CLINICAL LABORATORY IMPROVEMENT ADVISORY COMMITTEE (CLIAC)
NEXT GENERATION SEQUENCING (NGS) WORKGROUP

SUMMARY REPORT

Workgroup Charge

Provide input to CLIAC for consideration in developing recommendations to CDC, CMS, and FDA for assuring the quality of next generation sequencing based testing in clinical laboratory settings.

Workgroup Tasks

- Identify challenges in applying the existing regulatory framework
- Identify challenges and gaps in guidance
- Consider and suggest strategies to address the identified gaps and challenges
- Consider and suggest strategies for assuring workforce competency

Workgroup members used the following questions regarding the application of NGS-based tests for clinical practice to guide discussions. Workgroup members were asked to identify challenges/gaps and suggest strategies to address them.

Workgroup Discussion Questions and Discussion

1. What consultation or other assistance does the laboratory provide to help clinicians order the appropriate tests for their patients?

Workgroup Agreement

- An evidence base is needed to understand the appropriate test to order. If there is no data on whether something is useful, it is very hard to know what to recommend. The community needs outcomes data from studies to determine clinical utility. One of the challenges is defining the outcome, are outcomes just medical outcomes or do they involve looking at the patient and the family as a whole. A top goal for those in the genomic space is trying to encourage people to include at least minimal outcome measures in any research study performed. Physicians should report outcomes to a central database but this would likely need a form of reimbursement to achieve consistent reporting.
- There is a need for better proactive measures such as guidelines or education of providers and education to patients to assist providers with understanding the tests that are available. There is a need for a better understanding of clinical utility. Approaches include long-term outcomes studies, meta-analyses of existing literature to determine trends, institution-based test utilization review mechanisms and one on one discussions between laboratory directors and ordering physicians. There are both funded research and professional society educational initiatives needed.
- There are several guidelines available for oncology testing. Since pathologists direct most testing, they ensure that the test ordered is appropriate. In infectious disease, there are few guidelines on when to order NGS tests. Guidelines should be developed to educate providers about what NGS tests are available, and when they are appropriate.

Workgroup Discussion and Comments

Personnel

- Conversations often occur at the director level but some laboratories hire other staff such as genetic counselors to help ordering clinicians. Genetic counselors should keep current with the appropriate training to assist clinicians in the appropriate test selection.
- Licensure of occupations like genetic counselors can vary and it is a challenge to ensure that the laboratory is not violating any one of 49 different states' laws that is not the home state. The issue of licensure across state boundary lines is difficult, because with a more complex task, physicians do need advice. This is an issue for germline, somatic mutation, and infectious disease NGS tests and results.
- Laboratories need adequate staffing for insurance preauthorization and laboratory consultation with a genetic counselor so the clinician can make an informed decision about testing.

Clinical Utility/ Diagnostic Yield

- There is good data for the neonatal period, the children who have been seen by and diagnosed by clinical genetics, NGS testing provides a close enough diagnosis to shape management and diagnosis made faster than by molecular testing. The test yields less additional benefit in terms of changes in acute management but has significant long term care utility. The biggest changes in management occur for children where nobody, a priori, suspects a genetic disorder. A diagnostic yield is much lower in those cases, 10% to 15%, as compared with closer to 80% in the kids that are seen by clinical genetics. There is a need to move away from the idea that a diagnostic yield is a marker of a good test. A good test should be a test that leads to a change in care, particularly one that was not previously expected.
- There is an analogous situation in cancer. People will say that 80% of patients will have an actionable finding, but then the number of cases where the information is used to direct management is much lower. Again, the challenge is that no standardized definitions of these terms are used.
- The clinical utility is going to vary depending on the cohort used.

Education/knowledge

- A 2018 report from the National Academies of Sciences, Engineering, and Medicine (https://www.nap.edu/read/25094/chapter/1) concerning reporting test results to patients out of a research lab that is not CLIA certified. Many providers that spoke at that conference said that such genetic test reports provided by research groups are difficult to understand.
- Some laboratories report heterozygous patients as having a pathogenic variant for an autosomal recessive disease and when a provider sees “pathogenic” on the report, many do not understand that patient is a carrier for and not suffering from the disease.
- Rare disease patients may have the same or overlapping tests ordered from many different places. A problem for the industry is that in ClinVar, a variant is classified as a “variant of unknown significance”, "likely benign”, and “pathogenic” by different laboratories due to variations in interpretation. Users think that the data is from three different patients but it is all the same one, because nobody is helping physicians order the right test. Thus, one patient is getting many different sorts of testing.

Needs

- Different groups define diagnostic yield differently. If diagnostic yield is the outcome or this is how well this test is doing but the diagnostic yield is defined differently, it does not help clarify the issue. In addition, diagnostic yield can be misleading. A standardized definition of diagnostic yield would be helpful.
- Patients, clinicians, community doctors need more clear communication and available information. Clinicians may order a test because the patient wanted it based on information from the internet. Additionally, patients and many community physicians do not have the means and knowledge to distinguish hype from fact.

2. What consultation or other assistance does the laboratory provide to help clinicians understand and use NGS-based test results?

Workgroup Agreement

- Laboratories should reach out to providers to determine the need for a molecular tumor board or tumor-board style discussion about unusual or atypical results. This enables a discussion of the findings that are not necessarily reportable. Sometimes significant findings have not been validated and are not in the report but a discussion with the providers is warranted. Practices that have Electronic Health records (EHRs) associated with it, must put those results into the health record, just like any other laboratory result.
- Hospitals and laboratories should release results to patient portals, but need to give providers an adequate period of time to communicate with the patient prior to release. This allows the provider time to discuss results with the patient and allow the patient access to their information. Many pediatric institutions do not release any genetic results to their portals and the patient has to come into medical records.
- There is a need for an unbiased workgroup to look at ways for communicating germline genetic data in a standard and structured format to include the genomic variant and include limitations, the interpretation and the phenotypic information that was used for that interpretation.

Workgroup Discussion and Comments

Personnel

- It is important to have the staff that have the ability to assist front line physicians in the practice of medicine and have the conversations with patients about their test results.

Report Information

- If the relevant information is not at the top of the first page, it likely may not get read. The structure of the reports is critically important.
- Sending paper reports and faxing are still common practice. Reports are re-transcribed or sent in accordance with Health Level Seven International (HL7) formatting into an electronic medical record (EMR) that loses all formatting and hampers the ability to back up the information.
- When reports are emailed or faxed, the likelihood that they end up getting into the EMR as machine readable data from their original scanned form is close to zero. This means that

the second time somebody orders the same test, nobody knows it has already been done. This is bad for the patient and healthcare spending.

- There can be challenges with patients getting hold of providers who have the expertise to explain genetic test results
- A tumor board model works well for intra-institutional when you know the doctors and you can talk to them about the cases. Challenges with it are that it requires a significant amount of health care provider time and therefore does not scale well. Having a code or a billing code that would allow for reimbursement or some recognition that this is a clinically-significant activity that involves provider time.
- Results need to be returned so they are not too late to act on. Additionally, sometimes results are not ending up in the hands of parents, in the case of pediatrics, and if the providers are not acting when there's a treatable diagnosis in a timely fashion, we have no business doing the test.

Needs

- Requirements are needed specifically for when an abnormal result is returned or for when a result has a clear, obvious follow-up that could be done. Proactively make calls when delivering those reports. This could help resolve the situation where clinicians do not understand what the test results mean.

3. What are the challenges with developing and performing NGS test validations?

Workgroup Agreement

- Test validation should include from sample collection through to reporting, including not just “easy-to-reach” samples, those previously validated, but also the edge cases and the more difficult samples, this would help understanding of the true test limitations. Quality assurance and quality control remains an ongoing process, and test limitations are better understood as more testing is completed.
- Current professional guidelines for validation exist that are of varying usefulness dependent on the use case, but the recommendations for validation should be driven by the use case for the test in the clinical context and not by NGS as a technology. Validations should be set up and performed with appropriate input from individuals that understand bioinformatics, statistics, and clinical informatics.
- The informatics pipelines should be run in a controlled environment with appropriate version control, with appropriate quality control for both the software and the hardware. There is a need for CLIA to recognize that software as a service in the cloud is a part of modern laboratory testing, and the current regulatory framework does not address this problem adequately.

Workgroup Discussion and Comments

Guidelines

- Many guidelines exists, but gaps exist due to the enormous methodology and different clinical applications that NGS encompasses. Creating an all-inclusive guideline is challenging as there is a fear in being restrictive.
- There are significant numbers of publications from professional organizations that articulate the guidelines for validation and revalidation. The issue arises due to laboratories not having the correct expertise to interpret those guidelines in a hands-on way to develop the validation studies.
- The technology is evolving so quickly that the guidelines are unable to stay current. Many of the guidelines available are already out of date.

Performance Characteristics

- Evaluation of some assay performance characteristics (e.g., lower limit of detection for somatic variant detection in cancer) can be challenging to perform across a target region of greater than 100 kb.
- Determining which performance characteristics to apply for a particular method is going to be application specific when validating a test that uses NGS as its core methodology.
- Much focus is placed on the false positive issue whereas the false negative can be clinically more important than false positives.

Validation Issues

- Microbiology is complicated by the fact that NGS will detect microorganisms that can be challenging for a gold standard, such as culture, to detect. If virulence factor or antibiotic resistance genes are detected, it can be difficult for laboratories to determine which organism they belong to.
- NGS technologies have the potential for detecting different types of variants. There should be a clear definition of your test and what is being detected, the different types of variants, and the limits of detection. There should be some minimum standards for what a test can reliably detect.
- There should be a minimal standard for validations for exomes and genomes based on agreed upon standards of what a basic exome or genome should detect for example agreed minimal size of CNVs that should always be detected, are mitochondrial rearranges included with mitochondrial SNPs.
- The usefulness of a test result for a particular patient is dependent on the clinical context and prior probability. One of the things that laboratories tend not to focus on is validating the phenotypic information and the information coming in on the front end. Then there is no validation on the communication of the results in an understandable way to the provider.
- A common area of discussion, especially for somatic testing, is the number and type of specimens to include in 1) the validation as a whole, and 2) determining the individual assay performance characteristics (e.g., sensitivity, specificity, reproducibility) as part of the validation.

- In terms of performing validations, they have to be structured so that quality metrics are defined and monitored during the tests. Those metrics can be used to establish a quality assurance foundation that can then be monitored in the day-to-day operations of a test. Validation should be leveraged to establish the quality in order to build the foundation for quality assurance.
- One of the main points of validation is to understand the limitation of the test and clear communication of those limitations is needed. In instances where there is a finding that cannot be validated, the communication needs to be that there is a finding but it may not be valid. Confirmatory tests can help with those findings, but, in some cases, there may not be any confirmatory tests and there remains an uncertainty. Ultimately, in the practice of medicine, there is some uncertainty and it is the clinicians call.
- Reagents and instruments are expensive so the validation plan has to be well thought out to find a balance between cost and performing a sufficient number of runs with enough samples to adequately establish the accuracy and reproducibility of the assay.
- A test based on NGS methodology has both a wet lab and a dry lab component, i.e., the bioinformatics and the test has to be an integrated validation of those components. There is a gap in CLIA about the complexity of bioinformatics.
- Validation should be overseen by individuals that are clinical professionals boarded in pathology or clinical genetics.
- In general, the biggest problem with validating NGS assays is the scale of these assays. Because many genes, many regions, and many types of variants are included in a single test, it becomes quite difficult to identify samples that represent all the possible combinations that we expect to detect. Depending on the intended use of the assay, it is often not possible to identify samples that contain all the reportable variants for which the assay is intended. There needs to be some acceptance that not every reportable variant can be "validated," and method-based validations are acceptable.
- Consider a way that materials are generated to be able to test the limits of the technology and to be used in test validations and also potentially as proficiency testing (PT).

<u>Needs</u>

- There should be rigor involved in the laboratory inspection process with regards to the tests that are brought into clinical practice. There needs to be qualified inspectors who understand NGS to actually review the documentation of validation and make assessments as to whether or not they are sufficient.
- Updated and detailed guidance is needed on revalidation and what is required when there are minor changes.
- Access to positive controls is a real challenge, because with rare diseases the actual amount of DNA available from an individual with that variant is limited.
- In order to share controls, there should be a degree of protection or consent in place. It would be useful to have a uniform agreement along with a uniform clearinghouse for positive controls such as the Genetic Testing Reference Materials Coordination Program (GeT-RM).
- A source for a sufficient number and breadth of less commonly examined variant types (e.g., gains/losses, fusions for somatic assays) or newer markers (e.g., microsatellite instability, tumor mutation burden, mutational signatures) is needed.

- There is a need to develop and maintain a database of sequence variants identified by NGS. The major challenge is harmonization and curation of these databases. A significant amount of time is required to collect data on sequence variants to accurately classify them for pathogenicity and to catalog that information in a searchable database.

4. What are the challenges for clinical laboratories in performing NGS quality control and quality assurance?

Workgroup Agreement

- Having daily run quality control (QC) samples that contain every variant type is difficult if not impossible and QC becomes unrealistic and uneconomical. A combination of run metrics and actual QC samples needs to be designed that gives the best assurance of high quality runs.
- There is a need to define the key quality assurance and QC metrics that should be evaluated and monitored for each run as well as analyzed for shifts and drifts would benefit laboratories performing NGS.

Workgroup Discussion and Comments

Guidelines

- The Association for Molecular Pathology (AMP) and College of American Pathologists (CAP) issued the guideline, *Standards and Guidelines for Validating Next-Generation Sequencing Bioinformatics Pipelines* available at https://jmd.amjpathol.org/article/S1525-1578(17)30373-2/pdf. Table 4 of that guideline lists an extensive amount of different quality control metrics associated with monitoring bioinformatics processes.
- *Designing and Implementing NGS Tests for Inherited Disorders – a Practical Framework with Step-by-Step Guidance for Clinical Laboratories* reviews different types of quality metrics that can be used to measure quality assurance. Available at https://www.sciencedirect.com/science/article/pii/S1525157818302563?via%3Dihub.
- Even though metrics exist, sometimes the metrics we need are not any of the ones provided in publications.
- There are lack of guidelines and agreement on what QC metrics to evaluate and expectations of what is needed for evaluation. Even with agreement on appropriate QC metrics, the technology changes so quickly that expectations/requirements will change.

Quality Control Metrics/Quality Assurance

- Trying to establish a longitudinal quality assurance program that monitors all quality metrics is a very resource intensive process.
- Monitoring plays an important role in quality. Monitoring for NGS is different than monitoring for other non-NGS testing. In NGS, multidimensional datasets are utilized and each quality metric can represent a certain step in the testing process.
- An assessment should show a criteria for every single quality metric being used and how the information is being used and if it is being used appropriately.
- A type of contamination that you see in NGS is bleed through of index sequences which could cause barcode misalignment. This type of contamination is different than a no

template control contamination and laboratories should assess and control for the possibility of barcode switching.

- Software developers should produce a code that monitors metrics over time that is easier to understand.
- NGS is a technology, it is not a test. QCs and assurances are built around specific performance requirements of specific tests/applications, not technologies. The quality controls/quality assurance processes need to be tuned to the specific intended use of the application. This varies greatly across germline/Mendelian, somatic, microbiology, NIPT, etc. No single answer/solution will work for all these applications.
- In infectious disease NGS testing, there are issues with reagent and laboratory supply contamination.
- Uncertainty around what quality metrics are relevant to the "edge" cases. As use cases are changed and test definitions are changed, sources for error and the ability to monitor for those sources of error changes because different things cause different errors. So when adding new things into the test, one needs to ensure current quality metrics are capturing errors.
- One benefit of NGS is that there is far more resolution and power to measure quality at multiple stages of this test than for most previous types of tests. Because of this, quality metrics can be established and refined to predict with very high confidence the quality of a call as described in *A Rigorous Interlaboratory Examination of the Need to Confirm NGS-Detected Variants by an Orthogonal Method in Clinical Genetic Testing* https://jmd.amjpathol.org/article/S1525-1578(18)30291-5/fulltext.
- Quality metrics are used to identify confident calls, calls that require orthogonal confirmatory testing, calls have failed quality standards, and identify contamination events. The challenge is that a fairly sophisticated and rigorous team of bioinformatician/statisticians is needed to produce these types of analyses.
- Because different laboratories may establish different thresholds for quality (and this may be appropriate, depending on the applications of the test). Also, regulatory guidelines have been developed for tests that typically do not have the possibility of measuring quality, there is a confusion of ideas about what is needed in terms of running controls versus using quality metrics and measures in the field. This results in the use of controls when other measures are far more indicative of sample performance, and the controls may not be at all predictive of the quality, or potential for contamination, of a specific sample.

<u>Needs</u>

- There is a need to use standard laboratory metrics to ensure that you are testing the right sample from the right patient.
- For microbiology, a challenge is that at least some of the metrics used are organism-specific and experience is needed to define the metrics. It seems like a good amount of experience is needed to determine the proper metrics. Could a laboratory performing NGS utilize the Individualized Quality Control Plan (IQCP) to determine the proper amount of QC needed?

5. What reference materials (physical and/or electronic) are important to include for developing validation and quality control procedures?

Workgroup Agreement

- There are reference materials available, both physical specimens and in silico. There is a need for an increased variety to include "edge" cases. Different types of variants with corresponding metadata are needed to challenge NGS assays and pipelines.
- The Medical Device Innovation Consortium (MDIC) Clinical Diagnostics Cancer Genomic Somatic Reference Samples Project and many other efforts are making headway for the somatic material landscape, but something similar is needed for the germline space.
- A process should be defined to ensure availability of clinical specimens in addition to the collections of panels of pathogens, and other needed reference materials.

Workgroup Discussion and Comments

Currently Available Reference Materials

- The Genome in a Bottle Consortium has selected several genomes to produce and characterize as reference materials. The National Institute of Standards and Technology (NIST) has developed NIST Reference Materials from these genomes, which are DNA extracted from a large homogenized growth of B lymphoblastoid cell lines from the NIGMS Human Genetic Cell Repository at Coriell Institute for Medical Research.
- The complexities involved in 16S rRNA community profiling and shotgun metagenomics methods pose significant challenges for microbiome research. Significant biases can be introduced during sample preparation, DNA extraction, PCR amplification, library preparation, sequencing, and/or data interpretation. To address these biases and provide a measure of standardization within microbiome research and applications, the American Type Culture Collection (ATCC) has developed the ATCC® Microbiome Standards Collection, a set of mock microbial communities comprising fully sequenced, characterized strains that can be used as universal controls for assay development and optimization.
- The CDC's GeT-RM goal is to coordinate a self-sustaining community process to improve the availability of appropriate and characterized reference materials.
- The MDIC Clinical Diagnostics Cancer Genomic Somatic Reference Samples Project is a public-private partnership to guide the development of reference samples that can be used to develop and validate NGS-based oncologic tests. MDIC published a landscape analysis of available reference materials for somatic indications https://mdic.org/resource/srs-landscape-analysis-report/.

Needs

- In silico reference materials represent an opportunity to supplement physical reference materials. Laboratories are willing to share in silico samples under a sample swap agreement, but need a way to identify which laboratories have the samples that are needed.
- A knowledge exchange that laboratories can sign up for and use to query other users for reference materials and samples.

- All the types of variants that are intended to be detected, in all the states (e.g., homozygous, heterozygous, mosaic, somatic, quantitative) should be included in validation. Because many genes, many regions, and many types of variants are included in a single NGS test, it becomes quite difficult to identify samples that represent all the possible combinations that can be detected. This is also true of traditional Sanger sequencing which uses a limited number of samples and extrapolates (based on the detection of known variant and non-variant sites) performance. The challenge is getting samples with truly difficult, but clinically relevant variants.

6. What proficiency testing programs or alternate assessment schemes are available for NGS?

Workgroup Agreement

- In silico PT, such as the one CAP offers, where the participating laboratories send their aligned files to CAP, CAP introduces mutations or variations in the files, and the files are sent back to the lab for analysis. This type of program should be recognized as a potentially useful way of doing PT, particularly, when bioinformatics is involved.
- The split business model for NGS needs to be recognized so that PT for these cases do not represent PT referral.
- There is a need for a formal recognition of a laboratory sample exchange as a PT method. The guidelines appear to suggest that a real human sample is inferior or second best. However, in some cases a real human sample is actually a better PT sample than some performance synthetic PT.

Workgroup Discussion and Comments

PT Resources

- College of American Pathologists (CAP) PT programs
- CDC PulseNet PT (food safety available to public health laboratories)
- Regional consortium round robins
- Internal blinded testing of previously tested samples (alternate assessment)
- Association of Biomolecular Resource Facilities NGS Study (ABRF-NGS)
- PrecisionFDA
- Proficiency programs and alternate assessment strategies exist but there is an opportunity to improve awareness of some of these programs.
- Commercial PT programs are a little bit too easy. While they may be meeting the checkbox of proficiency testing, they may not be meeting the challenge or the spirit of proficiency testing. Encourage those commercial PT manufacturers to be a little edgier while keeping in mind there are challenges for the manufacturer to collect and make these proficiency testing samples.
- The typical methods used to make proficiency samples, such as spiking or splitting, may or may not work or, at least, not be sufficiently challenging because of the technology of NGS. Splitting your sample in the same laboratory is almost too easy. It sounds like the informatics really identify it as a PT sample. Similarly, with spiked samples, it is just obvious that it is spiked and you know where it is, what to look for.

- There may be some alternate solutions such as laboratory sharing where it is a couple of labs doing the same specimen and seeing if they get the same results. Laboratories could also do a "data exchange" of files for PT of the bioinformatics piece. It may not be ideal but it may be an opportunity to overcome some of the matrix issues in all the laboratories, particularly in the microbiology world.
- The availability of some of the data sets for proficiency testing of a portion of the process. It is the earlier stage stuff that is hard.

Samples/PT

- CLIA does not currently recognize the distributive model. When using the distributive model, there is a risk of engaging in PT referral, even though it is treating the PT specimen like a patient specimen. A possible solution is to consider defining the test workflow to include all the entities in that chain of handling the patient sample and/or data. Those entities that are part of the SOP would not be considered referent labs.
- A pure culture does not allow testing the extraction from the native matrix nor does it allow testing of the host response. Some PT is too easy because you can see the spike-in. A genome sample can be biased by taking it from a dried blood spot but you only know that if you take it from a dried blood spot and if you have an appropriate sample.
- There is a gap for somatic interpretation because the guidelines are still relatively recent and complicated. It is not clear how to report variants associated with lower levels of evidence. Variants associated with companion diagnostics and those that are in guidelines can be readily identified and interpreted, but it becomes more challenging to evaluate and categorize other variants.
- HLA testing is a highly specialized area with unique PT needs.
- Every laboratory and laboratory director is scared of failing a PT, which means that laboratories want to pick the easy cases for PT.
- There is no economic incentive for trying to look at more challenging cases. In fact, there is an economic disincentive as well as the fear of failing a PT.
- Split sampling within the same laboratory, with the way the bioinformatics works and the way data is retained, does not assess the director's abilities or the system. Data analysis and interpretations are stored and saved and a variant that has been seen before pops to the top.
- In addition, other proficiency testing surveys for common heritable diseases, mitochondrial disease, pharmacogenetics, cell-free DNA screening for fetal aneuploidy, cell-free circulating tumor DNA, molecular oncology, and hematologic malignancies may generally be used by laboratories performing NGS (along with other methods).

Suggested Solutions

- Distinguish between proficiency at the analytic phase (detecting from sequencing or from sample to variant) versus the interpretation phase. It makes sense to also have that kind of split for infectious disease NGS because there is a great lack of not being able to do their own bioinformatics. Labs are stuck with not having PTs available to them. If they were at least able to do a portion and it would be an extreme improvement on the infectious disease side. Interpretation can be uncoupled by providing in silico abundance profiles.

- PrecisionFDA recently uncoupled the two portions of testing on a recent infectious disease challenge. They assessed the analytic capacity to determine the percent of reads that correspond to each genome.
- Exome interpretation PT is difficult because cases are often published in one form or another. Soon after somebody has cracked a case, the information is available in some public forum, like ClinVar and it is no longer possible to do a clean interpretation.
    - The Critical Assessment of Genome Interpretation (CAGI) group manages to sequester some 10 to 12 cases prior to any publication to give other people a crack at interpreting them.
    - There is another one for genomes, called Mutational Annotation & Genome Interpretation (MAGI) group exchanges samples as a round robin but is mostly interested in the edge cases**.**
    - PrecisionFDA focused initially on germline, and may have added some somatic samples which is more challenging. Liquid biopsy would be more challenging still.

Needs

- There is a need for more microbiology NGS PT.
- "Edge" cases, structural variants, and copy number variations (CNVs) are needed.
- PT/alternate assessments for bioinformatics/data science are needed.

7. What are the challenges to developing, establishing quality control, and implementing a bioinformatics pipeline within a clinical laboratory setting?

Workgroup Agreement

- The role of the bioinformatician should be defined in regards to required education, training, experience, and competency.
- Well characterized reference material, positive controls, in silico data is limited, especially for somatic copy number variants, unusual mutations, and rarely encountered variant types/pathogens.

Workgroup Discussion and Comments

QC Metrics

- The Association for Molecular Pathology (AMP) and CAP issued a recommendation for validating NGS bioinformatics pipelines which includes a list of QC metrics for standardization, https://jmd.amjpathol.org/article/S1525-1578(17)30373-2/pdf. However, later papers suggest that there may be other metrics that are more useful, because this is still an evolving field guidance needs to be flexible https://jmd.amjpathol.org/article/S1525-1578(18)30291-5/fulltext.
- Many QC metrics are dependent upon having access to appropriate controls. There are cost aspects and statistical assumptions with including positive and well controls. Positive controls or well controls on every plate determine something that is practical and cost effective. In using a well control, there is a statistical assumption that batch effects are significant only on the sequencing batch for that particular assay. The CLIA QC requirement for test systems states that a positive and negative control is required for each day of

testing. Some state and accreditation organizations require that every run has to have a positive and negative control, but that isn't practical in NGS. When performing sequencing, good sequence results can serve as an internal control. The same comparison can be made with NGS and metrics such as Qscore, coverage, etc. can be met to serve as the internal control. The CLIA guidelines for NGS controls should be different than the requirements for other non-NGS based tests.

- It is difficult to get a sufficient number of samples to make a significant statistical claim about sensitivity and specificity.
- There are software and hardware version control issues and challenges with automatic software updates. What the requirements are for establishing equivalency of testing through validations.
- Robust sets of metrics and parameters should be identified that always produce accurate and reliable results, and that can accommodate the majority of the situations and data. Any set of data producing metrics outside these established thresholds should either be rejected by the pipeline or at least inform the users of the potential failure.

<u>Costs</u>

- Finding and hiring personnel with adequate training and experience in creating bioinformatic pipelines is difficult.
- Costs are involved in the choice of technology or infrastructure used for data security.
- NGS is not just one pipeline/technology and different algorithms are used that need to be consistently updated.
- Having a clinical bioinformaticist is critically important for maintaining quality testing.

<u>Personnel</u>

- In traditional laboratory testing, there is the medical technology field. Laboratories hire people to work at the bench that have degrees in medical technology with both a core of knowledge and training, and intern or intern-like practicum experiences. There is no equivalent in the field of bioinformatics. There is no formal certification program.
- There are personnel issues to address such as the minimum requirements for individuals performing bioinformatics and how competency is demonstrated and documented. Professional organizations and government agency dialogue needs to occur to develop a path towards a certification for bioinformaticist.
- If personnel requirements are changed, then grandfathering of current personnel will need to occur.
- It is difficult to find informatics and bioinformatics personnel that have been trained or exposed to the unique aspects of clinical laboratory testing to ensure that the testing is performed according to those standards.
- Inspectors need to have NGS expertise to perform the type of inspection needed for laboratories performing NGS.

Bioinformatics Pipelines

- Bioinformatics comprises multiple activities and occupies a very large space. From acquiring the data to making a variant call to variant annotation and interpretation.
- Variant calling can be well defined with a given reference such as precisionFDA which is an open source cloud-based next generation sequencing data platform developed by the FDA which allows researchers to upload and compare data against references. The more open and available the better, to promote sharing and development of bioinformatics frameworks. There are technologies to facilitate this such as cloud-based compute and storage environments, Docker and others, so it is now possible to make a fully portable, reusable, shareable, and validated pipeline.
- The aspect of variability is not limited to public databases, it is also for the technology and the infrastructure that supports the pipelines and their deployment. Challenges are not only from costs of maintaining these databases, but also of finding the right personnel who are able to keep up with the changing landscape.
- Ensure that the people developing the pipelines are people who have the right skill set or you are going to end up with errors without the proper skills to help find the problems.
- The bioinformatics pipeline is heavily dependent upon the quality of the database used to provide data interpretation. The ability to decipher the good from the bad is a challenge for even the experts. Those without expertise are at a complete disadvantage to understand the results.
- For validation purposes, any change in a pipeline might require re-validation.
- The names and versions of each bioinformatics tool used, as well as any parameter that influences the output of the pipeline, should be kept somewhere with the data. This will help to compare results across labs or over time.
- Assuring the quality of outside data would require some type of additional review by the bioinformatician. Public repositories vary in the quality of data curation, and mis-annotations in e.g., GenBank®are then propagated. Annotations are either outdated or plain inaccurate because it is assumed that similar genes always have similar functions.
- The bioinformatics has to match the sequencing, high quality bioinformatics from plug and play tools are not achieved.
- Account for every possible situation, variation in data and exception, which the pipeline can encounter. This means multiple rounds of validation and running simulations to determine how the pipeline will react. Implement multiple failsafe procedures in the pipeline to warn the users in case something happens outside the tested parameters, which could compromise the accuracy of the analyses. Train the end-users in being able to understand the diverse metrics produced by the pipeline and identify situations when the pipeline may have been faulty in the analyses.
- Communication between the clinical laboratory and the bioinformaticians regarding the specifics of a pipeline, what results it should produce, and what format the results should take are challenges in establishing a bioinformatics pipeline. Quality control in a clinical setting is not necessarily translatable to a bioinformatics pipeline. Quality control for bioinformatics analyses is still largely subjective and based on the user's needs or specific goals. Therefore, defining universal guidelines seems inappropriate. Implementing a bioinformatics pipeline within a clinical setting requires educating the end-user of how to

implement it, the meaning of various summary statistics, and the limitations of the analysis. Outdated software or software versions are basically "locked-in" for the duration of the pipeline's use unless it is revalidated.

Needs

- For many clinical laboratories, the major challenges relate to lack of personnel with expertise in bioinformatics and limited detailed guidance available for implementing a bioinformatics pipeline.
- Well-developed pipelines for commercial, academic, public health and clinical sectors would be useful.
- Standardized training data should be made available that can be used to validate the output of each individual pipeline. These data should be species- and/or problem-specific and include typical, expected outcomes as well as unusual cases (e.g. contaminated samples, insufficient reads). This will allow different labs to implement their own pipeline and ensure that their results are acceptable.
- It would be beneficial to assemble a group of subject matter experts in the NGS bioinformatics pipeline who are available to go and assist other laboratories.

8. What are the challenges associated with using and assuring the quality of external data (e.g., data annotation) to inform the bioinformatics analysis?

Workgroup Agreement

- Advocate applying the same kind of standards that are used for ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) for other databases to parse out all of the insignificant or not meaningful results. There is a need for improvement in the clinical annotation and the ethnicity for those databases and an increased ease of submitting data to databases, such as ClinVar and the standardization and formatting of it.
- A mandate for data sharing is needed perhaps linked to laboratory accreditation. For laboratory accreditation organizations to support an accreditation mandate, it would have to be a requirement in the CLIA regulations. There needs to be transparency on the intended use of the data from programs such as the All of Us Research Program (https://allofus.nih.gov/) and the Million Veteran Program (https://www.research.va.gov/mvp/). The data from these programs should be built and curated in a defined way so that it is usable. Some level of standards or regulations should be established for published datasets.

Workgroup Discussion and Comments

Changing Pace of Data

- No metadata or phenotypic data provided such as the nature of patient, origin of the data, how data produced, etc. is typically given to inform the bioinformatic analysis.
- Suggest performing the variant interpretations and allow individual laboratories to use the interpretations in their reports.

- There is value in looking at one database in combination with other pieces of information or metadata that the different databases provide to come to the conclusion. The data integration part is poorly applied.
- There are many types of data annotations some of which are straightforward facts, like reference position, codon, amino acid change, and frequency, however some are assertions of clinical implications, such as ClinVar, Human Gene Mutation Database (HGMD), and in silico predictors like Sorting Intolerant From Tolerant (SIFT) and Polymorphism Phenotypine v2 (PolyPhen-2). For the former annotation types, these typically are versioned and are produced by large consortia and are associated with peer reviewed publications and transparency about methods that enable an assessment of them.
- For databases that assert clinical implications, such as ClinVar or HGMD, re-evaluate every assertion internally using the ACMG guidelines and reviewed by appropriately credentialed clinical and laboratory personnel. This is critical to do because the databases (and publications on which they are based) may be out of date; information about genes and specific variants changes over time, and an assessment of clinical implications requires both up-to-date, accurate external information, as well as internal information (including potentially the clinical context and presentation of the individual being tested). Thus, the assessment of clinical implications of variants is a professional activity that is guided by professional medical guidelines, and while many externally available databases are useful, they are not used in lieu of internal professional re-assessment every time the variant is seen.

<u>Validating Data</u>

- The variant interpretation can vary a great deal not only because of the sequencing technology being used, but because of the quality of the software/database used to annotate the data and the skill of the individuals interpreting the information. There are multiple sources of potential error that can be introduced throughout the course of the workflow, so adequate controls need to be established to monitor each step. This may be challenging at times since it may be difficult to standardize.
- Few variant databases have adequate oversight and quality control to accept data at face value without curation. For example, ClinVar classifications are frequently based on a single submitter so bioinformatic pipelines which filter out variants based on ClinVar classification may miss identification of potentially relevant variants.
- Most databases are for research use only. Centogene has CentoMD® a curated mutation database of rare diseases and the National Institutes of Health's Clinical Genome Resource (ClinGen) is a curated database for use in precision medicine and research. The ClinGen Variant Curation Expert Panel (VCEP) curated variants are a resource of human variants that have been interpreted for their potential association with disease. Test developers can use expert variant interpretations from ClinGen to support clinical validity.

<u>Needs</u>

- There is a challenge with the assumptions that are made when using data. When making assumptions, there needs to be an understanding of how rare or common is the disease, the phenotype, the environment, etc. There needs to be an expertise and qualifications requirements for personnel looking at the data.

- There is a need for higher quality population frequency databases that are curated transparently by individuals with a knowledge of how the data was processed, where the individual came from, and the disease and health state of the individual.
- Very few guidelines exist on how to prepare the data for databases as compared to those on how to use the data.
- Guidance documents address curation of variants, but do not touch on population allele frequency data. Most variants are excluded in analysis pipelines based on population frequency.
- There is a need for a centralized infrastructure and mechanism to share and use data without violating the Health Insurance Portability and Accountability Act of 1996 (HIPAA) regulations and there should be a mandate that this data must be shared.
- A standard consent for exome data sharing or whole genome sharing is needed.
- The Million Veteran's Program genomic database and the All of Us Research Program should be utilized as a resource for shared data.

9. What are the laboratory practices and challenges to NGS software and data management with respect to:

a. Data sharing (e.g., for QC, populating external databases, availability to patients)

Workgroup Agreement

- Sharing data is important not only for the patient but also for the larger community to broaden the medical knowledge. However, there are a number of issues and nuances that require more thought. Sharing data with patients is required by law but due to the nature of NGS, three different files could be released. The raw data file, FASTQ, is very large and minimally processed. The BAM file is aligned with the reference genome and is also very large. The VCF file contains the variant nucleotides and is much smaller compared to the other two files. Some laboratories have standard procedures for releasing raw data and others are still determining how to move forward. Appropriate federal regulatory generalized language should be developed that could be shared with patients who are requesting their genomic data, or their NGS data, to alert them of the benefits and the risks of taking that data for further analysis outside of the clinical lab and the limitations of the type of data file they receive.
- Logical Observation Identifiers Names and Codes (LOINC) has been mandated for several federal regulations for electronic interoperability, and there are limitations for LOINC around genomic results. Additional expertise in NGS molecular diagnostics and bioinformatics should be represented in the HL7 Clinical Genomics Working Group to look at how to plan mandating federal interoperability and examine various methods, including Systematized Nomenclature of Medicine – Clinical Terms (SNOMED CT) and LOINC.

Workgroup Discussion and Comments

Files

- According to HIPAA, the patient requesting personally identifiable information (PII) must receive the test report, the full variant information, and other information in the record set

concerning the test. Most have interpreted this as all of the file types but there are concerns such as:

- The test panel may only be part of the original panel so the raw data has more information than the clinical laboratory actually analyzed. In this case, should all the raw data be released?
- The raw data can be used by a third party or application that make different interpretations and the original laboratory cannot guarantee the quality of those subsequent calls.
- Data may include regions that the clinical laboratory has not validated and is not part of the clinical interpretation. But another lab reusing the data may include that region and there should be common data standards.
- Other challenges are how data should be encrypted and how to effectively communicate to the patient that different parties who analyze the data have different methods and standards to determine what variants are important.

Data Sharing

- Data sharing among colleagues is key to the advancement of science, but that requires curation and detailed metadata to put the data into context. Patients or their lawyers are in most cases insufficiently trained to fully understand the intricacies of the data.
- Also, hospital "permission to treat" forms cover the hospital doing almost anything they like with your data, including letting it be used by other parties without further consent by the patient.
- LOINC has been mandated for several federal regulations for electronic interoperability and there are limitations for LOINC that have been previously registered with them as well as with multiple federal agencies. Continuing down the LOINC path may restrict reporting data in a meaningful way.
- The cost of sharing data, storing data, is extremely high.

Needs

- Sharing relevant information while respecting patient privacy is extremely challenging. There is concern about if active consent from the patient or caregiver is required or if passive consent is sufficient. There is a need to think about ways to allow for more transparency on what is allowed including genomic and other clinical data, possibly through patient portals and Electronic Health Records (EHRs). Two examples are hospital labs filling out consent forms which typically have an opt-out system but the family will never see that form so they do not have the ability to choose.

b. Data and software storage/retention/upgrades

Workgroup Agreement

- Policy should be informed by what would be the long-term use of the data, such as patient health, mining for important clinical data, etc. Current CAP accreditation requirements indicate that a laboratory must store sufficient data files to allow for reanalysis of a patient sample for a minimum of two years. A survey of the laboratories performing NGS testing

should be performed to determine the goals of data storage, how long data storage must be maintained, and what is the current state of practice for data storage. If possible, follow-up with a survey of these three points from the larger community.

Workgroup Discussion and Comments

Storage/Software

- IT infrastructure, files stored, retention time, lossless vs lossy compression (no data loss vs some data loss), latency (how quickly lab can retrieve stored file), federal, state, local/organization regulations are all factors when determining the cost.
- Cloud-based storage can be used in a HIPAA-compliant manner, but this requires ensuring that all appropriate physical, network, and process security measures are in place and followed.
- For bioinformatics, many do not have a system to test upgrades before they take them into production and vendors sometimes will assume permission to upgrade software unless explicitly told not to without the lab validating it ahead of time. There should be regression test suites, preferably automated, for all pipeline changes. Unit testing on a piece of code that has been changed is advisable to see the ripple effect or the impact on all downstream systems.
- Sequencing instrument software may also be affected by upgrades that can negatively impact results.

Re-verification/re-validation

- For files, verification is required when compressing or encrypting data to ensure results have not been impacted.
- After an upgrade or changes to the bioinformatics pipeline, what previous versions need to be retained? Are previous versions of primary, secondary, and tertiary analysis pipelines needed to be retained such that a previous system can be reconstituted to run data through it? There are technologies out there, especially with container infrastructure, where you can actually do that.

Needs

- There is a need for clarification on what the minimum standards for storage would be, for example, at least a GVCF (genome variant calling file) or equivalent should be stored. The responsibility to reanalyze and how often can affect this decision.
- Guidance is needed on what types of file(s) would be HIPAA-compliant and how state statutes of limitations on medical records impact policies, especially regarding pediatric patient data. For example, some states require medical records be kept until age of majority plus 7 years.
- Additional information is needed to determine what files to store; raw data vs processed vs intermediate files. Policies and procedures need to be developed for re-analysis of previously tested samples as software changes and databases are updated.
- After a system upgrade, there should be guidance or best practices regarding the number of reverification samples required.

10. What are the real world and recommended practices and challenges in reporting clinically significant secondary findings that are not related to the test that was ordered?

Workgroup Agreement

- Secondary findings are not the same as incidental findings. These challenges of secondary findings exist outside of the oncology space. A **secondary finding** is when a test for a specific purpose is ordered and information about other genes that have clear action ability could be assessed from the test. The ACMG has recommended we additionally offer that information to patients. An **incidental finding** is when a specific test is ordered for a specific reason and we are looking only in those genes. However, while looking in those genes, we found a variant that has different implications. Relevant agencies and the committee need to be careful about the appropriate use of secondary and incidental as not being interchangeable. They do have very significant differences in both consent and legal frameworks within relevant states. Encourage the committee to be specific with what they mean and to be cognizant of the fact that there are established medical laws and practices around how to manage this. This is not unique to NGS or to genetics and be careful about genetic exceptionalism when thinking about this.
- An unbiased workgroup should be created to review and refine the list of what would be appropriate returnable findings. The scope may be widened to provide guidelines for the implementation of the list and what is the right way to disclose in the proper context, recognizing the local law challenges, if a test is validated or not for the finding, and other nuances that have been observed.

Workgroup Discussion and Comments

Secondary Findings

- The gene list is relevant to particular disorders.
- Some laboratories may limit the reportable findings to that list and not going beyond that if they feel that it is appropriate.
- Laboratories may not report mutations of clinically diagnosed disorders, such as neurofibromatosis, because it is not on the ACMG list. This leads to confusion and may cause the provider to think the results are incorrect because there was a sample switch or other issue.
- Who should follow-up with the patient about these types of findings, the provider who ordered the test or someone else? The provider who ordered the test may not have the expertise or knowledge to be able to advise the patient appropriately. Physicians are obliged to offer this to their patients but then they really don't feel prepared to know how to follow up appropriately. It would be useful to provide more information to the clinicians of resources available that can help their patients follow up.
- There are many unusual microbes that may be found and it is difficult to understand what their value may be in a clinical context.

Consent

- Typically in infectious disease, there is not the whole consent process that genetic testing has. The laboratory is testing for a known pathogen or panel of pathogens. It would be a paradigm change to require consent because of these potential incidental findings and it would be very difficult due to the time-critical nature of the testing.
- There can be state laws that do not allow certain results (like HIV) to be released without having informed consent from the patient. The laboratory can tell the provider that it would be a good idea to get HIV testing and then release the results. This is not to prevent people from knowing their results; it is to make sure that the patient has access to counseling services and that's the only way they can think of to do it.
- There are certain default positions that we have to take while recognizing autonomy and this is very context-specific. It really depends on the time and the situation as much as anything else. Even in germline genome-wide sequencing there cannot be blanket statements.
- Testing to diagnose or treat somebody, is very different both legally and morally, than choosing to go and look for risk factors or other problems that may happen in the future. So specifically in California, there are different consent requirements.

Needs

- There is a need to review and refine the ACMG list and incorporate anything that labs and providers have learned over time.
- Overall, consent is a challenging process to go through. One has to keep in mind State regulations, legal frameworks, patients, along with the conflict between the 'duty to disclose' versus 'the patient's right not to know.' Some laboratories use the opt-in, opt-out mechanism.
- It is challenging to be able to provide secondary or incidental finding in the appropriate clinical context.

11. What training and competencies are performed or considered essential to demonstrate that qualified personnel are testing specimens and analyzing data?

Workgroup Agreement

- CLIA requirements for competency assessment can be applied to the wet bench side of NGS testing, but are difficult to apply to the "dry" bioinformatics side.
- There exists a gap on personnel requirements for the bioinformaticians as the current CLIA personnel requirements do not fit clinical informaticians. There will need to be a way to distinguish those that are part of the analytical process and those that are not to determine if they should meet CLIA requirements. For those that are part of the analytical process, CLIA personnel regulations would apply and currently CLIA does not have personnel qualifications for bioinformaticists.
- An information gathering survey should be developed for laboratories performing NGS including state agencies to collect job descriptions, and qualification requirements to define the roles of informaticians in NGS assays and understand their educational background. The

data will promote an understanding of the current field and what is considered acceptable personnel requirements.

Workgroup Discussion and Comments

Personnel

- Bioinformatics personnel should be split into categories, those that are software developers who develop the pipeline, the data scientists that manipulate and pull the data, and those that do the analysis in terms of the clinical interpretation.
- An issue with a bioinformatics terminal degree is that you are not well versed in the biology side nor the computer science side.
- A determination should be made to distinguish if the people who are developing pipelines are doing it as part of a clinical laboratory or if they are producing a product that is used by a clinical laboratory. If they are producing a product, then that is an FDA-regulated in vitro diagnostic. The program should be validated not the writer of the program.
- A bioinformatics team that designs, prototypes, and assembles software as part of the clinical laboratory do not fit into the current CLIA requirements, but should have assessments and degree requirements such as a minimum of a master's, sometimes PhD terminal degrees, in bioinformatics.
- It would be potentially premature for there to be federal regulations around personnel requirements when there is no specified certification that bioinformaticists have to have for this body of knowledge. It would be premature for the federal government to mandate what is required for these individuals to be able to be competent without having any competency associated.
- An example of what might qualify as an analytical role in post-NGS and pre or post-Sanger confirmation, is a job description called QA Analyst. This position would look at the traces, the chromatograms from Sanger, and look at the NGS pileups to ensure the variant calls were correct. They are not bioinformaticists nor computer scientists but they play a critical role in the operational process.

Competency Assessment

- Personnel performing wet bench experiments for NGS are required to be licensed and perform competency assessment based on CLIA or other accreditation agency requirements.
- For the wet lab component certified technicians are used and require internal training, and annual competency assessments.

Needs

- Under the current CLIA regulations, there does not exist a personnel degree requirement for a bioinformaticist. CLIA regulations lack provisions for recognition of others with specialized knowledge.
- A recommendation is needed to form a workgroup to define the different subcategories, and sub-disciplines of NGS testing personnel. This group could sort through the types of personnel at the level of CLIA, CAP, and individual states requirements.
- Samples are needed for edge cases to test the pipeline to determine competency.

12. How does the laboratory determine the total annual testing volume for NGS?

Workgroup Agreement

- The majority of the workgroup members indicated that test name/report is how they determine the total annual testing volume for NGS.
- Common Procedural Technology (CPT) code stacking is still a problem.
- Code by accession (e.g. trios). Depending on how you bill, you may or may not bill CPT codes for additional genomes of the parents.

NEXT GENERATION SEQUENCING (NGS) WORKGROUP ROSTER

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Peer Reviewed Publications on Guidelines, Standards and Best Practices for Germline and Somatic Variant Diagnostic Testing Based on Next Generation Sequencing (Including Validation, Variant Interpretation and Proficiency Testing)

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Other Guidelines and Documents for Next Generation Sequencing

1. Guidelines on Next Generation Sequencing from American Society for Microbiology (ASM): Applications of Clinical Microbial Next-Generation Sequencing Clinical Tests (April 2015) http://www.asmscience.org/docserver/fulltext/ngsreportweb.pdf?expires=1549998336&id=id&accname=guest&checksum=79BE1C3D78CE3D21B55BA13C4B831A20
2. Guidelines on Next Generation Sequencing from the American Society for Histocompatibility and Immunogenetics (ASHI): Standards for Accredited Laboratories Next Generation Sequencing Guidance. Section D.5.2.11 (January 2018). https://cdn.ymaws.com/www.ashi-hla.org/resource/resmgr/docs/accreditation/standards_/20180525_2018ashi_standardsa.pdf
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