GENE EXPRESS, INC.
GEI

July 23, 2003

Dockets Management Branch (HFA-305)
Food and Drug Administration
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Rockville, MD 20852

To Whom It May Concern:

This is a response from Gene Express, Inc. to the 2/27/03 FDA Draft Guidance issued on 4/21/03 entitled "Multiplex Tests for Heritable DNA Markers, Mutations and Expression Patterns; Draft Guidance for Industry and FDA Reviewers. Gene Express, Inc. (GEI) very much appreciates the effort the FDA put into this important project.

This response includes the following

1. A recommendation for careful definition of important terms.
2. A description of why StaRT-PCR™ technology developed and marketed by GEI provides a less burdensome approach to multiplex gene expression testing that satisfies the requirements of the applicable statutes and regulations.

Recommended Definitions

1. Internal standard. An internal standard is a reagent that behaves exactly as the analyte being measured. Thus, a reference (or normalizer or housekeeping) gene would not be an internal standard for a target gene because it would not behave the same way as the target gene in the test. In contrast, a shortened DNA template that amplifies with the same efficiency as the native template for a gene would serve well as an internal standard in quantitative RT-PCR.
2. Normalizer gene (also referred to as a reference gene, or housekeeping gene. Sometimes inaccurately referred to as an internal standard). A gene that does not vary significantly from one sample to another and therefore serves to control for the amount of cDNA loaded into the assay.
3. Sensitivity; according to the International Union of Pure and Applied Chemistry (McNaught, A.D., Wilkenson, A., Eds. International Union of Pure and Applied Chemistry Compendium of Chemical Terminology, 2nd Edition. Online version, 1997, www.chem.qmw.ac.uk/iupac/bibliog/gold.html) sensitivity is the signal strength obtained in response to addition of analyte. For example, with a sensitivity of 100%, the signal doubles every time the analyte concentration doubles.
4. Lower detection threshold; the lowest amount of analyte (in this case, molecules of cDNA) that may be detected by the method.
5. Linear dynamic range. The range of values over which the method is linear

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2003D-0120 C17

StaRT-PCR Technology Provides Less Burdensome Approach

By including a standardized mixture of internal standards in each gene expression measurement, StaRT-PCR addresses many of the issues regarding performance characteristics raised in the Quality System regulation (QSR) as set forth in 21 CFR 820, and 21 CFR 814.2 (b)(4)(v).

Gene Express has prepared standardized mixtures of internal standards comprising nearly 600 genes. The amount of time and money necessary to accomplish this was far less than that necessary to develop and produce commercial microarrays. Thus, it is not burdensome to prepare an internal standard for each gene, combine the internal standards for multiple genes into a single standardized mixture of internal standards, and then include a small aliquot of the standardized mixture in each gene expression measurement. Because sufficient amount of the standardized mixture for 1 trillion assays is prepared, this will be enough for 1,000 years at present levels of consumption. Thus, this will enable measurement of all gene expression in relation to a single standard, for many years.

The inclusion of a standardized mixture of internal standards in each gene expression measurement eliminates false negative values and enables a statistically insignificant level of false positive values. Variation in all components of the gene expression measurement is controlled, including reaction-to-reaction variation, variation in quality and/or quantity of PCR reaction components, well-to-well variation in thermocycler efficiency, and sample- to-sample variation in presence of inhibitors.

Lower detection threshold (referred to as sensitivity on page 5 of Draft report); Due to quality control measures at Gene Express, Inc. the lower detection threshold for nearly all genes is less than 10 molecules/PCR reaction. Reproducibility; The first carefully controlled inter-lab study on reproducibility of gene expression measurement revealed that StaRT-CPR has a less than 50% CV. Since implementation of a Caliper automatic microfluidic device in the Standardized Expression Measurement (SEM) Center at MCO, the CV is now less than 15%. We continue to make improvements.

Validation of cut-off, reference range, or medical decision point. StaRT-PCR has 100% sensitivity (as defined by IUPAC). This, combined with low CV enables detection of very small differences in gene expression. Thus, StaRT-PCR data (as compared to microarray data) allow smaller differences in gene expression to distinguish one diagnostic group from another

Assay range; also referred to as linear dynamic range. The linear dynamic range for StaRT-PCR is the full range of gene expression (over 6 orders of magnitude). In contrast, the linear dynamic range for microarrays is 2.5-3 orders of magnitude. The linear dynamic range for other QPCR methods often is sub-optimal because the primers for most genes have not been evaluated for efficiency using a known amount of cloned cDNA. Because the linear dynamic range of a microarrays is less than the range of possible values, it is necessary to evaluate a serial dilution of the sample. Otherwise, some values will exceed the linear dynamic range and be recorded as

a falsely low value, and others will be below the lower detection threshold and erroneously reported as negative.

Specificity; The preparation of internal standards for StaRT-PCR is a carefully planned process. Primers for each gene are evaluated on Oligo software to ensure that they do not cross-hybridize with any other gene evaluated.

Summary

Gene Express believes it is necessary, and would not be burdensome, to include a gene-specific internal standard in each gene expression measurement. Equally important, it is important that the internal standard included in each gene expression measurement be in a standardized mixture of internal standards.

The presence of an internal standard within a standardized mixture of internal standards in each gene expression measurement controls for every cause of variation.

With StaRT-PCR, this quality control device is included in the method. In contrast, existing microarrays do not include an internal standard for each gene measured. Some QPCR methods include a single internal standard in a few tests, however, the value of a standardized mixture of internal standards is far greater and is necessary to keep CV low and to enable development of a standardized expression database.

Currently, we are designing standardized microarrays in which StaRT-PCR products are used as the analyte applied to the microarray.

Sincerely,

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Chief Science and Medical Consultant

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