COLLEGE of AMERICAN PATHOLOGISTS

November 14, 2022

Matthew Fickie, MD
Senior Medical Director for Medical Policy
Highmark
120 5th Avenue
Pittsburgh, PA 1222

Dear Dr. Fickie,

The College of American Pathologists (CAP) is opposed to Highmark's new credentialing requirements for next-generation sequencing (NGS) testing that impose independent third-party validation of NGS results and/or variants of uncertain significance (VUS) interpretation. ***The purported scientific basis for the use of this proposed proficiency testing methodology is contrived, misapplied and misrepresented, and further confounded by an apparent conflict of interest. For these reasons, we believe this new requirement would not only fail to improve patient care but would distract laboratories from clinically relevant aspects of testing that could improve quality. Therefore, we request that Highmark abandon this requirement***. The CAP requests a meeting with you to further discuss the issues included in this communication.

Specifically, the CAP disagrees with the recommendation for use of the Center for Genomic Interpretation (CGI) or a similar group for validation of laboratory standards, and the assertion that certifications from the CAP and Clinical Laboratory Improvement Amendments (CLIA) are not sufficient guarantees of quality from high-complexity laboratories. We welcome this opportunity to provide Highmark with information to support our request for Highmark to withdraw this requirement. We also welcome this opportunity to provide further information on both the CAP Accreditation and the CAP Proficiency Testing Program. In addition, the CAP would like to provide additional details regarding observed shortcomings of CGI and the SPOT/Dx pilot study that was used to justify the use of CGI's products. Finally, the CAP is concerned with the burdens placed on laboratories that conduct NGS testing to require new credentialing requirements that lack merit.

CAP Accreditation

The CAP is a deemed accreditor on behalf of the Centers for Medicare and Medicaid Services (CMS), under the CLIA law, with a longstanding and respected record of serving the public by overseeing the quality of laboratory operations and proficiency testing. The CAP is approved for all the specialties listed in the CLIA regulations that were first published in 1992. As an approved accreditor, the CAP Accreditation Program is re-evaluated by the CMS at least once every six years. The CAP has always maintained the maximum approval limit since initial approval in February 1995. There are six other CMS-approved accreditors, some of which are not eligible for all CMS specialties. The accrediting organization (AO) must maintain acceptable performance or be reassessed as a deemed AO. The CAP Accreditation has always been well below the federally established discrepancy limit.

The CMS reviews the details published in the CAP Accreditation Checklists prior to annual publication. The CAP Accreditation Checklists consist of 21 discipline-specific documents including Molecular Pathology. The first CAP Molecular Pathology Checklist (MOL) was published by the CAP in 1993 and the NGS section of the MOL checklist was first introduced to the laboratory community in 2012. The NGS accreditation requirements were developed by molecular pathology experts who donated their time to create a standard for the emerging technology when there were very few laboratories performing this testing on patient populations.

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The CMS also conducts annual validation inspections in CAP accredited laboratories by State Agency surveyors and evaluates and tabulates any discrepancies related to the CLIA regulations.

Challenges to CGI Assertions

CAP PT samples already include the same kind of *in silico* challenges proposed by CGI. In addition, CGI's assertions about the lack of precision of NGS testing lack sufficient supporting data. The main data that has been provided in support of CGI comes from the SPOT/Dx pilot, an industry-sponsored small pilot study of 19 labs testing a handful of contrived samples at one point in time, with numerous methodological flaws and biases. The CAP has significantly more data on laboratory performance than the SPOT/Dx pilot, assessing hundreds of laboratories at a time over multiple years. The CAP has transparently published this data on laboratory performance, as well as trends in performance over time.

As noted previously, the CAP PT program is designed and evaluated by panels of diverse, mostly volunteer experts from around the country, not from a single, or limited number of individuals who may have financial conflicts of interest. This is a greater depth of unbiased expertise from which to draw when making determinations about the importance of variants, expectations for their clinical detection and interpretation.

Variant interpretation is the practice of medicine; it requires incorporation of clinical and other laboratory data, in context, to arrive at an appropriate clinical recommendation. We agree it would be ideal if NGS were like an automated chemistry analyzer, requiring only manufacturing controls so that a sample can be put on an instrument and with the metaphorical push of a button, results automatically populate the medical record, no interpretation necessary. However, this is not reflective of the current state of genomic medicine, and therefore should not be subject to proficiency testing. Furthermore, no entity, including CGI, can reasonably claim to serve as the gold standard for the interpretation of variants.

CAP Proficiency Testing Program

The CAP's PT program was available as a CMS approved PT program when the CLIA PT regulations were implemented in 1994 and has maintained approval every year since. The CAP molecular PT programs are designed and reviewed by panels of 20-30 diverse, experts specializing in genomic medicine from multiple sectors of the specialty, who are blinded to the identities of the performing labs. This structure renders CAP incapable of exerting favoritism when evaluating the quality of labs' performance. CAP launched their first wet specimen NGS germline proficiency testing (PT) program in 2015 and then added somatic solid tumor and hematologic NGS PT programs in 2016. The original In Silico Bioinformatics PT programs were launched in 2016 with multiple other PT programs (undiagnosed disorders for exome and trio analysis) being added in subsequent years.

SPOT/Dx Pilot Study

The SPOT/Dx pilot study has been inappropriately used by CGI to support their marketing campaign that claims a need for consensus performance standards that go beyond routine proficiency testing. This industry sponsored pilot had stated goal of addressing a perceived gap in the standardization of personalized medicine laboratory testing for targeted therapies in cancer.

The CAP was asked to support this study, which quickly revealed numerous flaws in the study design as well as conflicts of interest that appear to be major contributors to the biased analysis and interpretation of the pilot results. The authors of the SPOT/Dx pilot technical manuscript have

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attempted to use a limited set of extreme samples with ultra-rare variants and low variant allele frequencies to make broad generalizations about overall laboratory performance.

The CAP attempted on several occasions to address the biases in this pilot while it was underway. However, the authors of the study declined the CAP's input, leading all CAP contributors to withdraw as participants in the pilot. Some of the most significant methodological flaws and biases in the SPOT/Dx pilot include:

1. **Most false negative errors were seen with variants engineered at, or below, the limit of detection of most assays.** The nature of next generation sequencing is to slightly underestimate the variant allele frequency (VAF) of mutations because mutant sequences are more likely to be filtered out (i.e., removed from analysis) in informatics pipelines than are wild type sequences. This has negligible impact, except at assay decision cutpoints were filtering out of even one variant molecule could flip a sample across the decision threshold. By engineering variants at 5% VAF - exactly the cutpoint for many of these assays - this slight underestimation (say 4.97%) resulted in a false negative result for labs that apply a hard cutoff (rather than rounding) for reporting.

2. The **variants chosen to evaluate LDTs were enriched for multinucleotide variants (MNV) - that are exceedingly uncommon in the genes tested (KRAS, NRAS).** MNV's make up less than 0.1% of all mutations in colorectal cancer, yet MNVs made up 22% of the variants in the pilot., , including some variants that have never been seen in nature. The inability of some LDTs to consistently identify these variants was misrepresented as an indication that these LDTs would harm a significant segment of the colorectal cancer population.

3. **Aside from being rare, MNV's are susceptible to misidentification due to a very specific limitation of bioinformatics processes that required a customized solution in the early years of NGS, when this study was launched.** Many standard informatics processes at that time interpreted MNV's as 2-3 separate SNV's (single nucleotide variants), which required manual review and a merging process to combine into a single MNV. This was a known limitation at the time, and most laboratories had a process for this for the BRAF gene, where MNV's are common, but not for all genes, including the KRAS/NRAS genes, where MNV's are not common (as above).

4. **The error associated with the MNVs did not generate false negative results, but rather misclassification of the specific sequence change in the gene, which would have no bearing on patient management or outcome.** For example, a KRAS G12E MNV mutation would be reported as a KRAS G12D SNV (single nucleotide) mutation. These mutations are treated identically and have the same prognostic implication, so there would be no clinical consequence to this misidentification. However, this was misrepresented as a potentially harmful error.

5. **The laboratories participating in SPOT/Dx were held to a standard defined by unproven claims by the Praxis assay manufacturer, not its actual documented performance.** The package insert from the Praxis assay manufacturer, in which they present the evidence supporting their claims, lists the variants that they claim to be able to detect, and also the data supporting their claims. Reviewing the supporting data shows no evidence that the great majority of the claimed MNVs were ever tested during their assay validation. Only one sample, with one MNV, was documented to have been tested by the Praxis assay.

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6. Variables involving its design and intent were introduced into the pilot that were not made clear to the participating laboratories; **they were told about the Praxis assay, but it was a very high-level summary and did not go into the appropriate needed details, nor were they provided the package insert with the Praxis assay claims.** Laboratories were misinformed that the study was intended to determine the suitability of different types of reference materials for their assays. Had they known that they needed to detect MNVs at VAF's of 5%, they could have employed the appropriate manual data analysis procedures to enable a fair assessment of assay performance.

Unnecessary Burden on Laboratories

In addition to relying on a flawed study, the new credentialing requirements place an unnecessary and harmful burden on laboratories. These additional requirements would take significant time, resources, and education to implement, on top of the sufficient guarantees of quality currently provided by the CAP Accreditation and the CAP Proficiency Testing Program. Laboratories across the country are currently facing tremendous administrative and reporting burdens while continuing to work in a strained environment because of the COVID-19 public health emergency and increasing respiratory illnesses. Additional unnecessary changes risk interfering with laboratories' ability to provide important care and services to patients. Now more than ever, patients and their treating physicians need to rely on the expertise of pathologists and the availability of appropriate testing.

Next Steps

The CAP is eager to work with Highmark and other stakeholders to understand any concerns about the quality of proficiency testing programs. These PT programs continuously evolve to meet the changing needs of laboratories and the CAP welcomes feedback on their design. We are also requesting a meeting to discuss our concerns with the implementation of Highmark's new credentialing requirement. For more information, please contact Pamela Wright, the CAP's Senior Director of Economic and Regulatory Affairs at pawrigh@cap.og.

Sincerely,

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