

Session 2A
Metrics for Universal Standards:
Expression Arrays

***Metrology and Standards Needs for
Gene Expression Technologies***

Universal RNA Standards

NIST Workshop

Stanford University

March 28 & 29, 2003

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Goal

- Establish a reference/control/standard enabling evaluation of data quality
 - Minimum standards/references for evaluating integrity of labeled sample, hybridization and data collection

Points for Discussion

- Function of the standard: Data the standard would provide
 - Metrics for evaluating sample and experimental variability and bias
 - Quality (integrity/purity) of starting material
 - Quality of processed (labeled/amplified) sample
 - Hybridization performance (intensity, sensitivity, specificity)

Points for Discussion

- Metrics for evaluating platform variability and bias/Manufacturing process
 - Identification of array defects, quality control of substrate
 - Integrity of feature location, quality control of probe manufacture
 - Cross contamination of probes
 - Image scanning variability and bias/limitations
 - Sequence annotations consensus

Points for Discussion

- Features of the standard
 - Practical utility and general applicability across/among platforms
 - Ease in translation of metrics/units from platform to platform
 - Useful for accepted normalization/scaling methods
- Quality control manufacture of the standard
 - Access to standard/cost

Critical Issues

- Sample procurement and handling
 - RNA/DNA integrity
 - Understanding the variability and bias introduced by different handling methods, tissue specific differences etc.
- Clearly identify the purpose of the control
 - Ensure that the control measures what it is intended to measure
- Platform controls VS. sample controls.

Critical Issues

- Controls must be quality controlled, reproducible, cost effective, versatile
- Equally useful and informative across microarray platforms.
- Precision use of terminology
 - Sensitivity, accuracy, intensity, specificity

Specificity from Method Comparison Truth Table

concordant negatives
negatives of gold standard*

Where precision, reproducibility and the coefficient of variation (SD%) of the analytical method are closely related

* Ideal gold standard is “True negatives”



Sensitivity from Method Comparison Truth Table

positives detected
positives gold standard

In absence of specificity info, sensitivity is meaningless

* Where the ideal gold standard is “true positives”



Intensity

Fluorescence measure

intensity \neq sensitivity



Accuracy

$$\frac{\text{\# concordants}}{\text{\# tests}}$$

reproducibility \neq accuracy

Predictive Value

Is a function of sensitivity, specificity and prevalence of a given analyte in the test population

- **Of a positive result:**

concordant positives
positives from test method

» $[\text{Called Pos} - (1 - \text{Spec.}) \cdot (1 - \text{true pos rate}) \cdot \text{Population Size}] / \text{Called Pos}$

- **Of a negative result:**

concordant negatives
negatives from test method



Session 2A Panelists

- **Rick Hockett**

- *Description of a Microarray Validation Plan and the Challenges Encountered*

- **Gianfranco de Feo**

- *The Use of RNA Standards in Microarray Experiments*

- **David Gerhold**

- *Evaluation of Microarray Platforms Using "Pooled Spikes"*

- **Bob Setterquist**

- *Producing Quality RNA Samples and Standards: A Commercial Perspective*

