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

To Whom It May Concern:

Thank you for the opportunity to submit comments to the US Food and Drug Administration (FDA) June 23, 2011 public meeting on "Ultra High Throughput Sequencing for Clinical Diagnostic Applications – Approaches to Assess Analytical Validity." The Association for Molecular Pathology (AMP) is an international medical and professional association representing approximately 1,800 physicians, doctoral scientists, and medical technologists who perform laboratory testing based on knowledge derived from molecular biology, genetics and genomics. Membership includes professionals from government, academic and community medicine as well as the *in vitro* diagnostics industry. Included within AMP's primary mission is the advancement and education of others about the field of molecular pathology. We thank FDA for the opportunity to share our next generation sequencing (NGS) expertise with the Agency. The subsequent comments address the assessment of analytical validity for diagnostic applications of NGS consistent with the scope of the meeting. However, we are also eager to provide additional input regarding practice issues of clinical validity and clinical utility in the future.

FDA needs to partner with professional associations:

AMP is currently analyzing the complex technical and medical issues associated with the clinical introduction of ultra high throughput sequencing, as well as the associated ethical, social and legal implications, and we are actively working to develop professional practice guidelines for the clinical use of NGS. We have an important reservoir of experience and expertise within our organization, and encourage FDA to allow us to collaborate with you in ensuring that this technology is safely, effectively and appropriately used for the benefit of patients.

Different standards are needed for different types of applications:

Analytical validation requirements for NGS will vary based on the clinical application at issue. For example, panel tests for Mendelian genetic disorders, whole exome (or genome) sequencing for heritable conditions, mutation detection in tumors, transcriptome analysis, and pathogen identification are heterogeneous in their analytical as well as medical features. Performance of,

and coverage needs for, a given platform are likely to differ depending on the nucleic acid and DNA regions analyzed, the type of variations interrogated, the relative allele proportions of particular variants, and whether quantitative or qualitative results are desired. Evaluation should consider the performance effects of relative GC content, homopolymeric and other regions of repetitive sequence, homologous gene regions (pseudogenes), and DNA structural variants, in addition to potential differences in the detection of single nucleotide changes, whether transversions or transitions, copy number variants, or gross chromosomal changes. This necessitates flexibility and individualization in the development of validation protocols, guidelines, and controls on a (clinical) application-by-application basis.

Accuracy of a sequencing result refers to the closeness of the NGS reported sequence, and the true sequence in the absence of variants. In practice, NGS sequence accuracy can be determined by comparison of the NGS to a gold standard method that is itself highly accurate and reproducible such as Sanger sequencing. The required coverage for a particular platform and variant type at a specific level of detection can be established using sample dilutions containing known proportions of variant and wild-type alleles. Assay controls should include a range of variants, and participation in formal proficiency testing should be encouraged whenever possible. Process controls like NA12876 used in NIH's 1000 Genomes Project and sequenced with multiple platforms and the synthetic ERCC RNA transcripts from the National Institute of Standards and Technology are examples of potential standard reference materials.

FDA should assess analytical validity and bioinformatics separately:

The analytical validity of a NGS instrument may be intrinsically high, although data conversion and analysis software may have design flaws or performance limitations. For example, even with accurate raw sequence determination, analysis software may have difficulty identifying insertions and deletions at sufficient coverage, distinguishing pseudogenes or identifying allele dropout. Optimal FDA review of a test system would focus on the analytical validity of the instrument and the performance of the bioinformatics software independently as well as a complete system.

Some aspects of analytical validity fall within the practice of medicine:

NGS platforms may have differing features and performance characteristics that influence their relative desirability for specific clinical testing or particular settings. For example, the read length of some platforms may be too short to enable accurate assessment of large trinucleotide repeat expansions. Others may not be able to distinguish medically relevant *cis*- and *trans*-mutations or alleles. Finally, optimal patient care requires the ability of molecular pathologists and other laboratory practitioners to select individual platforms or technologies based upon the clinical question at issue, and their professional judgment of the most suitable technological approach.

Thank you again for the opportunity to submit comments regarding assessment of the analytical validity of ultra high throughput sequencing. AMP welcomes the continued opportunity to work with FDA on the preceding and other laboratory issues for the benefit of our patients.