ASSOCIATION FOR MOLECULAR PATHOLOGY
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November 25, 2015

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

Comments re: Docket No. FDA-2015-N-2881 submitted electronically at www.regulations.gov

To Whom It May Concern:

Thank you for the opportunity to submit written comments as part of the workshop on "Standards-Based Approach to Analytical Performance Evaluation of Next Generation Sequencing In Vitro Diagnostic Tests". 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.

Earlier this year, AMP released a proposal to update the CLIA regulations that enhances transparency, ensures quality, and preserves innovation. 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.

Develop Both Design Concept Standards and Performance Standards:

AMP believes the best approach is a hybrid that utilizes both a design concept standard and performance standards. Developing standards to guide the principles of design and validation will help assure the analytical validity of an NGS test procedure. Indeed such efforts, independent of FDA, have already resulted in an important set of carefully considered standards that many clinical laboratories follow. AMP, along with the

College of American Pathologists (CAP), the American College of Medical Genetics and Genomics (ACMG), the Clinical Laboratory Standards Institute (CLSI), and other organizations have already produced laboratory accreditation requirements and practice guidelines that are used to ensure high quality performance of NGS-based test procedures. Examples of peer-reviewed best practice guidelines relating to NGS published by or in collaboration with AMP in 2015 include "Reporting Incidental Findings in Genomic Scale Clinical Sequencing: A Clinical Laboratory Perspective: A Report of the Association for Molecular Pathology"[i], "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology."[ii] In addition to efforts by AMP, there are also a number of CAP publications related to NGS validation standards. [iii,iv,v]

The CAP Molecular Pathology accreditation inspection checklist contains a section solely dedicated to NGS, which is updated on a yearly basis by subject matter experts in response to changing technology and advancing medical and technical knowledge. The CAP NGS accreditation requirements include principles and guidance on validation of NGS tests including recommended quality metrics for test process steps and documentation variance and exceptions. In addition, CAP has begun providing method-based proficiency testing specifically intended for laboratories that perform next generation sequencing. However, it is worth noting that such requirements do not specify concrete performance standards as the unique clinical applications of NGS in each individual case makes it very difficult to arrive at a single set of universally applicable performance standards that would ensure high quality testing for every possible NGS application. AMP also has numerous active working groups developing best practice guidelines that address multiple facets of the clinical NGS validation process, including but not limited to analytical validation, bioinformatics pipelines, and NGS-detected variant reporting:

- Development of Analytical Validation Standards for Next-generation Sequencing (NGS) Detection of Somatic Variants – Chaired by Dr. Lawrence Jennings and in collaboration with CAP.
- Developing Standards for Next-generation Sequencing (NGS) Bioinformatics Pipeline Validation: single nucleotide variants (SNVs), small indels (<=21bp), multiple adjacent (complex) variants occurring within 21 bp of contiguous length and multiple (>=3) variants identified in different alleles at the same or overlapping genetic coordinates – Chaired by Dr. Somak Roy with organizational collaborations pending.
- Intragenic Deletion/Duplication Assessment – Chaired by Dr. Madhuri Hegde.
- Variant Interpretation Test Across Labs (VITAL) – Chaired by Dr. Elaine Lyon.
- Interpretation of Sequence Variants in Somatic Conditions (Cancer) - Chaired by Dr. Marilyn Li and in collaboration with ACMG, CAP, and ASCO.

These NGS subject matter expert groups convened and supported by AMP, often in collaboration with other professional organizations, are actively engaged in developing peer-reviewed, literature-based, best practice guideline resources for the clinical molecular pathology community and other stakeholders. Dialogue with other medical professionals and their organizations, patient advocates, reimbursement and regulatory groups such as the FDA is welcome during this development process. The clinical molecular professionals on these AMP supported working groups volunteer their time and expertise to drive the development of these guideline documents with one overarching goal – improving patient care –and represent the most appropriate resource for guidance in this area of clinical practice.

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.

Need for Standardized Reference Materials:

The modern healthcare system offers great potential for personalized and effective medical care. However, the recognition and implementation of advances in medical research may be hindered by a lack of certified reference materials. Molecular assays provide the cutting edge for many individualized therapies in oncology, transplantation, infectious disease and genetics, but the production of certified reference materials has fallen far behind the technical capabilities of these assays. Reference materials are important to ensuring the necessary sensitivity, specificity and level of reproducibility of intra- and inter-laboratory test results. The best approach to achieve consistent and comparable quantitative data amongst laboratories is by the use of internationally established reference reagents.[vi]

AMP professional committees have collaborated with NIST and the CDC previously to identify, characterize and make available reference materials. For example, AMP, the CDC and the genetic testing community successfully collaborated to address the need for characterized fragile X mutation reference materials by developing consensus characterizations of DNA samples from 16 cell lines with repeat lengths representing important phenotypic classes and diagnostic cutoffs in Fragile X pre-mutation alleles.[vii] DNA purified from these cell lines and a NIST standard are now also available for Fragile X pre-mutation sizing. These can be used for test validation, proficiency testing, and as controls or calibrators. In addition, AMP provided a detailed list of critical needs gathered from the experience of AMP members to NIST in June 2009. AMP and multiple AMP member laboratories have been and continue to be actively involved in NIST's Genome in a Bottle Consortium efforts to provide a high confidence human reference genome standard and other reference materials.

Additionally, within AMP, we have created a Clinical Practice Committee focused on increasing the speed with which the National Institute for Standards and Technology (NIST) and other stakeholders can prepare quantitative standards, which is critical to the national and international laboratory community and their ability to deliver accurate test results. The deliverable would be purchasable, standardized, platform-agnostic reference materials that would ideally be available for inter-laboratory comparison studies and purchase by commercial and clinical laboratory communities. For example, AMP has also participated in the Tapestry Network's Sustainable Predictive Oncology Therapeutics and Diagnostics (SPOT/Dx) Working Group and other efforts addressing development of reference materials for the clinical molecular diagnostics community. AMP encourages FDA to employ mechanisms that facilitate the development of standardized reference materials, including direct funding, as well as partnering with NIST and other experts to foster their development.

Thank you for the opportunity to submit these comments. If AMP may be of further assistance, please contact Mary Williams, AMP Executive Director, at mwilliams@amp.org.

Sincerely,

Charles E. Hill, MD, PhD
AMP President

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[i] Hegde, M et al. *Reporting Incidental Findings in Genomic Scale Clinical Sequencing-A Clinical Laboratory Perspective*. J Mol Diagn. 2015 March; 17(2): 107-117.

[ii] Richards, S et al. *Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.* Genet Med. 2015 May; 17(5) 405-24.

[iii] Gargis, AS et al. *Good laboratory practice for clinical next-generation sequencing informatics pipelines*. Nat Biotechnol. 2015 Jul; 33(7): 689-93.
[iv] Aziz, N et al. *College of American Pathologists' laboratory standards for next-generation sequencing clinical tests*. Arch Pathol Lab Med. 2015 Apr; 139(4):481-93.
[v] Gargis, AS et al. *Assuring the quality of next-generation sequencing in clinical laboratory practice*. Nat Biotechnol. 2012 Nov; 30(11): 1033-6.
[vi] Robertson JS. "International standardization of gene amplification technology." *Biologicals* 26:111-3, 1998.
[vii] Amos Wilson J et al. *Consensus characterization of 16 FMR1 reference materials : a consortium study*. J Mol Diagn. 2008 Jan;10(1):2-12.