General Comments

Description of Genetics vs. Expression
The distinctions between genetics vs. expression made in Section III of the draft guidance document may need to be refined to ensure clear understanding between the two types of genetic information. We have reviewed the revised wording that PhRMA has submitted for this section and support their approach. The wording is reiterated here for your reference.

III. Different types of information: DNA tests v. RNA tests

Information is qualitatively and quantitatively different for tests measuring DNA versus tests measuring RNA. Both DNA and RNA have the potential to provide information regarding the genetic state of germinal cells, somatic cells and tumor cells. Whereas measurement of DNA provides information about allelic state, gene copy or chromosome number, measurement of mRNA provides information about relative gene expression activity.

Because of DNA polymorphism, epistasis, response to environmental stimuli and varying efficiencies in biological processing, relative transcription levels of messenger RNAs (and ultimately proteins) vary within and among individuals. Also, levels of messenger RNA are measured as continuous variables and the level of any one or a few transcripts may or may not provide enough power to be used for a certain type of test. Consequently, for many analyses, gene expression pattern information from larger numbers of transcripts can be more informative than the level of any given transcript.

DNA information including genotype, gene dosage and karyotype information is more digital in nature than RNA expression information. Informative DNA analysis can identify specific alleles or mutations, specific whole numbers of gene copies and/or whole numbers of chromosomes. Results from these tests can be characterized as dichotomous, e.g. present or absent, trichotomous, e.g. homozygous A, homozygous B or heterozygous A, B, or categorized, e.g. haplotypes. For DNA analysis measurements of single base changes as well as patterns of SNPs can be highly informative.

DNA based tests: Genotype, gene dosage, karyotype
Process for interpretation of results from tests identifying allele or mutation state, gene copy number etc., should be relatively straightforward. Clinical studies should account for allele frequencies in unaffected populations and disease prevalence in the populations being studied.

mRNA based tests: Gene expression patterns
Expression patterns represent interactions between genes, pathways, and networks. Applications can include disease predisposition, disease class/subclass, prognosis, treatment response/monitoring information.

Multi-Center Guidance
There are many uses for multiplex / array technology that will require guidance from not only CDRH but also CDER and CBER. It would be very helpful to us from both a product development and regulatory perspective if there were a single, agreed upon FDA guidance document.

Scope of Guidance
We suggest that the guidance document be written in a broader way so that it will apply to most or all classifications of multiplex and array assays. We have created a matrix (table 1 below) which suggests regulatory pathways for various types of intended uses. This approach suggests a more stringent regulatory pathway for those products which have higher risk and lower relative benefit to the patient and a less burdensome approach for those in which many benefits and few risks can be delivered to the patient.

Table 1: Least Burdensome Regulatory Pathways Vary Depending on Intended Use

	Screening / Early Detection	**Predisposition**	**Initial Diagnosis**	**Classification / Disease Recurrence / Prognosis/Used as Adjunctive Information**	**Drug Choice and Dosage**
Example	Cardiovascular disease	Detection of early markers in patterns of diseases such as lung cancer	Gene sequence detection and identification tests for multiple infectious organisms	Gene expression patterns subclassifying lymphomas, enabling more targeted therapies	Drug response genes
Potential Safety Issues	Early indicators may give more options for prevention	Availability of information to outside sources as privacy issue. Unclear treatment decisions.	Accuracy of result (False negative/false positive) rate may relate to safety issues. Need to balance with benefits.	Could affect therapy choice but other information available. Few safety issues relative to enormous potential benefits.	Could affect therapy choice and dosage; depending on direction, may pose no additional safety risk
Benefit to Patient	High	Unknown	High	High	High
Regulatory Path	510(k) or PMA	Unknown	510(k) or PMA depending on disease state	510(k) based on scientific literature or clinical data	510(k) based on scientific literature or clinical data

We also suggest the wording in section 1.8 of the FDA document: Guidance for Submission of Immunohistochemistry Applications to the FDA (June 3, 1998) offers a useful precedent, adapted as follows:

Class I Multiplex / Array Products. Class I multiplex / array products provide the physician with adjunctive diagnostic information. These multiplex / array products are used after the primary diagnosis of a disease is made by approved diagnostic products and more general information regarding a patient's condition is desired. The types of information generated would include gene expression patterns or whole genome SNP profiling. An example of a Class I multiplex / array products used in this way are expression analysis arrays and SNP analysis arrays. Class I multiplex / array products are subject to general controls including current good manufacturing practice regulations. Class I multiplex / array products may be subject to 510(k) applications at the Agency's discretion.

Class II Multiplex / Array Products: Class II multiplex / array products are intended for the detection and / or measurement of certain gene expression patterns or specific polymorphisms in order to provide prognostic and predictive data that are not directly confirmed by another assay. These multiplex / array products provide the physician with information that may affect treatment but are not initial diagnostic results. An example of a Class II multiplex / array product is a product that gives dichotomous, trichotomous or categorized results.

Class III Multiplex /Array Products: Class III multiplex / array products are those products that do not meet the criteria for class I or II, or are devices that raise new issues of safety and effectiveness. An example of a Class III multiplex / array product is a product that is used for determination of disease predisposition, which may use either genotypic or gene expression information.

Clinical Truth

The FDA's reference to 'Clinical Truth' is of concern when contemplating least burdensome approaches to delivering safe and beneficial products to the public. We have referenced another draft FDA guidance document in this area: 'Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests.' The specific nature of the concern is that it will often be difficult or impossible to obtain a perfect standard for multiplex / array products since the information that is generated is unique compared with existing diagnostic information and may only be verified based on patient outcomes. In cases where the intended use poses few safety issues, the potential benefit is large, and patient outcomes may take years or decades to obtain, this approach clearly is not in the best interests of the public.

In addition, we question whether studies that incorporate an outside reference method (rather than testing of known specimens) are appropriate for all classifications of multiplex / array products. We suggest the clinical parameters for multiplex / array products be defined as noted below (similar to how IHC products have been defined).

Clinical Data: Class I Multiplex / Array Products. Performance characteristics of Class I multiplex / array products should be developed with analytical laboratory studies and clinical studies using known specimens. These specimens may be naturally occurring or prepared based on a consistent method for all specimens. Clinical validation using a

reference standard is not necessary for Class I products. Reference to scientific literature is sufficient if the benefits of delivering a product to the public outweigh the risks.

Clinical Data: Class II Multiplex / Array Products: Performance characteristics of Class II multiplex / array products should be developed with analytical laboratory studies and clinical studies using cohorts of clinical specimens. Clinical validation should use a comparative reference method when possible. The reference standard may be a cleared device, a laboratory method or standard of care, or a set of known specimens developed for this purpose.

Clinical Data: Class III Multiplex / Array Products: Performance characteristics of Class III multiplex / array products should demonstrated across the whole assay range in comparison to clinical outcomes, e.g., length of survival with or without therapy; difference in morbidity or mortality rates; or appropriate surrogate markers; etc. The sample size of the study should be statistically significant and justified.

Quality Control
There is very little information in the guidance document on how Quality Control of these assays should be addressed. More guidance in this area would be helpful and should include software and components. In particular, we would like to have a better understanding of how controls should be used both in the Quality Control area and in the laboratory. In addition, how many controls should be incorporated into the assay? For example, are a single positive and a single negative control sufficient? Are there expectations for how results should be reported to avoid interpretation issues?

Appendix II: Statistical Considerations for Analyzing Array Data
In general, this section would be more useful if there were equal discussion of all of the accepted methods, examples of the methods and references to published literature.

Specific Comments

Guidance Section	Current Wording	Suggested Wording
Title	Multiplex Tests for Heritable DNA Markers, Mutations, and Expression Patterns	Multiplex Tests for DNA Markers, Mutations, Polymorphisms and RNA Expression Patterns
II. Analytical Variation	Target and probe are used in various ways.	Provide definitions of these terms at the beginning of the document.
II.2 Analytical Variation	Assay components: ... instruments, and software.	Assay components: ... instruments and software if not previously cleared via a 510(k).
II.B.1 Analytical Variation	Assay Sensitivity	Clarify sensitivity in the area of classification. It would also be helpful to clarify the terms 'positive sample' and 'assay range.'
III.A Comparison studies using clinical samples	Comparison to another device: Results of comparison studies with another well-characterized or predicate device; usually reported as percent agreement.	Comparison to another device: Results of comparison studies with another well-characterized or predicate device including IHC and other 'pattern' products; usually reported as percent agreement.
IV.1 Clinical Evaluation studies	Informed consent ...	Please clarify if prepared (spiked) samples may be used in cases where statistically relevant numbers of clinical samples are not available.
IV.B.2 Clinical Evaluation Studies	Clinical Truth	Additional information on FDA's meaning of the term 'clinical truth' would be helpful.
IV.B.3 Clinical Evaluation Studies	Validated expression patterns	Additional clarification would be helpful in this section
	"verify with a second detection system (e.g. quantitative RT-PCR)	We suggest "verify with a second detection system as appropriate. If a second system does not exist, describe how data will be confirmed (i.e., multiple tests using the same sample.)"
Appendix II	(e.g., normal, diseased)	(e.g. normal, diseased, disease sub-type, treated)