Statement to the
Clinical Laboratory Improvements Advisory Committee on
Next Generation Sequencing (NGS)
in Clinical and Public Health Laboratories

The College of American Pathologists (CAP) appreciates the opportunity to provide comments to the Clinical Laboratory Improvements Advisory Committee (CLIAC) on the topic of Next Generation Sequencing (NGS) in Clinical and Public Health Laboratories. As the world's largest organization of board-certified pathologists and leading provider of laboratory accreditation and proficiency testing programs, the CAP serves patients, pathologists, and the public by fostering and advocating for excellence in the practice of pathology and laboratory medicine worldwide.

Clinical Laboratory Improvement Amendments of 1988 (CLIA) provides an adequate baseline to ensure the accuracy and reliability of clinical laboratory results therefore, a specialty or subspecialty for NGS is not necessary given the broad clinical applicability of NGS. However, specific updates to CLIA are needed to address and accommodate the changes in practice and technology. As a minimum requirement, proprietary or complex algorithms (including bioinformatics) used in the generation of a test result are analytical components of high complexity clinical laboratory testing and should be subject to the requirements of CLIA. Hence, any organization performing portions, including application of proprietary or complex algorithmic interpretations to generate an individual result, should be considered a clinical laboratory, whether that organization receives or processes physical specimens. In addition, proficiency testing (PT) should be handled with the same good laboratory practices as performed for other patient samples, including moving samples among multiple sites to complete all aspects of testing.

The CAP Accreditation Program continuously works to improve and update the checklist to reflect advances in NGS technology and the ever-growing diversity of clinical applications to which NGS is being applied. Since 2012, the CAP revised the NGS checklist items six times to add recommended metrics and quality control parameters for this dynamic field.

Regardless of where any of the test components are performed, laboratories should observe good laboratory practices throughout the total testing process. Hence, the CAP recommends the addition of personnel with expertise in bioinformatics, record retention requirements, software maintenance and revamping the PT requirements to test total testing process.

Bioinformatic Personnel

Bioinformatics expertise has minimal overlap with the expertise of a pathologist, laboratorian or geneticist related to NGS technology. Therefore, a category of bioinformatics should be added to CLIA for the personnel performing bioinformatics or pathology/laboratory informatics activities[1]. For instance, it should include positions such as Director of NGS Bioinformatics, Technical Supervisor of NGS Bioinformatics, Clinical Consultant of NGS Bioinformatics, General Supervisor

[1] Pathology/laboratory informatics activities sit at the intersection of information science, information systems, workflow and processes, as well as leadership and management in the clinical laboratories. These informatic activities are how data is acquired, structured, stored, processed retrieved, analyzed, presented and communicated then transformed into useable actionable information.

of NGS Bioinformatics, and Testing Personnel of NGS Bioinformatics. These personnel should possess education and experience in informatics, computer science, genetics or pathology specialty.

Record Retention

The CAP requires its accredited laboratories to retain data including the files used to analyze and generate reports. Specifically, sequence read files (eg, FASTQ, uBAM, BAM, CRAM) and variant calling files (eg, vCF, gVCF) must be retained for a minimum of two years. Importantly, longer lengths of retention may be necessary for specific clinical scenarios. For example, sequence read files on pediatric exome sequencing cases may require storage for several years for availability for re-analysis.

In addition, data files must be retained in compliance with applicable national, federal, state (or provincial), and local laws and regulations.

Also, the CAP requires that test reports for neoplastic conditions be retained for 10 years, and that test reports for constitutional disorders be retained for 20 years.

Software Maintenance

Clinical laboratories can maintain and use previous version of sequence analysis software but should use clinical grade standards with version control, perform validation, and determine data mitigation needs. Clinical grade standards for each of the NGS data analysis file types should be developed and maintained with version control for NGS analysis. New software and upgraded versions of current software should be thoroughly validated prior to implementation to a degree that is appropriate for the specific change(s) being made. Laboratories need to determine whether data migration from the prior version(s) of software to the new software is required. If it is not required or not possible to migrate the data, then laboratories should develop a data access plan to ensure that prior data can be accessed and re-analyzed as needed prior to implementing new versions of hardware and software. This plan can include making backups of prior data and ensuring that these files can be read on the updated/new system or keeping prior software versions. It is critical that data files should incorporate standard provenance metadata that indicate software and data processing library versions and ideally a processing pipeline description so that results can be associated with processing environments.

Distributive Testing Model PT

Also, an important quality metric in determining clinical laboratory testing accuracy and reliability is to perform PT. Laboratories should perform PT by observing the same good laboratory practices they do for patient samples, including moving samples among multiple sites to complete all aspects of testing. Doing so should not constitute intent to commit proficiency testing referral. The CAP PT program allows laboratories to evaluate their performance regularly and improve the accuracy of the patient results they provide. The CAP launched in 2015 PT for NGS where laboratories can test up to 200 variants in a method-based challenge using either gene panels, exome, and/or whole genome sequencing. The initial NGS PT program, designed to assess the ability of laboratories to detect germline variants, was followed by NGS PT for the detection of somatic variants and other NGS clinical testing applications. The programs can test “wet” and

“dry” bench components of NGS testing. Under the current regulatory paradigm, clinical laboratories are unable to test their total test system if portion of the tests are performed in a “distributive testing model” such as bioinformatics and cloud-based software computing. This makes it difficult to assess the complete process and discourages a good quality indicator.

Conclusion

The CAP supports the Agency’s goals of assuring patient access to quality testing by affording the least burdensome approach to oversight. CLIA is a very important tool that can ensure the integrity of clinical laboratory testing. As clinical laboratory testing continues to evolve, the CMS and interested stakeholders such as the CAP will need to work closely to ensure smarter regulations and policies.