

September 11, 2016

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

Re: Docket No. FDA-2016-D-0971 for “Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers; Draft Guidance for Industry; Availability.”

Submitted electronically at www.regulations.gov

To Whom It May Concern:

Thank you for the opportunity to submit written comments on the draft guidance entitled “Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers”. The following comments are made on behalf of the following organizations:

- The American Society for Microbiology (ASM) is the largest single life science association, with over 42,000 members worldwide. Many ASM members have primary involvement in clinical laboratory medicine including individuals directing clinical microbiology, immunology and molecular diagnostic laboratories, individuals licensed or accredited to perform such testing, industry representatives producing laboratory tests, and researchers involved in the development of new technologies.
- The Association for Molecular Pathology (AMP) is an international medical and professional association representing approximately 2,300 physicians, doctoral scientists, and medical technologists who perform or are involved with laboratory testing based on knowledge derived from molecular biology, genetics, and genomics. Membership includes professionals from the government, academic medicine, clinical testing laboratories, and the in vitro diagnostics (IVD) industry.
- The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the U.S. and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.
- The Infectious Diseases Society of America (IDSA) represents over 10,000 infectious diseases physicians and scientists devoted to patient care, disease prevention, public health, education, and research in the area of infectious diseases. Our members care for patients of all ages with serious infections, including meningitis, pneumonia, tuberculosis, HIV/AIDS, antibiotic-resistant bacterial infections such as those caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and Gram-negative bacterial infections such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, emerging infections such as Middle East respiratory syndrome coronavirus (MERS-CoV), Enterovirus D68,

and Ebola virus disease, and bacteria containing novel resistance mechanisms such as the New Delhi metallo-beta-lactamase (NDM) enzymes and others that make them resistant to a broad range of antibacterial drugs, including one of our most powerful classes of antibiotics, the carbapenems (carbapenem-resistant Enterobacteriaceae, or CRE).

- The Pan-American Society for Clinical Virology (PASCV) is an international society whose members perform laboratory testing for the detection, quantification, and characterization of viral pathogens. PASCV membership includes physicians, doctoral-level scientists, and medical technologists, representing academic medicine, clinical laboratories, commercial laboratories, the pharmaceutical industry, and in vitro diagnostics manufacturing.

This draft guidance includes information on FDA recommendations for the regulatory oversight of infectious disease next generation sequencing (ID NGS)-based tests. Our members are among the early adopters and users of NGS technology in a clinical setting, and have accumulated substantial knowledge and expertise as it relates to this novel and powerful technology. In order to meet the FDA's goals of creating a suitable and predictable pathway, the following comments should solely be viewed in reference to FDA oversight of in vitro diagnostics (IVD) manufactured for distribution.

NGS-Based IVD Tests in the Absence of a Known Pathogen (Agnostic Testing)

The development of NGS technology has enabled laboratories to explore the underlying genetic contributions of microbes to health and disease in an unprecedented way. Agnostic testing in particular holds the most promise because it does not require that a professional have a predetermined biomarker in mind that could limit the understanding of what may be contributing to a patient's clinical presentation. We encourage FDA to explore the following examples:

- Detection/discovery of a new pathogen belonging to a recently discovered Astrovirus clade causing fatal encephalitis in an immunocompromised adult.¹
- Detection of a known but relatively rare pathogen as a highly unexpected cause of encephalitis in a young boy.²
- A sizable fraction of bacterial isolates obtained from intensive care units identified as clinically relevant corresponded to previously undescribed species in a study using prospective whole genome sequencing.³

¹ Naccache SN, Peggs KS, Mattes FM, Phadke R, Garson JA, Grant P, Samayoa E, Federman S, Miller S, Lunn MP, Gant V, Chiu CY. 2015. Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. *Clin Infect Dis*, 60(6): 919-23.

² Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, Salamat SM, Somasekar S, Federman S, Miller S, Sokolic R, Garabedian E, Candotti F, Buckley RH, Reed KD, Meyer TL, Seroogy CM, Galloway R, Henderson SL, Gern JE, DeRisi JL, Chiu CY. 2014. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med*, 370(25): 2408-17.

³ Roach DJ, Burton JN, Lee C, Stackhouse B, Butler-Wu SM, Cookson BT, Shendure J, Salipante SJ. 2015. A year of infection in the intensive care unit: Prospective whole genome sequencing of bacterial clinical isolates reveals cryptic transmissions and novel microbiota. *PLoS Genet*, 11(7): e1005413.

- *Bartonella quintana* Aortitis was detected in a man with newly diagnosed AIDS who presented with months of back pain and fever.⁴
- Deep sequencing of 16S rRNA was used to implicate *Actinomadura madurae* as the cause of mycetoma in a diabetic patient when culture and conventional molecular methods were overwhelmed by overgrowth of other organisms.⁵
- Rapid 16S rRNA next-generation sequencing can catalog bacterial species in mixed specimens from which usable data cannot be obtained by conventional clinical methods.⁶

These tests exemplify the importance of further improving regulatory pathways for ID devices that use NGS technology. As such, we believe that the focus of the guidance should be on agnostic testing.

As FDA has noted many times regarding NGS-based testing in general, the possibility of identifying any number of variants renders the traditional FDA regulatory template for agnostic tests unworkable. While FDA states that the draft guidance would apply to both targeted and agnostic NGS-based IVD tests, a great deal of the information relayed in the document is panel-specific and sufficient information is not included for how FDA's thinking applies to agnostic tests. While we agree with the definition of agnostic testing provided on lines 257-262, we are concerned about how a "panel-based approach" can be used for evaluation of such an assay (Line 266). Such an approach would mitigate the potential power of this technology; namely, to diagnose infection without *a priori* knowledge of what the etiologic agent of infection may be.

As an example, it is unclear what FDA's expectations are with regards to LoD for agnostic tests. FDA should acknowledge that medically relevant organisms that have not been included in LoD studies may be detected by agnostic approaches. Reporting of such low-level results may be critical to patient care and as such, a framework for balancing the risk of false positives with the risk of false negatives must be more clearly addressed. Further, FDA should acknowledge that clinically relevant and irrelevant levels of organisms cause disease only in certain situations. For this reason, the professional that performs and interprets the test is a vital component of its ability to contribute valuable information to patient care. (Additional comments on the role of the professional are provided more fully in a separate section below.)

More importantly, concern that the level of risk is elevated when certain targets are included makes FDA's approach specifically for agnostic NGS-based tests unclear because agnostic tests could in theory detect any number of targets. We disagree that the inclusion of certain targets would elevate risk in such a way that a test would need to be classified as Class III. The detection of Hepatitis B and Hepatitis C virus, HPV, and HIV using NGS-based tests would represent a very small proportion of the potential pathogens detected. Furthermore, it is more than likely that a patient's positive status for

⁴ Lee SA, Plett SK, Luetkemeyer AF, Borgo GM, Ohliger MA, Conrad MB, Cookson BT, Sengupta DJ, Koehler JE. 2015. *Bartonella quintana* Aortitis in a man with AIDS, Diagnosed by needle biopsy and 16S rRNA gene amplification. *J Clin Microbiol*, 53(8): 2773-6.

⁵ Salipante SJ, Sengupta DJ, Hoogestraat DR, Cummings LA, Bryant BH, Natividad C, Thielges S, Monsaas PW, Chau M, Barbee LA, Rosenthal C, Cookson BT, Hoffman NG. 2013. Molecular diagnosis of *Actinomadura madurae* infection by 16S rRNA deep sequencing. *J Clin Microbiol*, 51(12): 4262-5.

⁶ Salipante SJ, Sengupta DJ, Rosenthal C, Costa G, Spangler J, Sims EH, Jacobs MA, Miller SI, Hoogestraat DR, Cookson BT, McCoy C, Matsen FA, Shendure J, Lee CC, Harkins TT, Hoffman NG. 2013. Rapid 16S rRNA next-generation sequencing of polymicrobial clinical samples for diagnosis of complex bacterial infections. *PLoS One*, 8(5): e65226.

these diseases would be known or could be confirmed through other types of testing. We believe that special controls and practice guidelines could be developed and put into place so that all ID NGS-based tests presented only an intermediate risk to patients. In particular, we believe it would important for it to be specified that the detection of variants associated with diseases that FDA considers to be Class III should be verified with additional testing. FDA should work with professional organizations, such as ours, to develop additional controls that could be employed to ensure that innovation can continue in this space.

In general, we appreciate FDA's intention to provide clarity on how the current laws can be applied to ensure accuracy and precision of ID agnostic NGS-based tests, but we believe that FDA needs to reformulate the draft guidance to address how each section applies to agnostic testing.

Targeted NGS-Based IVD Tests

As noted above, FDA emphasizes the risk of ID NGS IVDs several times in the guidance, but we maintain that all ID NGS-based tests may be able to be classified as Class II if the appropriate controls and practice guidelines are utilized. We encourage FDA to work with volunteer organizations such as ours to develop these criteria. Again, we believe one of these criteria to be that the detection of variants associated with diseases that FDA considers to be Class III should be verified with additional testing. More specifically relating to targeted tests, we believe that a multiplex PCR test or conventional Sanger sequencing could serve as a predicate device for NGS-based tests where there are known targets in a number of situations, and encourage FDA to explore this as an option.

Regulatory Flexibility and the Role of the Laboratory and Health Care Professional

Overall, we believe that FDA should be flexible with regards to the review of ID NGS-based tests. There are certain statements that fail to consider the reality of test development and the context in which tests are performed. There are several points that we would like to emphasize in particular.

The role of the laboratory and health care professional is essential to ensure proper test performance, and clear and timely communication of results. Performing and interpreting an NGS-based test is a complex process that requires a great deal of training and expertise. We believe that assurance regarding the involvement of qualified laboratory and health care professionals should enable the FDA more regulatory flexibility.

In this guidance, FDA's language suggests significant hesitancy as to conveying novel or emerging information to patients and their treating physicians. To provide regulatory clarity, and to facilitate the evaluation of novel variants, the guidance should state that manual variant and organism interpretation are not considered part of the test.

Inclusion of a detailed description of presumptive contaminants (e.g., skin microbiota) is complex and the requirement to provide this is almost impossible; what is a contaminant in one patient, may be a cause of true infection in another (e.g. *Propionibacterium acnes* in blood culture from a patient without indwelling devices vs. *P. acnes* in blood culture from a patient with prosthetic valve endocarditis). What constitutes a true contaminant should be left to the laboratory and health care professional performing

and interpreting the test and the patient's physician(s). Therefore, identifying contaminants should be outside the purview of this guidance document. However, there is need for a recommendation regarding the proper clinical interpretation and reporting of test results by the patient's ordering physician and associated MD consultants.

It is impossible for a test developer to validate "all specimen types" for which the agnostic test would be used. The power of agnostic sequencing relates to its ability to be applied to almost any specimen type. Moreover, even modifications to the specimen type for targeted assays are also made by the clinical laboratory using the test on an as needed basis. These modifications are within the practice of medicine, and this should be acknowledged as outside the jurisdiction of FDA.

FDA should define "emerging infectious agents." For example, *Corynebacterium striatum* is considered an "emerging pathogen" yet is frequently isolated in clinical laboratories. FDA states a test developer should include information about "additional measures that should be instituted if infection with a novel or emerging infectious agent is suspected based on current clinical and epidemiological screening criteria." This type of information is best developed and relayed to the treating physician via appropriate trained laboratory and health care professionals using the test. FDA should clarify the statement and consider providing examples to illustrate the point that the Agency is trying to make.

The draft guidance says, "The addition of these new sequence targets should be reported to the Agency at the time of emergence discovery and before diagnostic use." This requirement could impede the practice of clinical medicine in the event that such an agent were detected in a clinical specimen. For example, would the laboratory withhold the identification and susceptibility of a bacterial pathogen in a positive blood culture while FDA decided if the organism was "novel or emerging?" A famous example is the teenager who suffered progressive neurologic damage (NEJM) until leptospirosis was identified by NGS in the CSF. With appropriate antibiotic therapy, the patient, who had been ill for months, was cured within days. It would have been unethical to withhold the NGS results in such a case.

FDA should make changes to acknowledge that an interpretive component, which is within the practice of medicine and thus outside of FDA's jurisdiction, will be needed when algorithms are less well known. We support the inclusion of this caveat in labeling materials to ensure that laboratories using IVD kits are fully aware of this limitation. However, we do not think that it should automatically preclude the test from clearance or approval.

Lastly, it should be recognized that the inclusion of patient-specific information is a crucial component of interpreting test results and as a result, we recommend that FDA refrain from being too prescriptive about what results should, and should not, be included in the test report.

Use of Regulatory Grade Databases

Much of the guidance document focuses on the proposed requirement to use a database comprised of regulatory grade sequences, specifically the FDA-ARGOS database. While certainly the availability of an appropriately robust, comprehensive and agile database would be highly desirable, this database is in its infancy and is presently unsuitable for use. In particular, the power of agnostic NGS lies in its ability to theoretically identify any microorganism that may have been in part sequenced before – bacteria, viruses, fungi and even parasites. The FDA-ARGOS database thus far only contains bacterial entries that

are extremely limited in scope and depth of coverage. Furthermore, the guidance document refers to the identification of virulence and resistance genes, which would be impossible without sufficient database diversity to sufficiently cover the pan-genome of any organism contained therein. Finally, the FDA-ARGOS database does not yet catalog sequence variants associated with antimicrobial drug resistance, a critical application of NGS-based infectious diseases testing. While the FDA should be commended for taking on the endeavor of establishing a regulatory-grade database, restricting manufacturers and clinical laboratories to use of this database will impede development of this field and certainly negatively impact patients from receiving a timely diagnosis.

Given the nascent stage of development of such databases, we encourage the FDA to consider the negative effect any restriction (by FDA or others) would have on the advancement of NGS. If FDA were to restrict access to only specific databases, it would severely limit our ability to best serve our patients. It is imperative that qualified laboratory personnel maintain the ability to access and use any and all information available for a given sequence variant and use their professional judgment on how to weigh the available evidence. The information gleaned from a database is considered alongside a great wealth of other information before a report is issued to a treating physician and the patient, and FDA should not put forth recommendations that even inadvertently encourage laboratories to rely on a single source of information.

As noted by the American Academy of Microbiology (AAM) colloquium of NGS in recommendation 7.1, “these databases should not be a static collection of information but should allow for local, national and international data exchanges that are in line with agreed standards. Additional databases are not needed, but existing databases should establish quality metrics or curation strategies to promote confidence in clinical decision making”. Please provide information on how this recommendation can be met by use of the FDA-ARGOS database.

The lack of completeness of the FDA-ARGOS database also raises questions regarding the accuracy of its use as an alternative comparator particularly for agnostic direct from specimen sequencing where only a handful of contigs may be detected and could have significant homology with other closely related organisms. Please clarify if specificity can be adequately established using a database with such limited organism and strain representation.

We are also concerned with FDA inclusion of only high quality target sequences. This may inadvertently lead to the exclusion of important information that may be crucial in helping pathologists, treating physicians and the patients making treatment decisions. As an example, a full genome is not available for every described pathogen. The involvement of various experts should be incorporated into decisions made about whether a target sequence is “regulatory grade.”

Conclusion

Once again, we appreciate the opportunity to provide these comments on the present draft guidance. Given that there are substantial gaps in the draft guidance document, most notably regarding FDA’s thinking on agnostic testing, we recommend that FDA issue a second draft. We enthusiastically offer our organizations’ assistance and expert volunteers to resolve the issues discussed above. Please feel free to contact the following organization representatives for additional information:

- ASM: Kimberly Walker, PhD, 202-942-9262 or kwalker@asmusa.org
- AMP: Tara Burke, PhD, 301-634-7962 or tburke@amp.org
- APHL: Celia Hagan, MPH, 240-485-2758 or celia.hagan@aphl.org
- IDSA: Greg Frank, PhD, 703-299-1216 or gfrank@idsociety.org
- PASCV:
 - Alexandra Valsamakis, MD, PhD, 410-955-5077 or avalsam1@jhmi.edu
 - Matt Binnicker, PhD, 507-538-1640 or binnicker.matthew@mayo.edu

Sincerely,

American Society for Microbiology
Association for Molecular Pathology
Association of Public Health Laboratories
Infectious Disease Society of America
Pan-American Society for Clinical Virology