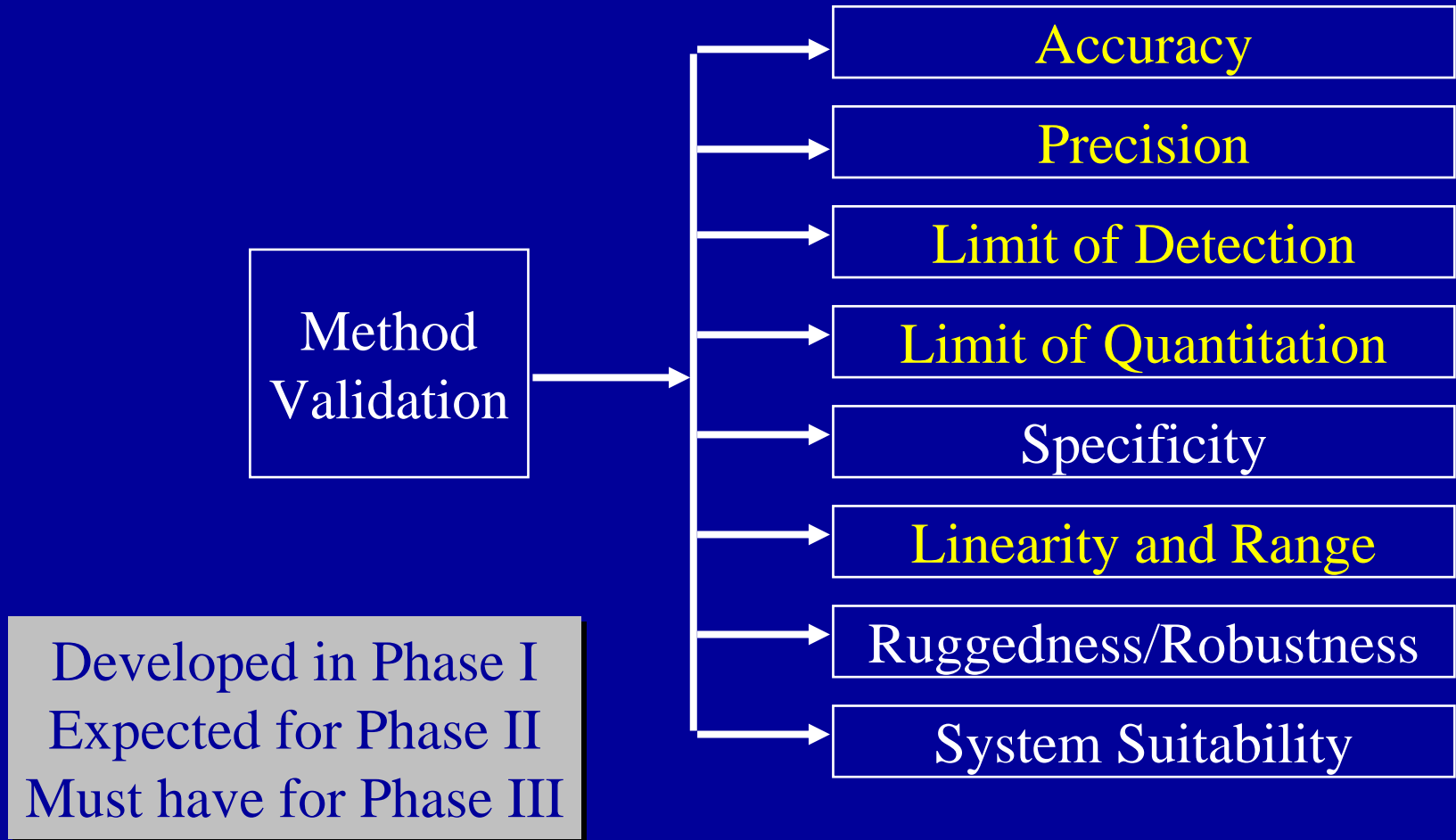


# Confidence of Measuring Response



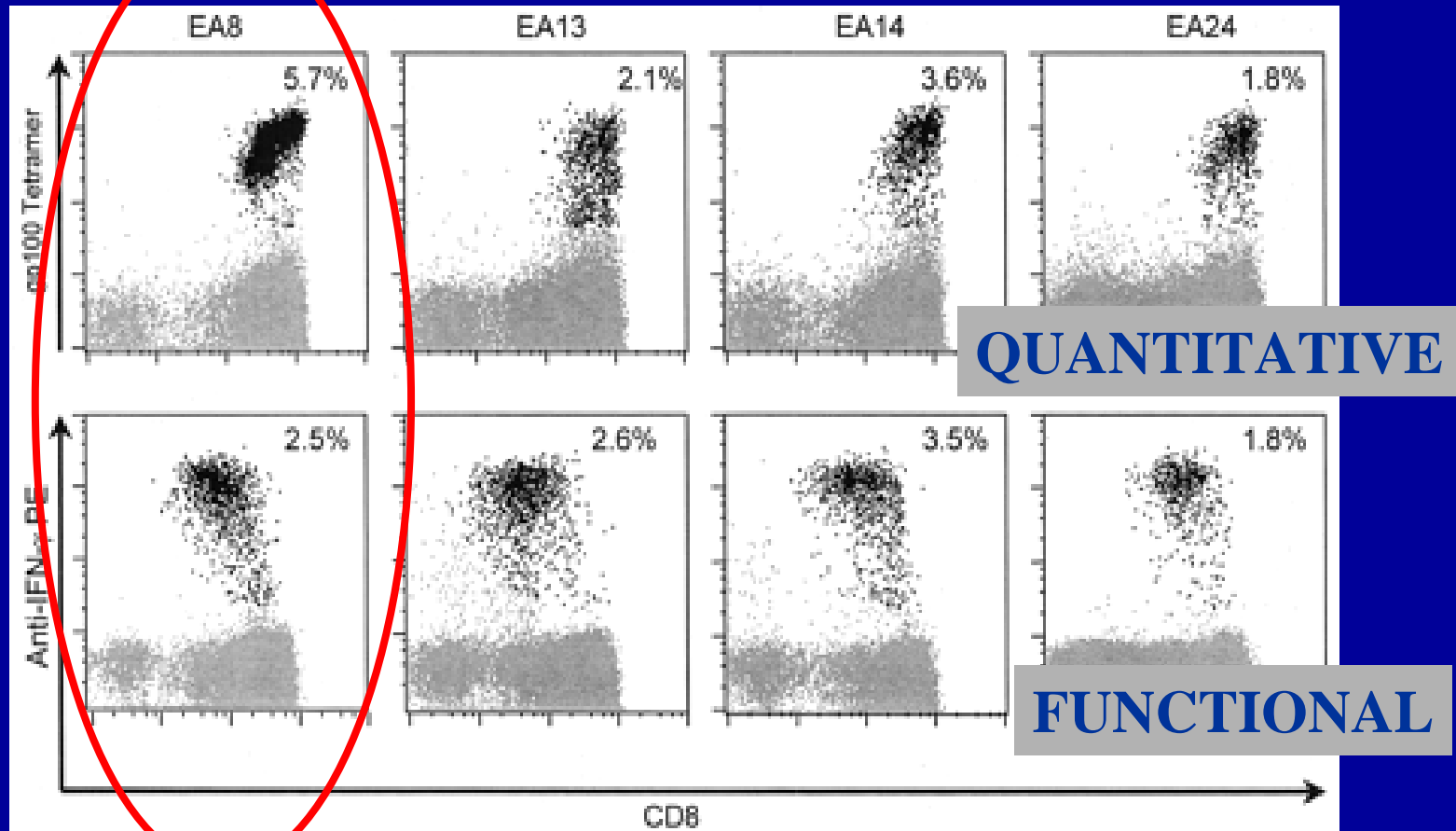
# Precision Industry Standards

Assay	Expected CV
Enzyme based assay	<10%
Binding (ELISA)	10-20%
Cell based assay	avg. 25%
Animal assay	20-50%

# Assay Accuracy

- Accuracy: the closeness of agreement between the measured value and an accepted reference value
- Determination requires a “gold standard” or an accepted method to which a new method can be compared
- T cell assays measuring immunity
  - No gold standard
  - No accepted method

# Assessing Accuracy by Comparison



Tetramer

CFC

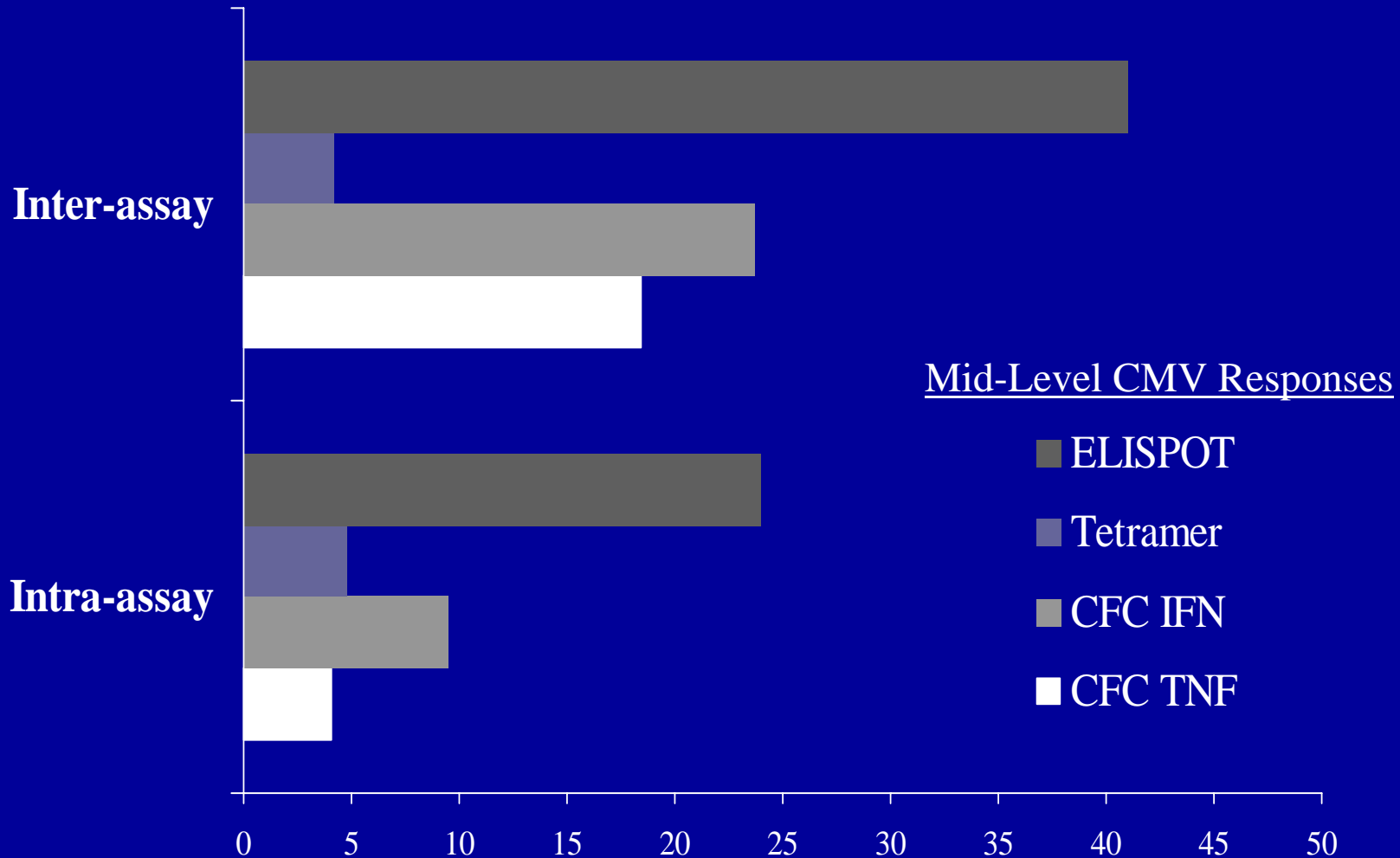
QUANTITATIVE

FUNCTIONAL

# Evaluate Precision

- Intra-assay Variability: Defines positive cutoff
- Inter-assay Variability: Defines whether samples need to be assayed at one time (cryopreservation)
- Biological Assay: Natural fluctuations in immunity (boosting immunity)

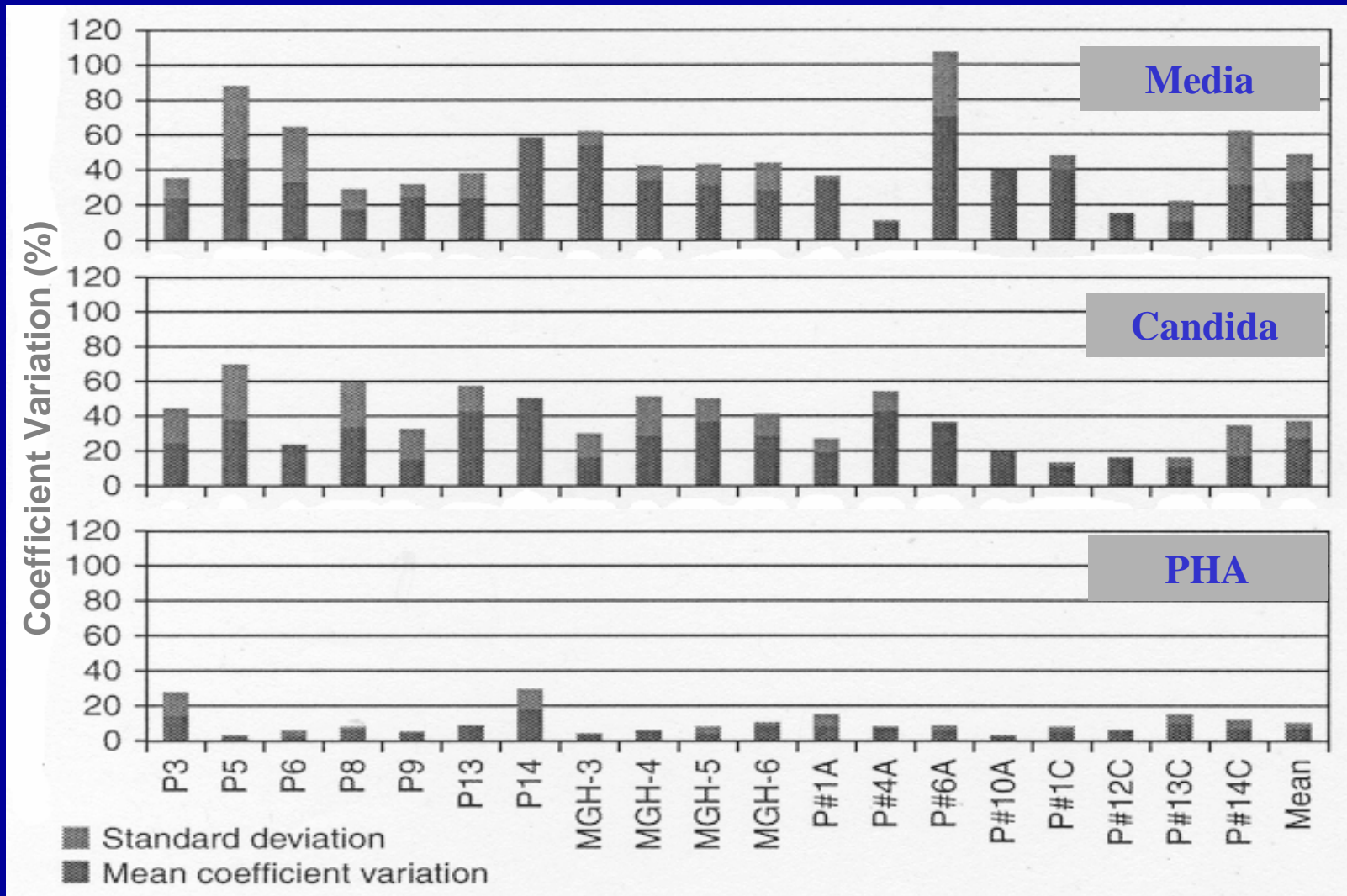
# Assay Performance



Average CV: 3 donors/3 runs

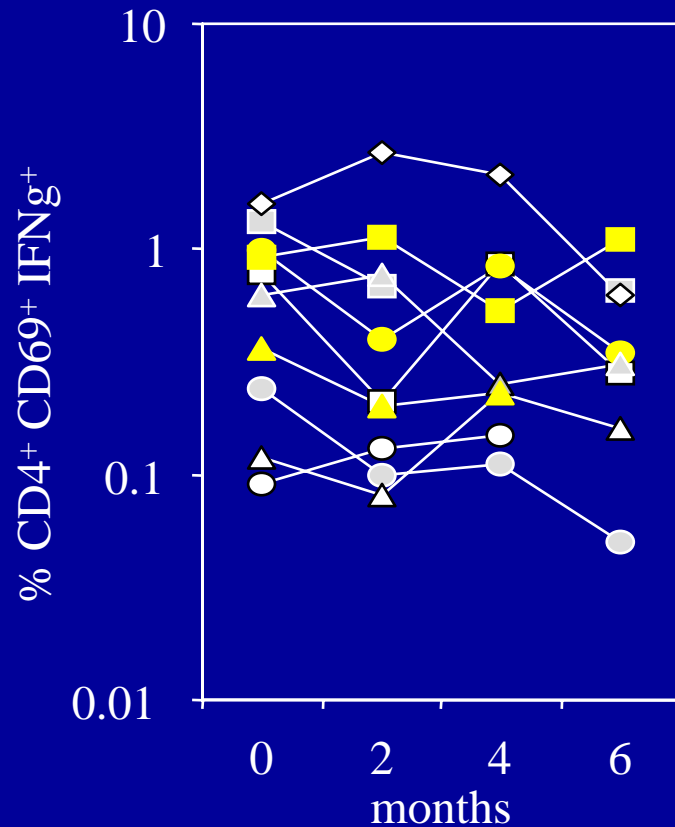
# High Variability in Functional T Cell Assays

## ELISPOT Intra-assay Variability



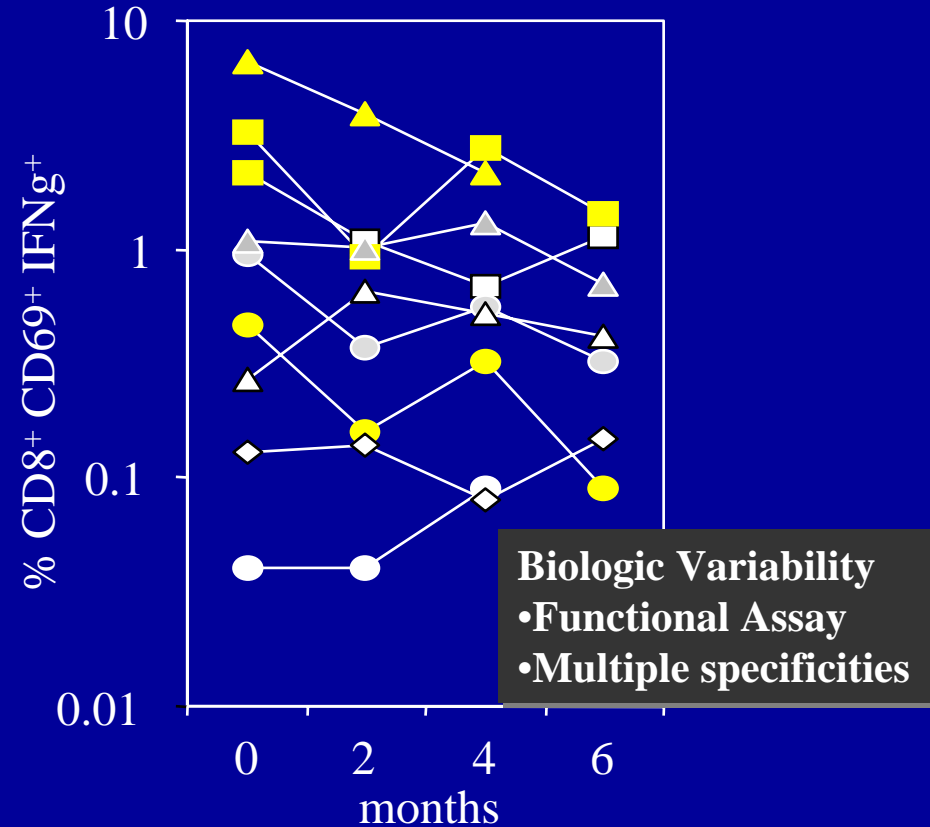
# Changes in CMV Responses Over Time

CMV lysate



median CV = 46%

CMV pp65 Peptide Mix



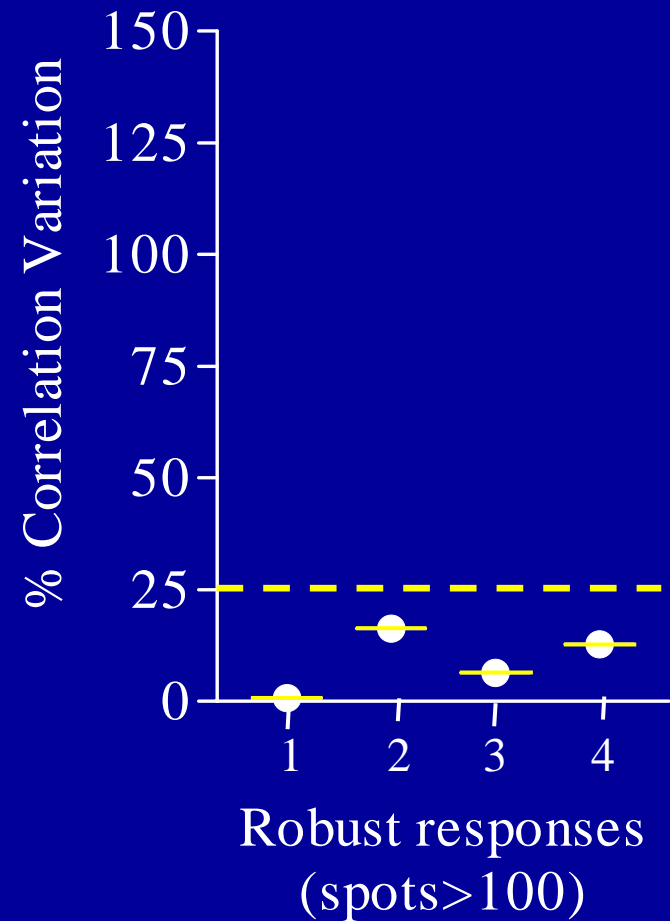
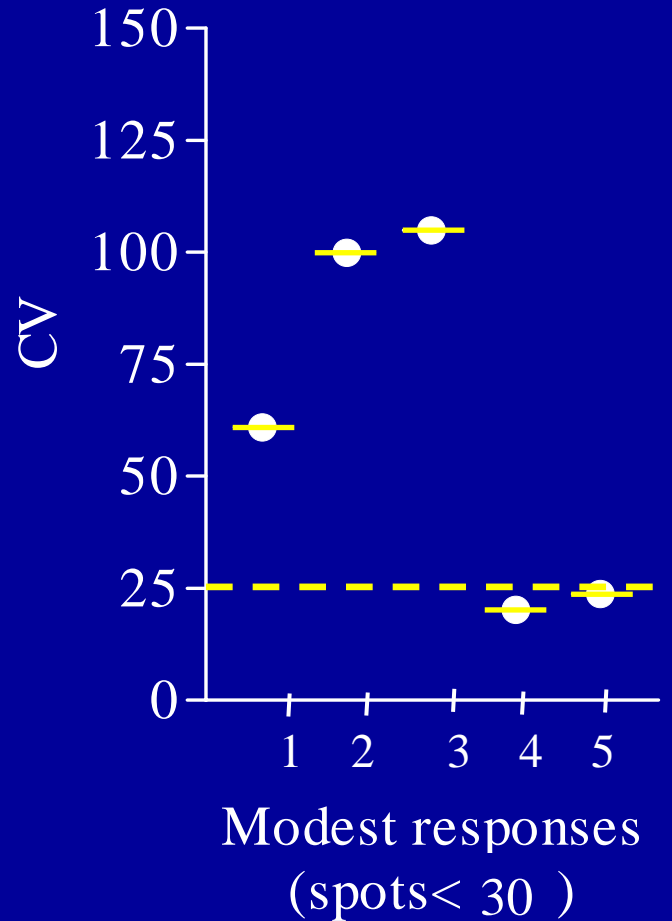
median CV = 51%



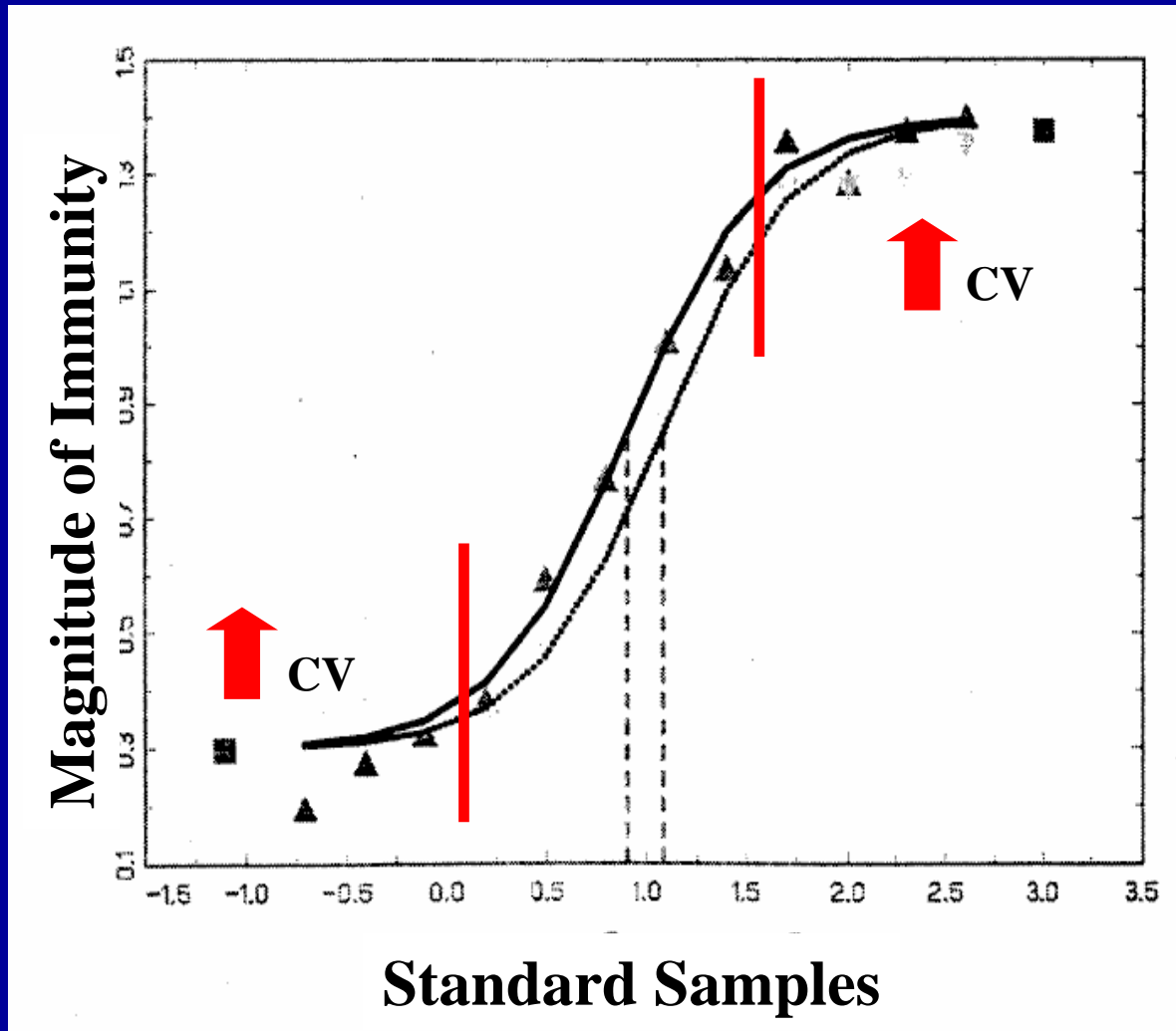
# Detection and Quantitation Boundries

- Limit of Detection: lowest concentration that can be distinguished from background
  - Not necessarily precise or accurate
- Limit of Quantitation: lowest and highest concentrations measured with accuracy/precision
- Linearity: results proportional to concentration
- Range: area between lower and upper limits of quantitation that is linear

# Variability at the Limit of Detection



# Range of *Reproducible* Detection



# Challenges to Cellular Assays

- Most sensitive may be least reproducible
- Establish performance; GLP
- Controls and standards
  - Reference population
  - Clones, beads, leukapheresis
- Low/Medium/High Response
- Choose standard antigens
  - CMV, EBV, mitogens
- Validation and QA Program
  - New technicians, reagent lot changes, quarterly performance ✓