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Federico Goodsaid, Ph.D.
Associate Director for Operations in Genomics
Office of Clinical Pharmacology
Office of Translational Science
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
10903 New Hampshire Avenue, Building 21, Room 4524
Silver Spring, MD 20903-0002

Comments Regarding FDA Guidance for Industry: Pharmacogenomic Data Submissions – Companion Guidance

Dear Dr. Goodsaid:

AMP is an international not-for-profit professional association representing over 1,500 physicians, doctoral scientists, and medical technologists who perform molecular diagnostic testing based on nucleic acid technology. AMP members practice their specialty in widely diverse settings: academic medical centers, independent medical laboratories, community hospitals, federal and state health laboratories, and the in vitro diagnostic industry. In this capacity, AMP members are involved in every aspect of molecular diagnostic testing: administration and interpretation of molecular diagnostic tests, research and development, and education. As the only professional association dedicated solely to molecular pathology, AMP provides national leadership for the advancement of safe and effective practice and education for molecular diagnostic testing in the health care industry.

We have reviewed the draft guidance and offer the following comments:

This guidance switches between RNA and DNA without a clear transition between sections (*i.e.* line 249). Individual sections of the document seem reasonable but overall the document's content seems unfocused. Some topics are covered in great detail and others not at all, such as massive parallel sequencing (*e.g.* Solexa or 454 technologies). Our understanding is that this document is to aid submissions for genomic classification of tumors. There is currently no section related to this.

Suggestions to improve:

The need to run external controls with every batch of specimens was overlooked. We recommend adding, "During validation of each assay, one must develop and implement a plan for using external controls, including the frequency/content/acceptable limits of each control, so that each of the critical outcomes is checked at a reasonable periodicity."

Also overlooked, except for a brief mention on page 7, is consideration of spike-in controls to complement endogenous controls for both DNA and RNA targets. We recommend adding,

"Spike-in controls of known amount and content should be used to check for adequate extraction, reverse transcription, amplification, labeling, hybridization, and data analysis. Limits on acceptable performance should be set."

This document focuses on a subset of the quality assurance parameters that every CLIA-certified clinical laboratory must meet. While CLIA regulations are difficult to read, a straightforward and somewhat more stringent list of CLIA requirements are laid out in checklists from the College of American Pathologists, particularly in the" Lab General checklist" for fundamentals of good laboratory practice, the "Molecular checklist" for gene expression profiles and other DNA and RNA-based assays, and the "Cytogenetics checklist" for CGH arrays. These checklists are freely available on the CAP website, www.cap.org.

Another reference for quality assurance documents is the Clinical and Laboratory Standards Institute, www.clsi.org. Some of the most relevant guidelines include "Collection, transport, preparation, and storage so specimens for molecular methods", and "Verification and validation of multiplex nucleic acid assays."

On page 14, we recommend adding, "For any studies in which humans will be treated/managed differently based on the results of their genomic tests, it is essential that test results be reliable and therefore testing must be performed in a CLIA-certified laboratory. For retrospective studies on human specimens, it is recommended but not required that testing be done in a CLIA-certified laboratory."

Proper gene nomenclature should be used to identify the genes or gene products that were tested and any genetic alterations that were identified.

Some specific comments are listed below:

Line 39 - Divide chapter into: "EXPRESSIONAL ARRAYS" and "PROTEIN ARRAYS". Describe "PROTEIN ARRAYS".

Lines 51-52 - Replace "some steps before and after RNA purification should be carefully planned to ensure quality during isolation and confirm high quality before use" with "some steps before and after RNA purification should be carefully planned to warrant high quality of extracted RNA before use"

Line 69 - Add: (remove the period (.)) "and that gloves are being worn at any time when RNA is handled."

Line 79 - Add: …and assessed for suitability and "interference in a downstream applications" in a pilot …

Lines 90-92 – Please clarify the bulleted points.

Line 105 - Add: …percent tumor, "presence of necrotic tissue (if applicable) and/or other tissues (e.g., muscles, connective tissues, blood vessels etc.)" in the sample …

Line 115 - Add … with RNA isolation procedures and "does not interfere with downstream RNA analysis" may be …

Line 123 - Add: … Gloves should be worn at all times.

Line 173 - Consider adding new chapter "RNA isolation from solid tumors":
Similarly to the section "RNA isolation form whole blood and PBMCs" a separate section should be devoted to RNA extraction from solid tumor depicting issues such as tumor tissue homogeneity, presence of non-tumor tissue in the tumor sample, assessment of tissue for necrosis etc.

Line 177 - Add: … or in TE buffer "addition of RNA stabilizing agents and" should be stored …

Line 180 - *RNA storage.* For long term storage, RNA samples could be stored at -20ºC in 75% ethanol.

Line 184 - Replace: …of "RNA samples" can be… With: …of "extracted RNA" can be…

Line 199- replace the word "clear" with "distinct"

Lines 215+, Section B, Page 6 - We agree that SOPs are useful and training is important not just for labeling but for all steps. Studies should be done to show that the chosen methods of specimen collection and handling, RNA extraction, cDNA preparation, gDNA amplification, and array hybridization, and data analysis, yield reliable and reproducible results. Consider developing quality checks at key steps during analysis.

Line 247 - Add… is maintained "and that all the reagents are stored and used" in accordance with the SOPs "and manufacture's recommendations"…

Line 277 - Replace …"microarray chip details"…With: … "microarray platform details:"…

Lines 283 and 287- replace the word "chips" with "arrays" or "microarrays"

Lines 467 - a common contributor to poor DNA quality was overlooked, namely cross-linking by formalin fixation.

Line 287 – change the word "customer" to "custom"

Line 288 - Add: … protocol, "sequence of used probes"…

Line 418 – please clarify Q-PCR and RT-PCR…real time PCR, Reverse transcriptase PCR. It is preferable to write out the abbreviation to not cause confusion.

Line 444 – What is CPD?

Line 450-452 - Please revise this paragraph. We would suggest the following paragraph: When DNA is isolated from blood, many of the reagents traditionally used by molecular biologists in the purification of nucleic acids, e.g., EDTA, detergents such as sodium dodecyl sulfate (SDS), phenol, ethanol and chaotropes such as guanidinium HCl, are inhibitory to amplification enzymes.

Lines 463, 486, 588 – please change the word "lab" to "laboratory"

Line 475 - Add: … methodology "and interpretation of obtained results"

Section IV, lines 489-508 – Please clarify why Proficiency Testing (PT) or Alternative Assessment (AA) is included in this document. PT/AA is a required function of clinical laboratories. It is not clear why this section is in the document to be used for data submission.

Line 550 - Add: …profiling experiments "(mRNA, proteins)", genotyping …

Line 551 - "*single-nucleotide polymorphism (SNP)*". I would suggest changing that term for more appropriate terms: **"sequence variant"** or **"allelic variant".**
See a paragraph below from the Human genome Variation Society, HGVS; http://www.hgvs.org/mutnomen/recs.html.

Mutation and polymorphism

*In some disciplines the term **"mutation"** is used to indicate "a change" while in other disciplines it is used to indicate "a disease-causing change". Similarly, the term **"polymorphism"** is used both to indicate "a non disease-causing change" or "a change found at a frequency of 1% or higher in the population". To prevent this confusion we do not use the terms mutation and polymorphism (including SNP or Single Nucleotide Polymorphism) but use neutral terms like **"sequence variant"**, **"alteration"** and **"allelic variant"**. Human Mutation (Vol. 19 (1) of 2002) contains several contributions discussing these issues as well as the fact that the term **"mutation"** has developed a negative connotation (see Cotton RGH - p.2, Condit CM et al. - p.69 and Marshall JH - p.76).*

Line 584 - Remove: the extra period (.)

Line 599 - Remove: the extra period (.). Add: space between the bullet and the text

Line 677 – Please remove the extra period (.)

Thank you for the opportunity to comment on this very important document. Please do not hesitate to contact Vicky Pratt, PhD, Clinical Practice Committee Chair, at victoria.m.pratt@questdiagnostics.com if we can be of further assistance.

Sincerely,

Gregory J. Tsongalis, PhD
President