



Association for Molecular Pathology

Promoting Clinical Practice, Basic Research, and Education in Molecular Pathology

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June 30, 2010

Association for Molecular Pathology Comments to FDA's Public Meeting on Array-Based Cytogenetic Tests: Questions on Performance Evaluation, Result Reporting and Interpretation

The Association for Molecular Pathology (AMP) is an international medical professional association representing approximately 1,800 physicians, doctoral scientists, and medical technologists who perform laboratory testing based on knowledge derived from molecular biology, genetics and genomics. Membership includes professionals from academic medicine, commercial reference laboratories, community hospitals, the government, and the *in vitro* diagnostics industry. AMP aims to educate others and advance the field of molecular diagnostics; we thank the organizers for the opportunity to provide guidance on how FDA considers array-based cytogenetic tests.

Background:

AMP believes that array-based cytogenetic tests provide a dramatically higher resolution than traditional karyotyping and with expansive capabilities to diagnose and identify causes of genetic syndromes due to chromosome abnormalities. Traditional karyotyping has been performed for over 30 years to identify chromosomal changes (deletions, duplications, translocations, etc.) with well established clinical utility. Array-based cytogenomic analyses have been shown to detect the same chromosomal abnormalities, but at a higher resolution. For some clinical disorders, cytogenomic microarrays have also shown superior technical performance when evaluated against traditional karyotyping, targeted FISH, or other molecular technologies. [\[1-3\]](#) The standard of care in molecular and cytogenetic laboratories as well as genetics clinics is shifting from traditional karyotyping to array-based cytogenetic tests, and this new technology has provided diagnoses that would have otherwise not been possible with older methods. Table 1 shows examples of microdeletion/duplication syndromes that have recently been recognized due to the improved resolution of analyses generated using cytogenomic microarrays.

Professional associations, such as AMP, are actively working to develop professional practice guidelines for classification and nomenclature, and to create databases to catalog results from array-based cytogenetic tests. They are also working to address the ethical issues with reporting incidental findings. Proficiency testing for technical and nomenclature challenges is available through the College of American Pathologists (CAP), with increasing number of laboratories participating.⁴ The FDA should partner with these professional associations to collaborate regarding the best manner to standardize the use and reporting of array-based cytogenetic tests. AMP believes that the interpretation and reporting of results of chromosome analyses—both traditional karyotyping and array-based cytogenetic tests—fall within the practice of medicine; therefore, while FDA should evaluate the technology platform, it does not need to review each possible copy number variation (CNV) result. Interpretation continues to evolve as described in the ACMG Standards and Guidelines:

“As with conventional and molecular cytogenetic studies, chromosome abnormalities of unclear clinical significance are sometimes uncovered by microarray analysis. These unclear results require

the cytogenetic analysis of parents or other relatives to fully interpret the abnormal finding. Through the testing of parents or by FISH confirmation studies, many of these genomic alterations can be clarified. Thus, the situations encountered by microarray analysis are not unlike those that were experienced early on in the clinical cytogenetics laboratory in the elucidation of chromosomal heteromorphisms, nor unlike the finding of a novel subtle abnormality by conventional G-banding.”¹

It is important to remember that all testing is performed in the context of the phenotype of the patient, and interpretation of laboratory data is a collaboration between the clinical scientist and treating physician. AMP believes that to advance the use of array-based cytogenetic tests, the molecular cytogenetic field needs to collect data on both the laboratory results and clinical information. Such a database will enable the community to continually assess the validity of and accelerate the understanding of the array results. AMP encourages the government to fund clinical research to further explore the associations between array findings and health information.

Answering the FDA’s Questions:

In addition to the above general comments, AMP would like to address many of the specific questions posted in the Federal Register Notice of the meeting.

1. Clinical significance

a. The resolution of array-based cytogenetic tests and the presence of copy number variations (CNVs) in the apparently healthy population poses challenges for result interpretation. What criteria should be used to determine the clinical significance of CNVs (e.g., when categorized as benign, pathogenic, or of unknown significance)?

Professionals have the responsibility to catalog and re-examine CNVs as additional clinical evidence is accumulated. AMP believes that the clinical significance of CNVs should be determined by professionals in the field as part of the practice of molecular medicine.

b. Should there be different requirements implemented for interpreting the clinical significance of deletions vs. duplications vs. translocations?

The strength of cytogenomics microarrays is in numerical analysis (copy numbers) of small chromosomal segments. Balanced translocations will not be identified with this method. For potential unbalanced translocations detected by cytogenomic microarray analysis, it may be appropriate to follow up the array testing with FISH to detect a more complex rearrangement (e.g. insertion). Additional testing, such as FISH, is not, however, necessary to make a call of a deletion and duplication by cytogenomic microarray results. FISH confirmation on interphase or metaphase nuclei can be challenging for small deletions and duplications that are detected by cytogenomic microarray, but below the technical detection limit of FISH. Each lab is responsible for setting a normal and abnormal range for classification of duplications and deletions.

2. Result reporting and interpretation

a. Should result output be limited to results associated with known syndromes that can be adequately validated clinically and analytically?

Any result of possible significance should be reported with its limitations of the interpretation and any recommendations for additional testing to confirm the significance noted.

b. What criteria (e.g., minimum overlap, size, etc.) should be used to conclude findings are indicative of known syndrome?

Criteria must be determined on an individual basis and gene of interest. ISCA is helping to define standards and guidelines with benefit of very extensive data sharing.

c. Should the performing, ordering and/or result interpretation of these tests be limited to certain professionals (e.g., clinical cytogeneticists)?

As with traditional karyotyping and FISH, physicians in the clinic order these tests when encountering a patient with a suspected chromosome abnormality. However, cytogeneticists, molecular geneticists, molecular pathologists and molecular cytogeneticists will be the primary individuals responsible for performing, interpreting and reporting the results. Findings should be interpreted in conjunction with clinicians within the clinical context of their patients.

d. How does FDA ensure that the results are interpreted correctly?

CGH array validations are similar to analytic validations of any LDT and incorporate the elements of accuracy, precision, analytic sensitivity and specificity, reportable range of results, performance of PT, etc. FDA will review and approve informatics and software interpretation tools.

3. Additional and confirmatory testing

b. Should a second follow up test (e.g., FISH) be required for result confirmation prior to reporting array-based cytogenetic results?

A follow up test is recommended for certain conditions, such as utilizing FISH to confirm unbalanced translocations identified by cytogenomic microarrays when appropriate FISH probes or other reagents are commercially available. Clinical laboratories should not be required to develop such confirmation reagents. Additionally, further tests may be useful to better characterize the nature of a chromosomal change (e.g. duplication is actually due to an insertion of the material), but are not necessary to confirm the finding.

4. Incidental findings

Laboratories are obliged to report clinically significant findings unrelated to the test order, when identified. How can the reporting of results for diseases or conditions outside of the indications for use be restricted?

This is a challenging ethical issue not limited to array-based cytogenetic testing that is currently being discussed and considered by many professional associations. Since the entire genome is being interrogated, there is an obligation to report abnormalities observed. This is especially important because the reason for referral is typically not limited to a specific syndrome. Additionally, reported observations enhance understanding of genome variants. For example, identifying monosomy 16p11-2-p12-2 (see table 1) by cytogenomic microarray allowed further studies to be performed to understand its role in autism.⁵ AMP believes the FDA should allow these organizations to complete their process and establish professional practice guidelines on how to handle the reporting of incidental findings.

5. Clinical evaluation for approval of array-based cytogenetic devices

a. Would validation of a group of CNVs associated with well-known syndromes be acceptable as a representation of all types of detectable CNVs?

Detection of well-known CNVs, whether or not associated with a syndrome, would suffice for identification/validation of CNVs.

b. If yes, then which syndromes should be included and how many CNVs would be a representative number?

It is challenging to identify a representative number as this would vary from setting to setting. Similar to the issue of reporting incidental findings, professional associations are working to develop professional practice guidelines. AMP encourages the FDA to allow the community to reach consensus on this topic and implement guidelines.

c. What should be used as the reference genome?

Reference genomes would be variable and several genomes could be utilized for validation of both CNV and genomic alterations associated with disease.

d. What studies should be performed to understand clinical specificity?

Validation with known characterized clinical cases should be used for sensitivity studies. Since testing indications are typically for developmental delay, studying unrelated individuals without delay can identify benign CNVs and help establish clinical specificity.

6. Use of database(s) in result reporting

a. How can the accuracy of information used in the determination of results be assured?

Assurance of accuracy of results is through procedures for analytic validation.

i. Who should develop and maintain a curated database of known/probable CNV changes and benign findings in the population?

A curated databases of known/probable CNV findings already exist and are being managed and coordinated by The Children's Hospital of Philadelphia (CHOP), Database of Genomic Variants (DGV), Database of Genomic Structural Variation (dbVAR), and The International Standard Cytogenomic Array Consortium (ISCA).

Conclusion:

AMP is grateful for the opportunity to provide this information to the FDA as it considers the oversight of array-based cytogenetic tests. While FDA should review the technology platform to ensure high analytical validity, the interpretation of the clinical significance of CNV findings and the laboratory's reporting practices fall within the practice of medicine and should be determined by professional practice guidelines generated through consensus building among professional associations. The vast number of potential CNV findings would make FDA review of each one impractical. Analogous to the interpretations of structural abnormalities seen on a MRI image, the interpretation of array results should be left to the trained, certified professional.

Thank you for the opportunity to provide these comments. AMP hopes to serve as a resource to the FDA and looks forward to continuing this discussion. Questions can be directed to Dr. Elaine Lyon, Chair, AMP Professional Relations Committee, c/o Mary Williams at mwilliams@amp.org.

Sincerely,

Karen Mann, MD, PhD
President

Genetic condition	Chromosomal region	Gene / locus	Minimum Length Detected	Detection rate by standard karyotyping	Detection rate by cytogenomic microarray	Frequency in patient populations that were screened (ascertainment bias)	Reference
Monosomy 1q41-q42	1q41-q42	multiple	1.17Mb	0.5%	~99%	0.07% of MR/DD patients	Shaffer et al (2007) Genet Med 9:607-616
Monosomy 2p15	2p15	multiple	570kb	0.0%	~99%	1.2% of MR/DD patients	Rajcan-Separovic et al (2007) J Med Genet 44:269-276
Monosomy 15q13.3	15q13.3	multiple	1.5Mb	0.5%	~99%	0.3% of MR/DD patients	Sharp et al (2008) Nat Genet 40:322-328
Monosomy 15q24	15q24	multiple	1.7Mb	0.5%	~99%	0.3% of MR/DD patients	Sharp et al (2007) Hum Mol Genet 16:567-572
Monosomy 16p11.2-p12.2	16p11.2 – p12.2	multiple	555kb	0.0%	~99%	1% of autism patients	Ballif et al (2007) Nat Genet 39:1071-1073
Monosomy 17q21.31	17q21.31	multiple	478kb	0.0%	~99%	0.3% of MR/DD patients	Shaw-Smith et al (2006) Nat Genet 38:1032-1037
Monosomy of distal 22q11.2	distal 22q11.2	multiple	1.4Mb	0.0%	~99%	5% of patients with conotruncal heart defects	Rauch et al (2005) J Med Genet 42:871-876

Table 1. Newly recognized microdeletion/duplication syndromes detected by aCGH technology

1. Shaffer LG, Beaudet AL, Brothman AR, et al. Microarray analysis for constitutional cytogenetic abnormalities. Genet Med 2007; 9(9):654-662.
2. Pickering DL, Eudy JD, Olney AH et al. Array-based comparative genomic hybridization analysis of 1176 consecutive clinical genetics investigations. Genet Med 2008;10(4):262-266.
3. Lu X-Y, Phung MT, Shaw CA et al. Genomic imbalances in neonates with birth defects: high detection rates by using chromosomal microarray analysis. Pediatrics 2008;122:1310-1318
4. CYCGH Surveys, 2008A, 2008B, 2009A, 2009B. College of American Pathologists.
5. Ballif BC, Hornor SA, Jenkins E, et al. Discovery of a previously unrecognized microdeletion syndrome of 16p11.2-p12.2 (2007) Nat Genet 39:1071-1073