generated by labs to derive thresholds.

The confidence intervals outlined in the section on “Accuracy” are unrealistic and as stated previously, there is no one size fits all approach for these metrics. Depending on a variety of factors that the laboratory can and should define upfront it will be possible to prescribe general categories of accuracy (e.g., for genes that are

well studied and have a high clinical sensitivity, it is imperative to reach maximum analytical sensitivity). Instead of stating that PPA, NPA, and TPPV be set at no less than a point estimate of 99.9% with a lower bound of the 95% confidence interval of 99.0% or all variant types reported, the FDA should expand this section considerably and lay out a series of scenarios with specific performance thresholds that have to be met. Further, AMP recommends incorporating the orthogonal confirmation layer here. It is acceptable for an NGS assay to have a FP rate provided that the laboratory guarantees confirmation, which will address that issue.

In the draft guidance, FDA recommended thresholds for reproducibility and repeatability that meet or exceed 95.0% for the lower bound of the 95% CI, calculated by conditions tested and genomic context, separately for each variant type. However, the agency failed to provide data to support these thresholds which on the surface, also appear to be unrealistic. For coverage, AMP assumes the 300x figure is a typo or otherwise incorrect.

6. How can bias and over-fitting be minimized or accounted for if known "reference" samples are used as comparators in accuracy studies?

Blinded specimens are useful to control for bias. The individuals performing accuracy studies should be blinded from the previous results of samples used in a validation. In fact, this practice is widely utilized and outlined in practice guidelines².

Thank you for the opportunity to submit these comments. If AMP may be of further assistance, please contact Tara Burke, AMP Senior Policy Analyst, at tburke@amp.org.

Sincerely,

Federico Monzon
AMP President-Elect

² Arch Pathol Lab Med. 2009 May;133(5):743-55