Implementing Traceable Calibration for Heterogeneous Analytes

David C Sogin
Abbott Laboratories
AdvaMed Representative

Diagnostic Tests Serve to Improve the Probability of a Correct Diagnosis

- How is the test going to be used
- What type of information
  - Qualitative or Semi-quantitative (numerical signal generated used to determine a positive cutoff)
  - Quantitative measurement of a biological activity
  - Quantitative value of the amount of material

Types of Biological Assays

- Use of living organisms to test a sample
- Measuring the biological function contained within the samples
  - Coagulation tests
  - Enzyme activities
  - Complement Fixation
- Identification of infected and disease free populations
- **Estimating the amount of a biological marker-usually protein**

Why do we care about Traceability?

Traceability is an approach to standardize assay results through calibration to a common reference material or reference method.

If we can measure the “true” value we should have comparability.

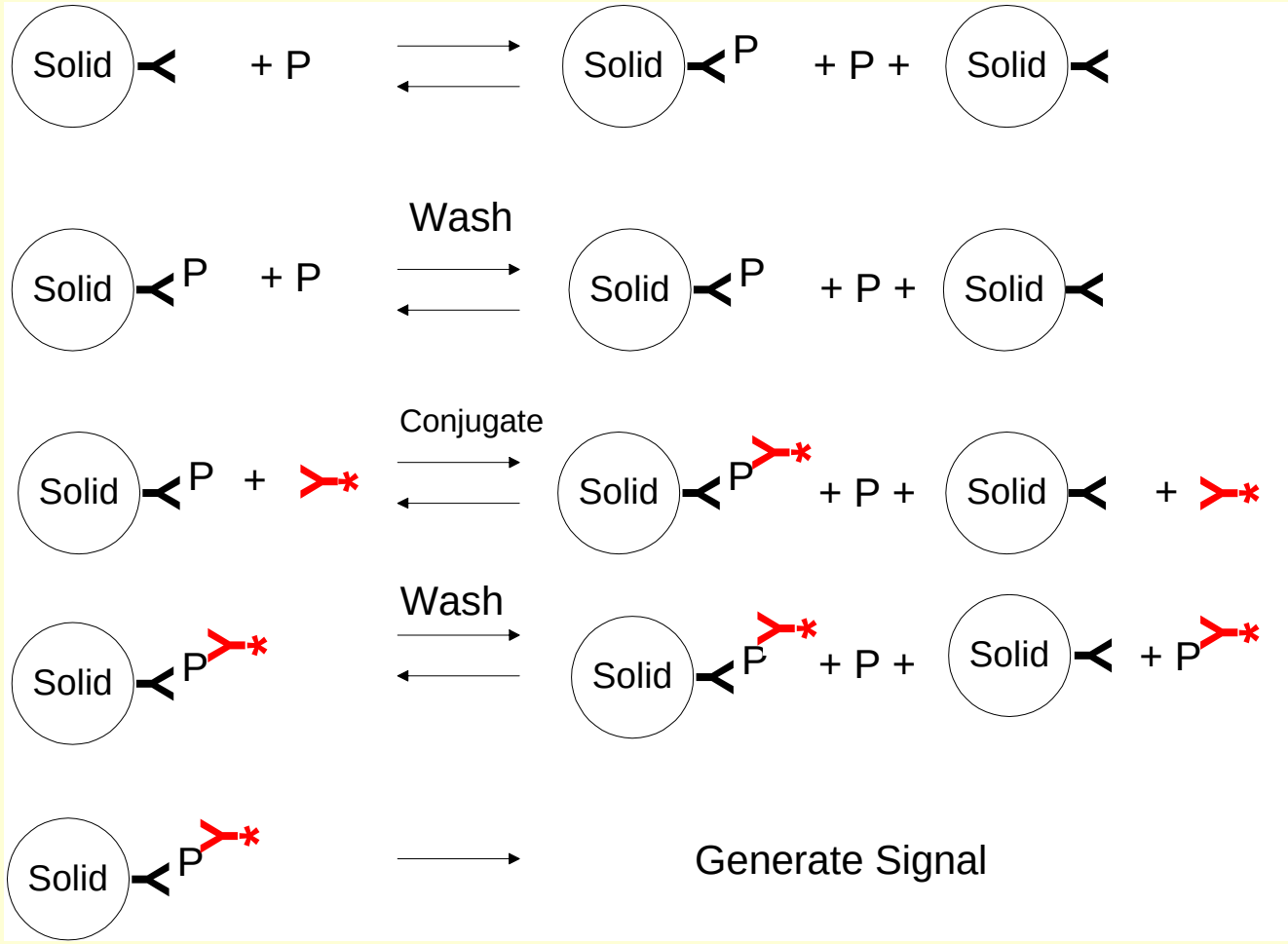
What do we Measure?

- A Protein is identified as a marker that can yield information on a disease state.
- Across different individuals a protein shares common attributes
- Usually no assay can measure the attributes as a whole
- Obstacles to defining a common attribute
- Need to define what is being measured

Defining the Measurand

- Define the analyte as Protein X with the measurand expressed as moles (or mass) per unit volume.
- The measurand we actually have for most immuno-assays using monoclonal antibodies is the following:
  - The amount of protein captured by epitope “A”
  - Detect the amount of epitope “B” detected by the signal generating end of the assay.

Immunological Reactions are Complicated but do follow Chemical Kinetics



Calibration Versus Standardization

- Belief is that traceability can result in comparability of results across time and place. - Usually works with small molecules
- With large biological markers, the standardization is often driven by the first assay to market
- All subsequent assays use the predicate device to drive standardization
- International standards that follow can fall into a “Standardization Trap”

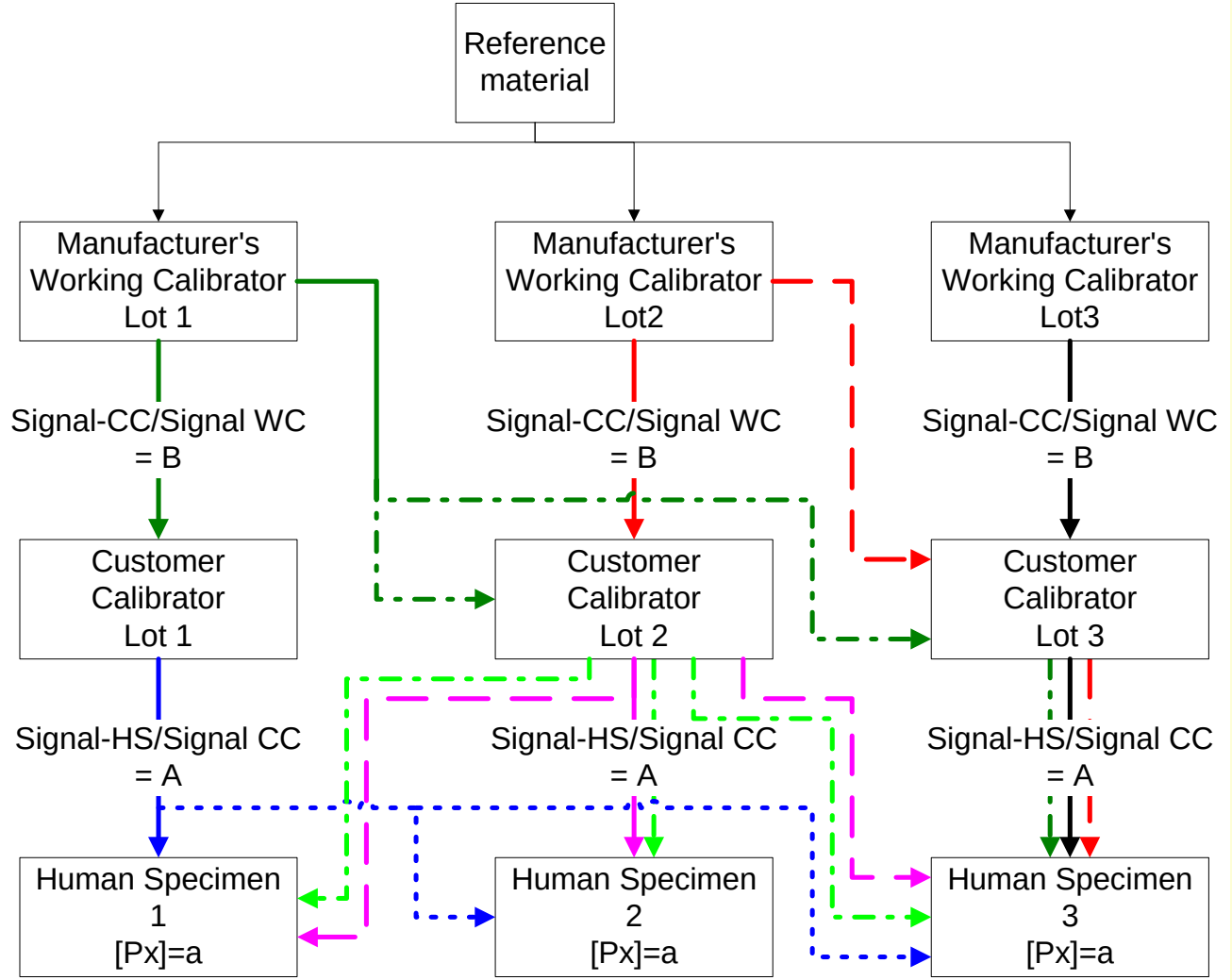
The Standardization Trap

- Have a collection of assays exist that do not have well anchored or traceable calibration.
- Introduce a material for use as the basis of calibration without taking into account the actual measurement equations that may have been developed to achieve some level of harmonization.
- Do not sufficiently characterized materials (including materials and matrix) or pay attention to their generation of signal in relationship to human specimens

Commutability

- An attribute of the calibration (reference) material not the result
- Manufactures must establish that their calibrators are commutable across different lots of reagents and instruments for which the calibrator is intended.
- The standard need not be identical to the intended analyte, it must simply maintain a constant numerical relationship with patient samples.
- International Standards must demonstrate that they are commutable to some level.

Commutability Starts with Specimens



Replacement Standards Create a Dilemma for Reported Results

Compare Standard	IMx % Difference	Architect % Difference
Edition 1 to 2	8-10	23
Edition 2 to 3	5-14	23-32
Edition 3 to 1	15-22	46-54

What we Ultimately Need for the Diagnosis

Laboratories require assays that will rank order patient samples with a consistent relative quantitative relationship. A recognized biological reference standard should facilitate maintaining that rank order through calibration of the available assays.

Traceable calibration may not be sufficient.

Ideal System for Calibration and Validation – Small Molecule Example

- Cortisol Standards from IRMM
  - Value assigned by LC-MS to within 2%
  - Not ideal since concentrations do not cover full range of the assay
  - A complete set of samples that have values assigned and can form the basis of a validation study

An Approach to Replacing Existing Standards

- Assign a preliminary value to a new standard.
- Manufactures prepare calibrators with both the new and earlier recognized standard.
- Provide a common set of authentic human specimens for the analyte with minimum additions
- All instrumentation to be run with curve fitting weights as appropriate but with any post calibration factors removed.
- Report results for the the panel samples based on both the old and new standards and relate to specimen panel.

We All Desire the Same “Result”

- Over space and time the same reported result will consistently indicate aid in an unambiguous diagnosis.
- Our customers believe in SI units. Their perception is one of authenticity.
- Manufacturers will actively participate in effort of standardization through calibration
- Probability of success increases with the degree of participation of all interested parties in planning, execution and interpretation of the results.

Thank You