September 8, 2009

AMP Comments on the draft report from the Agency for Healthcare Research and Quality (AHRQ): "AHRQ Draft Report on Quality, Regulation and Clinical Utility of Laboratory-developed Tests".

The Association for Molecular Pathology (AMP) is an international medical professional association representing approximately 1,500 physicians, doctoral scientists, and medical technologists who perform laboratory testing based on knowledge derived from molecular biology, genetics, and genomics. Since the beginning of our organization we have dedicated ourselves to the development and implementation of molecular diagnostic testing, which includes genetic testing in all its definitions, in a manner consistent with the highest standards established by the Clinical Laboratory Improvement Act (CLIA), the College of American Pathologists (CAP), the American College of Medical Genetics (ACMG), and the United States Food and Drug Administration (FDA). Our members lead and work at the majority of clinical molecular diagnostic laboratories in the United States and laboratories in many other countries. We are frequently involved in the development of novel molecular tests, and in the validation of laboratory developed or commercial assays.

We welcome the opportunity to provide comments and feedback to the "AHRQ Draft Report on Quality, Regulation and Clinical Utility of Laboratory-developed Tests".

First, AMP would like to express its appreciation for the acknowledgment of AMP and its resources in this document, and we commend AHRQ for its recognition of the (urgent) need for development of standards and reference materials. As the report points out, professional organizations and practice guidelines have responded in a timely and detailed manner to address issues in validation.

General Comments:

- The report suffers from a general lack of familiarity with established clinical laboratory regulations and practices regarding not only molecular LDTs, but all clinical tests. Repeatedly, the report draws conclusions and makes inferences without knowledge of extant regulations concerning the responsibilities of laboratory directors, without reference to available proficiency testing programs, without review of available proficiency testing data for many molecular tests, some of which have been in place for years. To the knowledgeable reader (i.e. one who works in a molecular diagnostics laboratory) these omissions create serious concerns about the credibility of this report and undermine its authority and utility. We strongly recommend the inclusion of laboratory professionals who are familiar with and operating under current regulatory guidelines for molecular LDTs.

- The report focuses on *molecular* LDTs but the title suggests a more broad report on all LDTs. We recommend that the title of the report reflect this distinction.

- Most diagnostic tests, particularly most molecular tests, have their origins as LDTs. One such example is HIV viral load testing. Whether or not LDTs become commercial products

depends primarily on demand, market size and intellectual property licensing issues. If LDTs were not as readily available as they are now, diagnosis of a many cancers, infectious diseases and genetic conditions would not be available to patients. Certainly, the rapid response in initiating the development of diagnostic tests for many emerging infectious agents would not be possible but for LDTs. A prime example of this is the role that LDTs played in the novel H1N1 outbreak earlier this year. The adaptation and validation of available molecular tests for Influenza allowed community molecular diagnostics laboratories to perform accurate and specific diagnoses for the new influenza strain, and markedly reduced the workload on the public health laboratory network. The one assay advancement by FDA under Emergency Use Authorization (EUA) was restricted to authorized public health laboratories. The only recourse to clinical laboratories throughout the entire 12 week episode was the use of laboratory developed tests for influenza. Indeed, in the coming months, the majority of novel H1N1 diagnoses in this country will be made in clinical laboratories using LDTs. To not recognize this is a failure to understand and appreciate the important contributions LDTs make to the advancement of medical science and clinical practice.

- This report attempts to compile and review all of the information on LDTs currently available for the Medicare population (>65 years old) including tests available, laboratories providing tests, regulations (CLIA, FDA, and others), proficiency testing available, etc. Unfortunately the review has relied entirely on peer-reviewed journal publications, which is not an optimal source for this topic since test validations are rarely published (see below, comment regarding page 18). Input from laboratory professionals and their organizations would have led to a much more comprehensive report. It is noted that consultations with FDA were conducted to enhance the report. We recommend a similar approach with laboratory professionals and their organizations. The membership of AMP could possibly be a great resource to fill this gap.

- There are inconsistencies in the report. For example, on the one hand the report appears to exclude heritable diseases, and on the other cites guidelines from the American College of Medical Genetics (ACMG), which are specifically directed toward heritable disease testing. The authors are also not clear about the applicability of published validations for various types of molecular assays. For example, the report appears to generalize information from articles on quantitative infectious disease testing or tuberculosis (TB) to the whole of molecular diagnostics.

- The report does not comment on controversial aspects nor does it provide any recommendations or conclusions concerning the oversight, quality and utility of LDTs. We recommend that these areas be addressed.

- We recommend that this report use standardized nomenclature for genes and genomic variations as documented by the HUGO Gene Nomenclature Committee and Human Genome Variation Society).

- While we are appreciative of the utility the report found in the AMP Test Directory, it should be emphasized that the Test Directory was developed not primarily as directory of clinical

molecular laboratory testing services, but as a resource for AMP members who, as experts in the investigation of disease at the molecular level, are frequently at the forefront in developing novel diagnostic test. The Directory was instituted as a vehicle for exchange of information and collaboration in order to promote standardization among laboratories and to promote development of uniform high quality proficiency for often very esoteric tests. In the report's tables it can be seen that many of the molecular LFTs are truly for esoteric diseases and the vast majority are offered by no more than two or three laboratories. The report would be greatly enhanced in recognizing the esoteric nature of many molecular tests, the evolutionary course of novel diagnostic medical tests from the research bench to the clinical laboratory, the contributions of clinician scientists and molecular pathologists, and the role AMP and the AMP Test Directory play in the development of LDTs as high quality clinical tests.

Specific Comments:

Introduction – There is a statement in the introduction that experts agree that clinical utility should be included in the validation process of a laboratory test. AMP does not agree with this, nor does FDA require such. Consideration of clinical utility is intrinsic to the assessment of clinical validity by the Medical Director, but clinical utility is fully understood only when experience with laboratory tests is progressively gained over time.

On page 6 (Question 5) the Food and Drug Administration (FDA) is incorrectly referred to as the Federal Drug Administration.

Page 4 – The authors may want to include array-based karyotyping methodologies, such as array Comparative Genomic Hybridization (aCGH) or SNP arrays. Although currently these methods are primarily used in the diagnosis of inherited conditions in pediatric patients, this technology is now in the early phase of use for diagnosis of oncologic disorders.

Page 5 – The authors discuss that clinical validity and clinical utility of any given assay are not assessed by CLIA. However, under CLIA, the medical director of the laboratory must approve the clinical validity of any LDT; CLIA inspectors are expected to assess whether and how well a laboratory director is performing the validity assessment. A laboratory director's responsibility for clinical validity should include:

a. At a minimum, documentation of information regarding clinical validity (including, as applicable, clinical sensitivity, clinical specificity, positive predictive value and negative predictive values) of the genetic tests that the laboratory performs using available information resources, such as literature references and professional practice guidelines.
b. Provision of the clinical validity information as part of the set of information the laboratory should provide to its clients prior to test selection and specimen submission.
c. Establishment of clinical sensitivity, clinical specificity, and predictive values based on internal study results, if information regarding clinical validity is not available from published references.
d. "Truth in advertising" by documenting whether the clinical claims in the references or information sources used could be reproduced in the laboratory, indicating test limitations

in all test reports, and informing users of changes in clinical validity values as a result of knowledge advancement.

(Reference: CDC's MMWR on Good Laboratory Practices for Molecular Genetics, June 2009).

Page 5 – The authors state that laboratories do not have to participate in proficiency testing. This is an over-reaching statement as none of the currently regulated analytes are molecular tests. Laboratories are in fact *required* to perform alternative assessment (AA) twice a year. For molecular assays, proficiency testing for a large number of tests is offered by the College of American Pathologists (CAP). The CAP establishes proficiency testing (PT) whenever there are a sufficient number of participants to justify it. In fact, the greatest obstacle to more widespread proficiency testing is the lack of control materials and the lack of economic feasibility of establishing PT for assays performed by only small numbers of laboratories. The CAP has recently established a mechanism to assist in such instances through its Sample Exchange Registry Service, in which the CAP coordinates sample exchanges between laboratories for relatively rare diseases, and for esoteric analytes for which formal proficiency testing is not yet established. As noted above, the AMP Test Directory was also instituted for this purpose.

Page 13 – Typo: AMCG, should be ACMG.

Page 14 – Dimech et al. recommend at least 100 positive and 100 negative samples be tested. This may not be possible in rare disorders, though the authors do indicate that a minimum of 20 positives should be tested. Sample size is a statistical measurement and should be treated as such. The number of samples used in a validation determines its statistical power, which is a measure of how much confidence can be placed on the results of the validation. Therefore, validation sample size is ultimately one of the most important factors in determining the analytical utility of the test. Unfortunately, definitive guidelines defining specific sample sizes cannot realistically be given as the requirement is so dependent on a wide range of factors including the nature and performance of the test, critical parameters, how the test will be used in practice and the confidence level required for clinical utility. The report also does not describe the option of a tiered risk assessment strategy with corresponding levels of comparative statistical analysis requirements which would reflect more stringent criteria for high risk testing. A large number of tools for determining sample size given certain input criteria (e.g. confidence interval) are freely available on the internet (e.g. www.statpages.org/Power#, accessed in August, 2009).

Page 16 – Analytical sensitivity – refers to 2 concepts (below). However, the authors only discuss Limit of Detection (number 2 below).

1. The ability of a test to detect a mutation or disease when that mutation/analyte is present

   Sensitivity = True positive ÷ (True positive + False negative)

2. Also used to refer to the lower limit of detection for the analyte of interest (i.e., the lowest concentration of an analyte that the assay can detect)

Page 16 – Analytical specificity – refers to 2 concepts (below). However, the authors only discuss cross reactivity (number 2 below).

1. The ability of a test to give a normal (negative) result in specimens without the mutation or analyte being tested.

Specificity = True negative ÷ (True negative + False positive)

2. Also used to refer to the ability of a test to detect the analyte without cross-reacting with other substances

Page 18 – The authors discuss that very few validation studies have been published, but do not address why. It is important to recognize that very few journals will accept validation studies as an article for publication. An accurate depiction would be that most laboratories do not publish their validations, so that there is little published evidence of validation of *individual* assays. One reason for this is that assay validation is deemed a routine professional activity. Most assays that are published in the literature are in some way novel.

Page 18 – The authors discuss a validation published by the Wadsworth Center. When discussing sensitivity and specificity, it should be clarified whether the discussion pertains to analytical or clinical sensitivity and specificity.

Page 20 – CYP2C9 is not in the Roche CYP450 Amplichip assay.

Page 25– Challenges in Assessing Clinical Utility of Molecular Tests
This paragraph is inaccurate and conflicts with later discussions about CLIA requirements to establish analytic validity, and does not acknowledge CAP's Laboratory Accreditation Program (LAP). As discussed above, the lack of published validation data does not mean that "for most molecular tests, especially laboratory-developed tests, the analytical and clinical validity have not been clearly established". All CLIA regulated laboratories need to establish analytical and clinical validity. This information is available at each laboratory and is reviewed during inspections to maintain accreditation by CMS, CAP, JCAHO and other organizations. As mentioned above, it appears that the authors did not consult with laboratory professionals who could have pointed to appropriate sources of information. Given that this is report is an evidence-based review, we recommend removal or revision of this comment.

Page 26 – It is unclear whether the authors are discussing analytical or clinical sensitivity and specificity.

Page 28 – The abbreviation ESBC should be spelled out

Page 31 – Often studies for CYP450 do not address all populations. Many of the variants have different frequencies in various ethnic backgrounds.

Page 38 – Proficiency testing – see comment above (page 5)

Page 38 – Clinical validity – see comment above (page 5)

Chapter 5: Some FDA special control documents that could apply to molecular assays were omitted (such as those for multiplex instrumentation, and replacement reagent).

Page 41 – Note that the ASR guidance was updated in 2007.

Chapter 6: The report states that the FDA and DTC do not have clearly defined internet promotion as labeling or advertising, but warning and/or untitled letters have in fact pointed to FDA's conclusion that labeling also can include websites and use of literature.

Page 48 – The CDC has had annual meetings, often adjoining the AMP annual meeting, since 2003.

Page 48-49 – This section states that the AMP currently facilitates sample exchanges among laboratories across North America for molecular testing and that a manuscript describing results from the sample exchanges is currently being drafted. This is an inaccurate statement that should be corrected, the systematic sample exchange is facilitated by the CAP and the used reference is not one of the AMP publications. AMP has performed sample exchange studies for specific molecular tests when it believes such a study would be useful to the molecular pathology community. When AMP conducts such studies, it can include laboratories outside of North America. AMP publications regarding sample exchanges and QC of molecular testing can be found at: http://www.amp.org/ (members section) and can be provided upon request.

Page 55 – The Personnel section is both confusing and inaccurate, since the document overall seems to pertain to molecular genetic laboratories and the study referenced was carried our specifically to address questions regarding biochemical genetics laboratories.

Page 49 – All molecular tests are non-regulated analytes. See proficiency testing comments above (page 5).

Page 71 – Please note that EGAPP (Evaluation of Genomic Applications in Practice and Prevention) has focused primarily on genetic-related testing; not necessarily on molecular infectious disease testing.

Epilogue – The implication that the NY State model should be emulated is concerning. Laboratorians who have experienced this process know its strengths and limitations and its potential to impede patient care via administrative delays. More specifically, to our knowledge there are no data confirming that the NY State process results in better results and better patient care outcomes for NYS-reviewed LDTs versus those in non-NYS labs that are CAP-accredited.

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