



**Association for Molecular Pathology**  
*Promoting Clinical Practice, Basic Research, and Education in Molecular Pathology*

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Dockets Management Branch (HFA-305)  
Food and Drug Administration  
5630 Fishers Lane, Room 1061  
Rockville, MD 20852

**Docket Number 2006D-0012: AMP's Response to Guidance for Industry and FDA Staff – Pharmacogenetic Tests and Genetic Tests for Heritable Markers**

AMP is an international not-for-profit educational society representing over 1,400 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. For the last several years AMP has provided national leadership for the advancement of safe and effective practice and education for molecular diagnostic testing in the health care industry.

AMP's Mission Statement identifies the Society as "dedicated to the advancement, practice, and science of clinical molecular laboratory medicine and translational research based on the applications of genomics and proteomics." Our goal is to represent all members regardless of the setting in which they practice because they are united in the end intent to provide high quality, relevant information for the purpose of directing individual and patient community health management. We acknowledge, however, that different perspectives may emerge from those widely diverse settings. In those instances, our primary responsibility is to comment from the standpoint of molecular testing laboratories and the patients they serve.

Our comments are listed below:

**Section II.** – We recommend revisions to include language highlighted in red.

Fundamentally, testing for pharmacogenetic polymorphisms and genetic mutations is the same and yields the same general types of results. The target populations, **intended use**, and how the test results are **interpreted** and used however.....

**Section III B. Device Design**

The FDA is recommending that the manufacturer provide a recommended method for DNA extraction. There are many commercial reagents for DNA extraction, we would recommend that either multiple extraction platforms are recommended or that the laboratories are recommended to provide their own extraction system and that minimum

and maximum DNA input requirements should be submitted to the FDA for device design. Also important is the specimen collection device. DNA can be extracted from a variety of materials using a variety of collection devices. We would recommend that laboratories use any collection device and that again, minimum and maximum DNA input requirements should be submitted to the FDA for device design.

**Section III C.1** - We recommend revisions to include language highlighted in red.

You should demonstrate the device's ability to accurately and reproducibly differentiate genotypes, alleles, or mutations using both the lowest and highest nucleic acid input concentrations recommended in product labeling. **The requirements for specificity, accuracy, and reproducibility are particularly important for genes that have known homologues, genes with high homology to another gene(s) or pseudogene(s) either within the same or different genetic family, and also genes that are or can be present in more than two copies (such as CYP2D6).** When fresh samples for rare alleles, genotypes, or mutations are scarce, we will consider the use of **archived, retrospective samples, or verified DNAs characterized from cell lines such as those available from Coriell Cell Repositories.** Although natural samples are preferred, we will also consider artificially prepared materials, such as plasmid DNA or amplified gene segments.....

The FDA recommends that natural samples be used and if not available artificial materials are permitted. While we concur with the sentiment of preference for natural samples, we are concerned that validation using artificial samples may cause an assay not to perform as expected in patient samples. We realize that homozygous samples and other rare variants may not exist and that manufacturers need to validate their platform. Therefore we would recommend that in the package insert for all mutations not validated with a natural sample be interpreted with caution. We would also recommend that manufacturers validate when possible closely spaced mutations that may interfere with assay performance.

**Section III C.2** - We recommend revisions to include language highlighted in red.

If you intend to provide reagents for specimen processing, you should demonstrate that the chosen sample preparation method consistently provides quality nucleic acid samples that yield reproducible test results for each specimen type with which your test is intended to be used. (See also Section 4, Precision.) If your sample preparation method involves preparation of an RNA intermediate, you should evaluate your procedure to ensure that residual contaminating genomic DNA is either absent or, if present, will not interfere with assay results. **If the RNA intermediate is to be converted to DNA, information on the efficiency of such conversion and appropriate controls monitoring the conversion should be described. RNA degradation during extraction and subsequent manipulation prior to the conversion to DNA should be addressed. If the sample is handled or stored as an RNA intermediate prior to the conversion to DNA, the stability in comparison to DNA should be addressed.** If you do not intend to provide sample preparation reagents in your kits, you should provide specifications for assessing the quality of the assay input sample so that users can validate their own sample preparation method and reagents. You should provide justification for these specifications in the submission. **A selection of nucleic acid isolation and purification methods or kits that the device has shown to yield reliable templates should be described. Recommendations on specimen collection, transport and pre-processing storage and handling, as well as**

information on the presence of additives (e.g., preservatives, anticoagulants, stabilizers) that may interfere with sample processing or with the use of extracted nucleic acid in downstream applications (e.g., hemolysis due to suboptimal storage or the presence of heparin in blood specimens which is known to inhibit PCR-based amplification methods) should be described. We also recommend that you carefully characterize sample stability and validate your storage and handling recommendations. Information on elution and storage buffers (e.g., composition, pH, stability) should also be available.

**Section III C.3** - We recommend revisions to include language highlighted in red.

You should investigate the range of nucleic acid sample concentrations that reproducibly yield acceptable results. Information about the source of nucleic acid (specimen type) and specimen processing (DNA extraction procedure) should be specified (e.g., because the presence of inhibitors might influence minimum input requirements). You should also determine the minimum amount of testable input DNA sample that provides acceptable performance and approximate the amount of patient specimen needed to generate this minimum amount of sample.....

We have noted that the nucleic acid template concentrations are not always a factor that affects the performance of pharmacogenetic and genetic tests, Such as the pH, the strandedness or grade of purity of the nucleic acid template (see CLSI MM17-P). However, we applaud the FDA's efforts in making sure that the manufacturer investigates a wide range of nucleic acid template concentrations and include information about the range so that the device reproducibly yields acceptable results.

**Section III C.5** - We recommend revisions to include language highlighted in red.

If you do not provide external controls or calibrators, you should indicate commercial availability of these materials or describe a method that users can follow to prepare them (or both). You should describe the reactions and functions monitored by internal controls. For different technologies, these controls may differ, but the controls should enable users to determine if critical reactions have proceeded properly. Controls should contain nucleic acid levels at the low and the high end of the input concentrations recommended in product labeling in order to adequately stress the system.

We recommend that manufacturers also indicate in analytical studies what should be done if controls are not available.

**Section III C.6** - We recommend revisions to include language highlighted in red.

- a description of specimen collection and storage prior to processing, preparation, including analyte levels, matrix, and how levels were established
- information about substances known to interfere with cut-off value determination

**Section III C.7** – We recommend adding the following to the list

- Influence of storage conditions prior to processing

**Section III C.9** - We recommend revisions to include language highlighted in red.

We recommend that you assess the potential for sample carry-over and cross-hybridization, and that you provide instructions in your labeling for preventing carryover and **reducing or eliminating cross-hybridization. Carry-over or cross contamination should be tested with the highest value of nucleic acid concentration range (or copy number) as determined by the analytical studies.**

**Section III D.2** -

There are many commercial manufacturers of thermal cyclers and DNA extractors, we would recommend that either multiple extraction platforms and thermal cyclers are recommended or that the laboratories are recommended to provide their own system and that minimal and maximum DNA input requirements should be submitted to the FDA for device design and that thermal cycling parameters be provided.

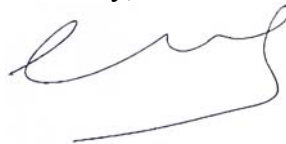
**Section III E.1** - We recommend revisions to include language highlighted in red.

For pharmacogenetic and genetic tests, we recommend that you validate your assay by performing studies that compare results obtained with your device to those obtained with bidirectional sequencing. Bidirectional sequencing is considered the reference method for sequence analysis (sometimes also referred to as the "gold standard"). For large deletions, rearrangement or insertion mutations, or other cases where bidirectional sequencing may not be an appropriate comparator, we recommend that you consult with OIVD to discuss an appropriate study design. In your description of this study, you should include your protocol (**including reagents and auxiliary instrumentation**), the sample types you used, any selection criteria you applied, and results. If the population tested is representative of the population for which the device is intended, results may be reported as clinical sensitivity and specificity.....

**Section IV Quality Control** - Edit the following to include language highlighted in red. We recommend that you provide a description of quality control measures that the laboratory should follow to help ensure proper device performance. **You should provide troubleshooting guidelines in case improper performance is observed.**

Thank you for the opportunity to comment on this very important document. AMP, members perform pharmacogenetic and heritable marker testing want to ensure the highest quality of laboratory testing for molecular pathology. Please do not hesitate to contact V.M. Pratt, PhD, AMP Clinical Practice Committee Chair at [victoria.m.pratt@questdiagnostics.com](mailto:victoria.m.pratt@questdiagnostics.com) if we can be of further assistance.

Sincerely,



Andrea Ferreira-Gonzalez, PhD  
President