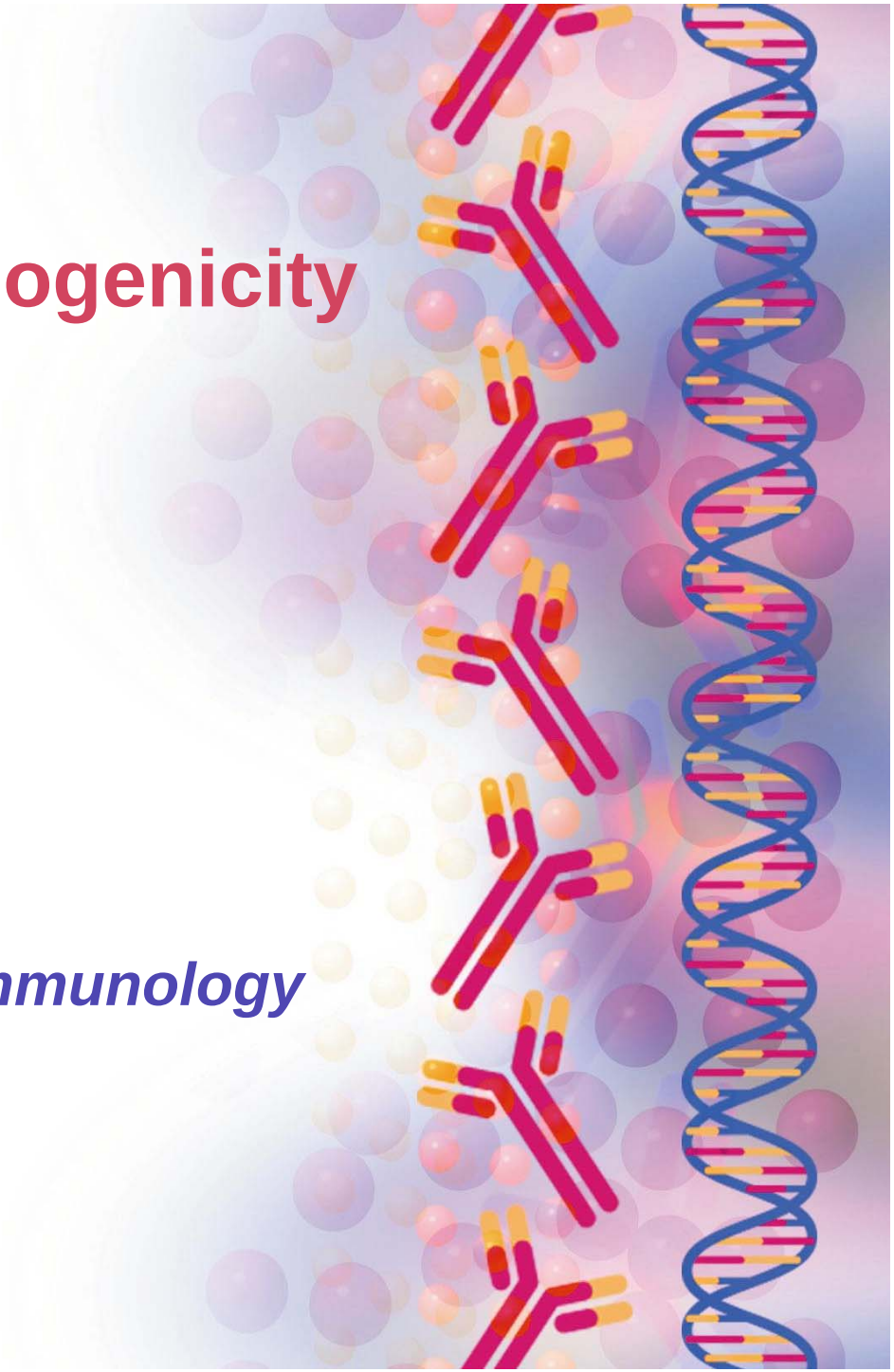
Evaluation of immunogenicity of pharmaceuticals



Geoff Hale

Chief Executive Officer
BioAnaLab Limited
Professor of Therapeutic Immunology
Oxford University

Guideline on Immunogenicity Assessment of Therapeutic Proteins.

EMEA/CHMP/BWMP/14327/2006

Mire-Sluis AR, Barrett YC, Devanarayan V et al. (2003) Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products.

J. Immunol. Meth. 2004; 289:1-16.

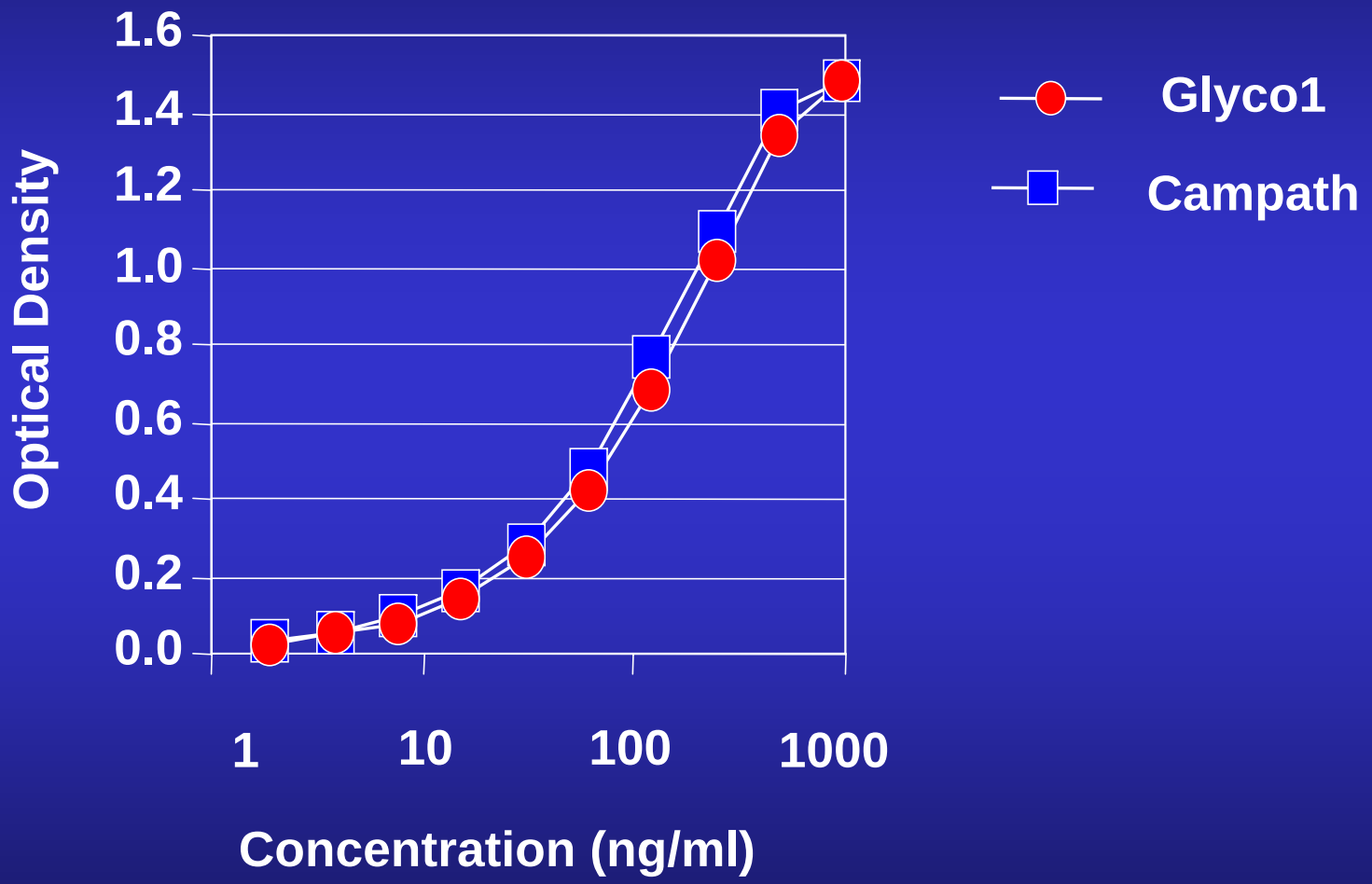
Principles

- Primary concern is clinical sequelae
- Assessment requires appropriate assays
- Wide range of technical difficulties
- Immunogenicity assays are quasi-quantitative due to lack of reference
- Titre-based approach is preferred
- Recommendations are based on experience and not a substitute for regulatory guidance or intended to stifle innovation.

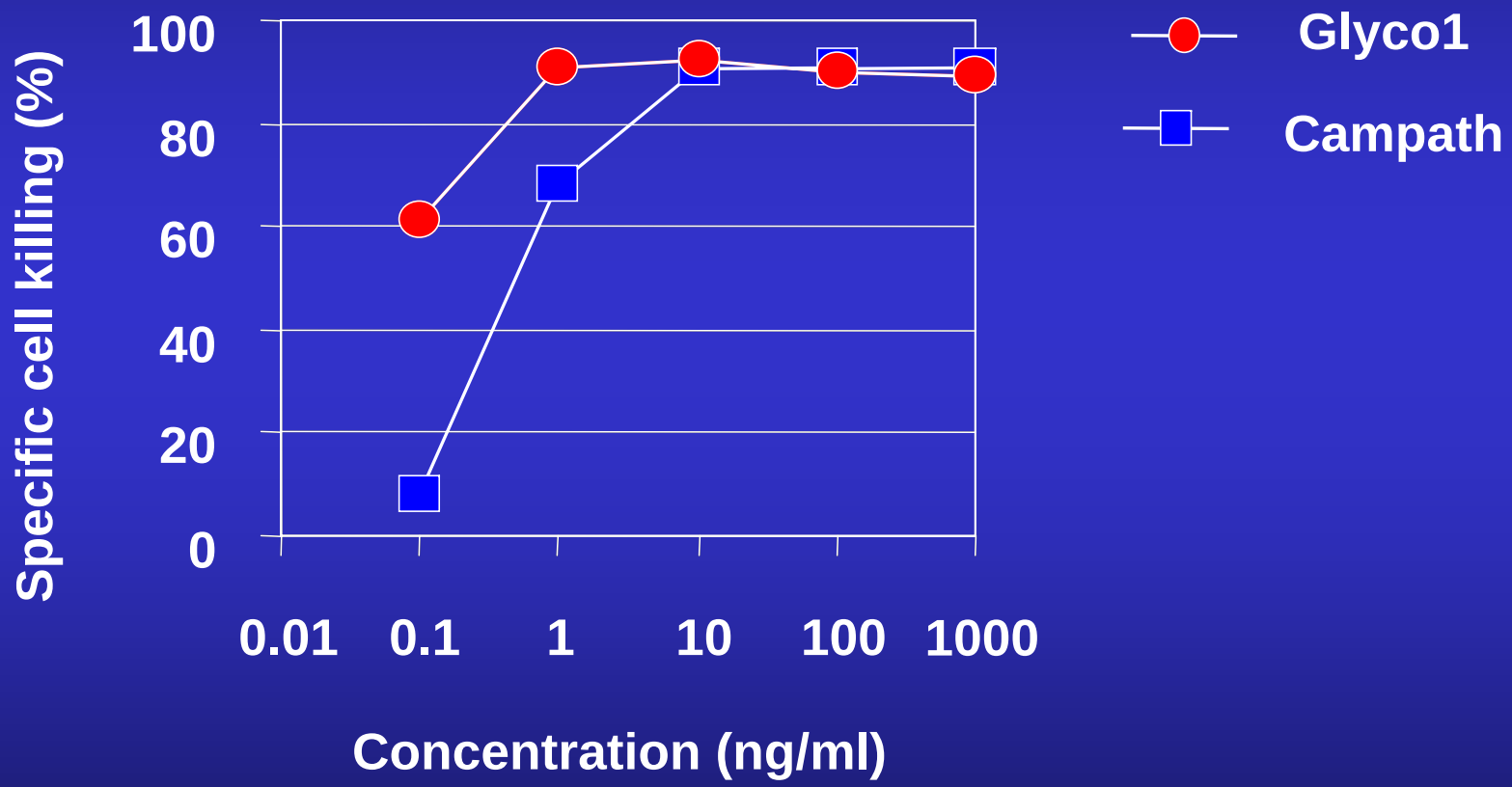
Topics

- Cut point and assay sensitivity
- Quantitation
- Confirmatory Assays
- Validation.

Binding to CD52 antigen (Antagonist effect)



Cell-Mediated killing (ADCC) (Agonist effect)



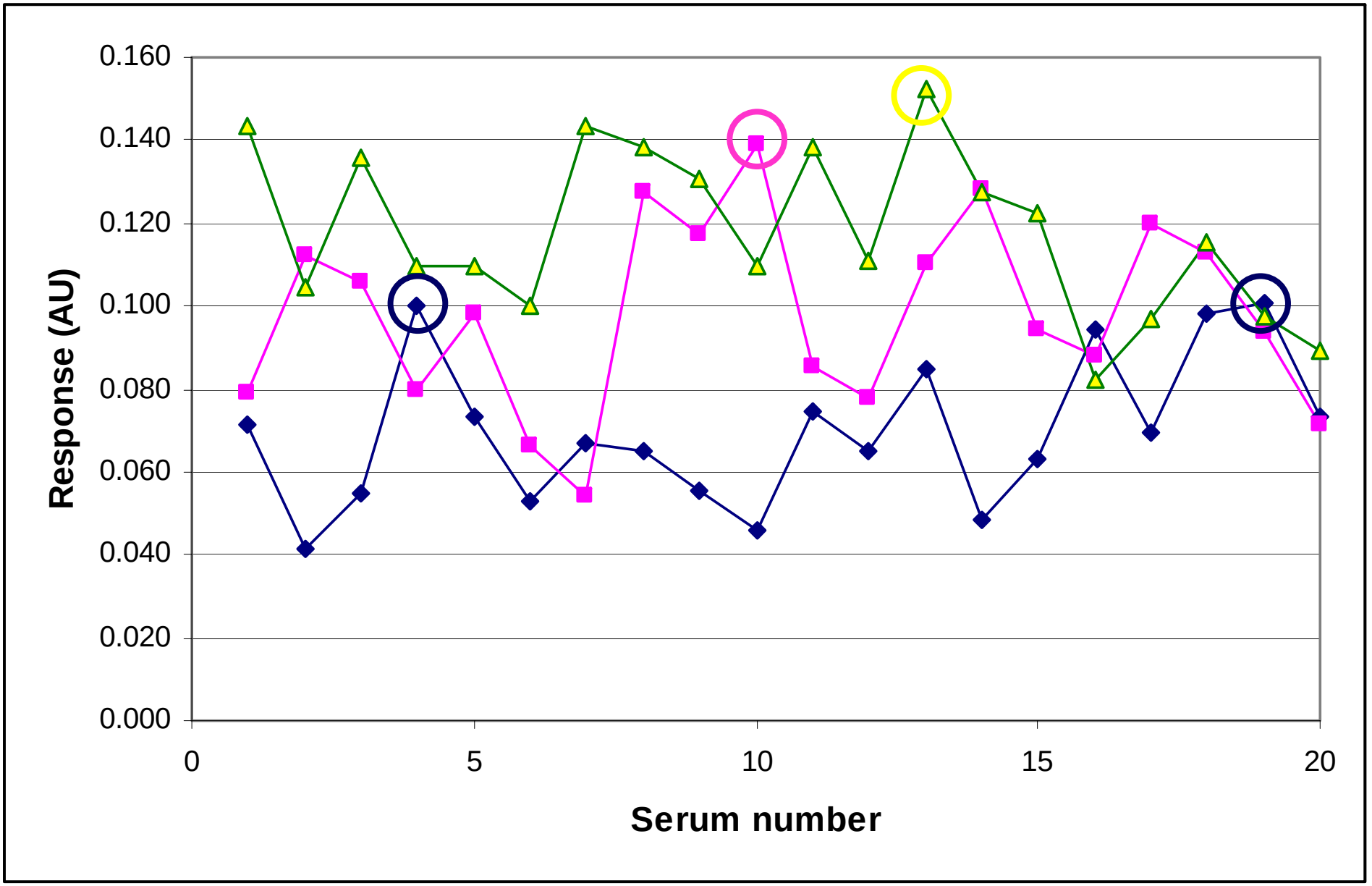
Screening Assay – Cut Point and Sensitivity

EMEA	Mire-Sluis	Clients
"Detection of some false positive results is inevitable" "eg 3SD above background"	"It is appropriate to have 5% false positives"	The cut point will be 1.645 SD above the mean
"Capable of detecting antibodies in all antibody-positive samples/patients"	"Strive for sensitivities near 250 to 500 ng/mL"	Sensitivity must be less than 500 ng/ml and the lower the better

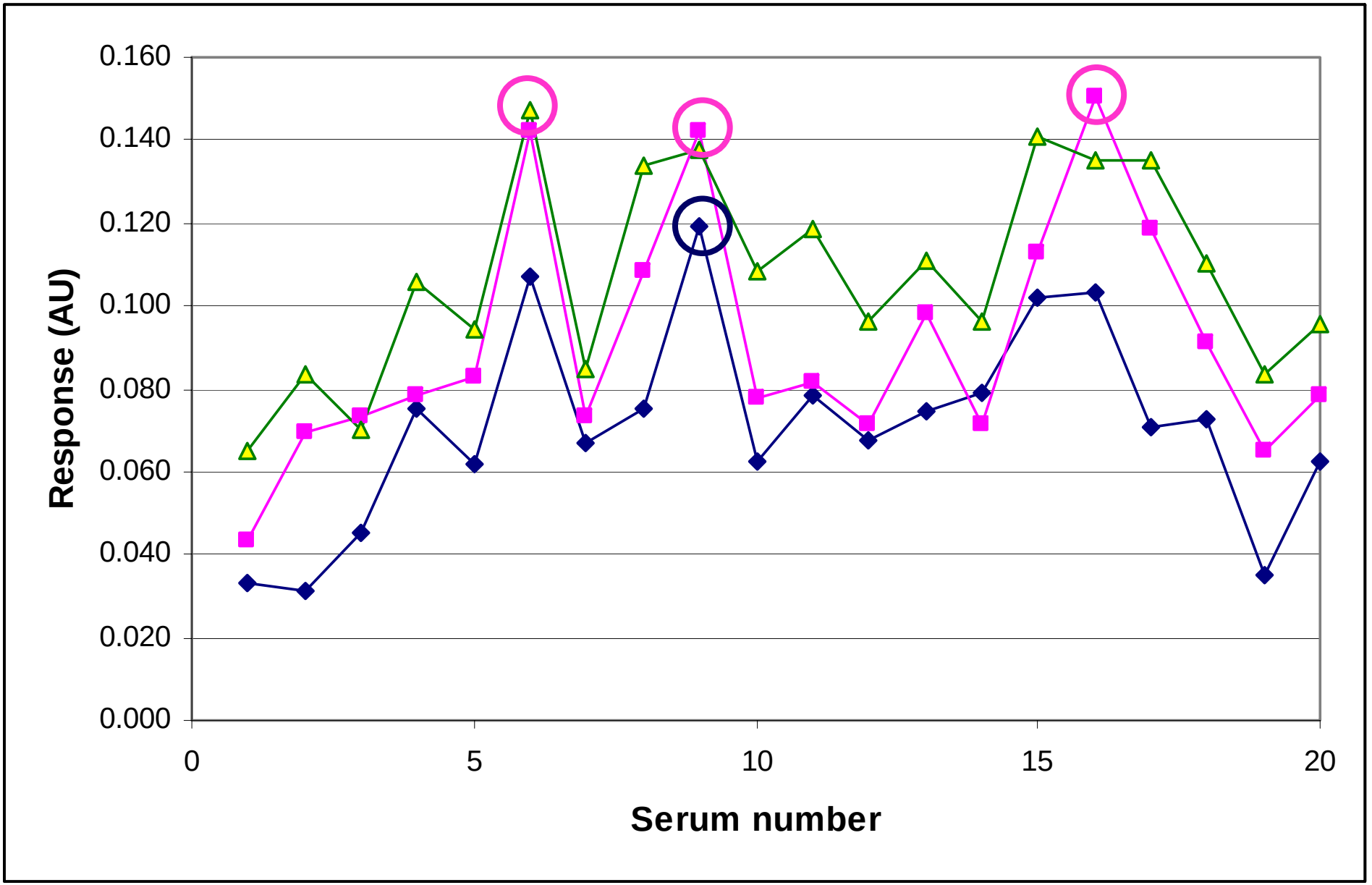
The following slides illustrate the effect of different levels of biological variability (Bio SD) and analytical variability (Assay SD) on the determination of “positive” samples, using sets of normally-distributed random data.

It is shown that even a small level of assay variability can lead to highly non-reproducible results and when the assay variability is comparable with the biologic variability, the cut-point methodology proposed by Mire-Sluis has no useful value.

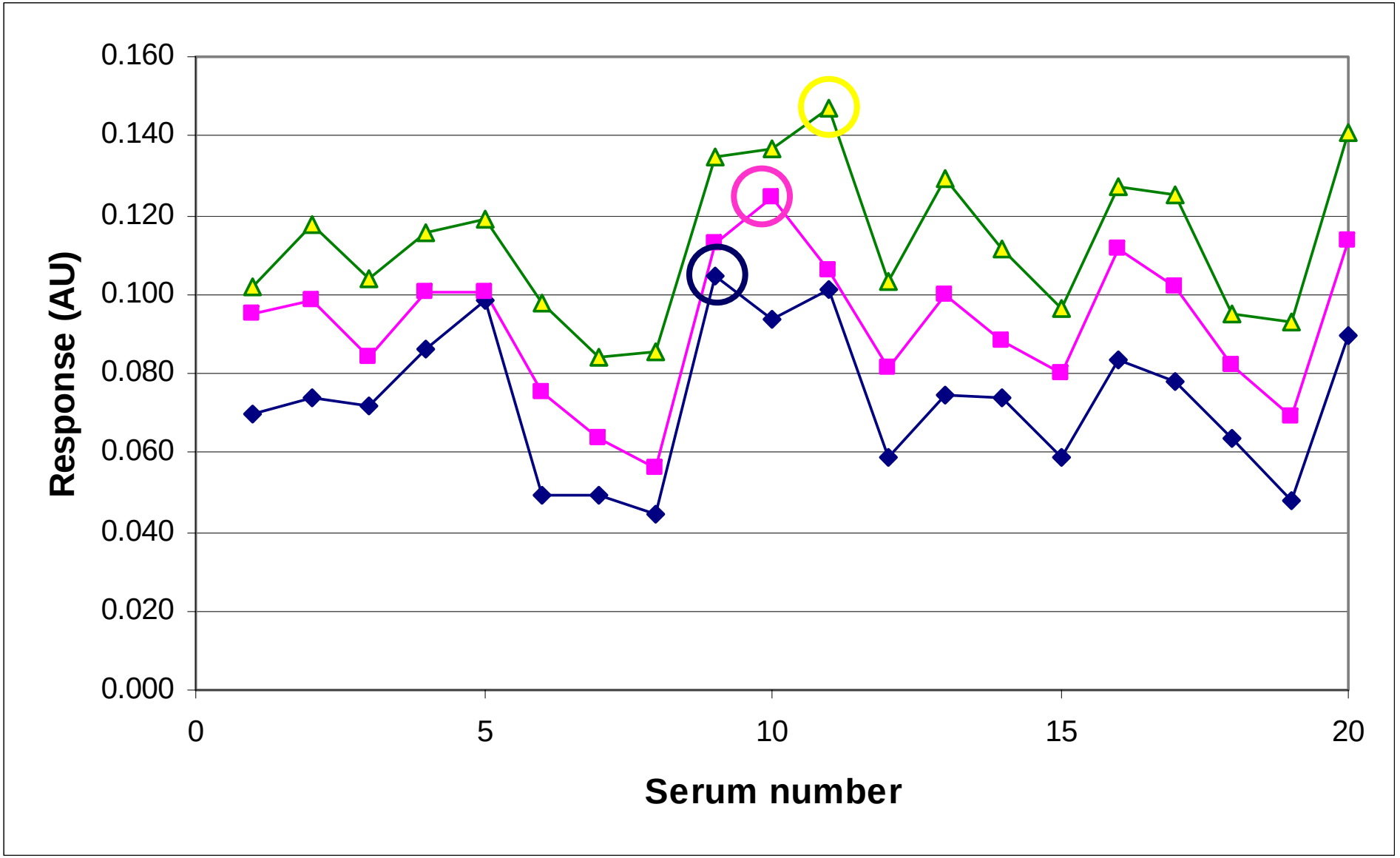
Bio SD=0.00 Assay SD=0.02



Bio SD=0.02 Assay SD=0.01



Bio SD=0.02 Assay SD=0.005



Cut Point and Sensitivity
- a case for “case by case”

Consider:

- Clinical consequences of antibodies
- Therapeutic concentrations of drug
- Likely frequency of antibodies
- Biosimilars – similar assay or not?

Quantitation of Response

EMEA	Mire-Sluis	Clients
“(Positive) samples need to be characterised in terms of antibody content (concentration/titre)”	“Due to the quasi-quantitative nature of immunogenicity assays, we advocate a titer-based approach”	Often require calibration curves and QC samples with same acceptance criteria as other ligand-binding assays

Confirmatory Assays

EMEA	Mire-Sluis	Clients
“Usually advisable to use a different assay format”	“Immunodepletion assay: a form of confirmatory assay”	Help!!

Different format?

- More sensitive
  - Why not use as screening assay?
  - May find positives among control samples which screened negative
- Less sensitive
  - Fail to confirm true weak positives

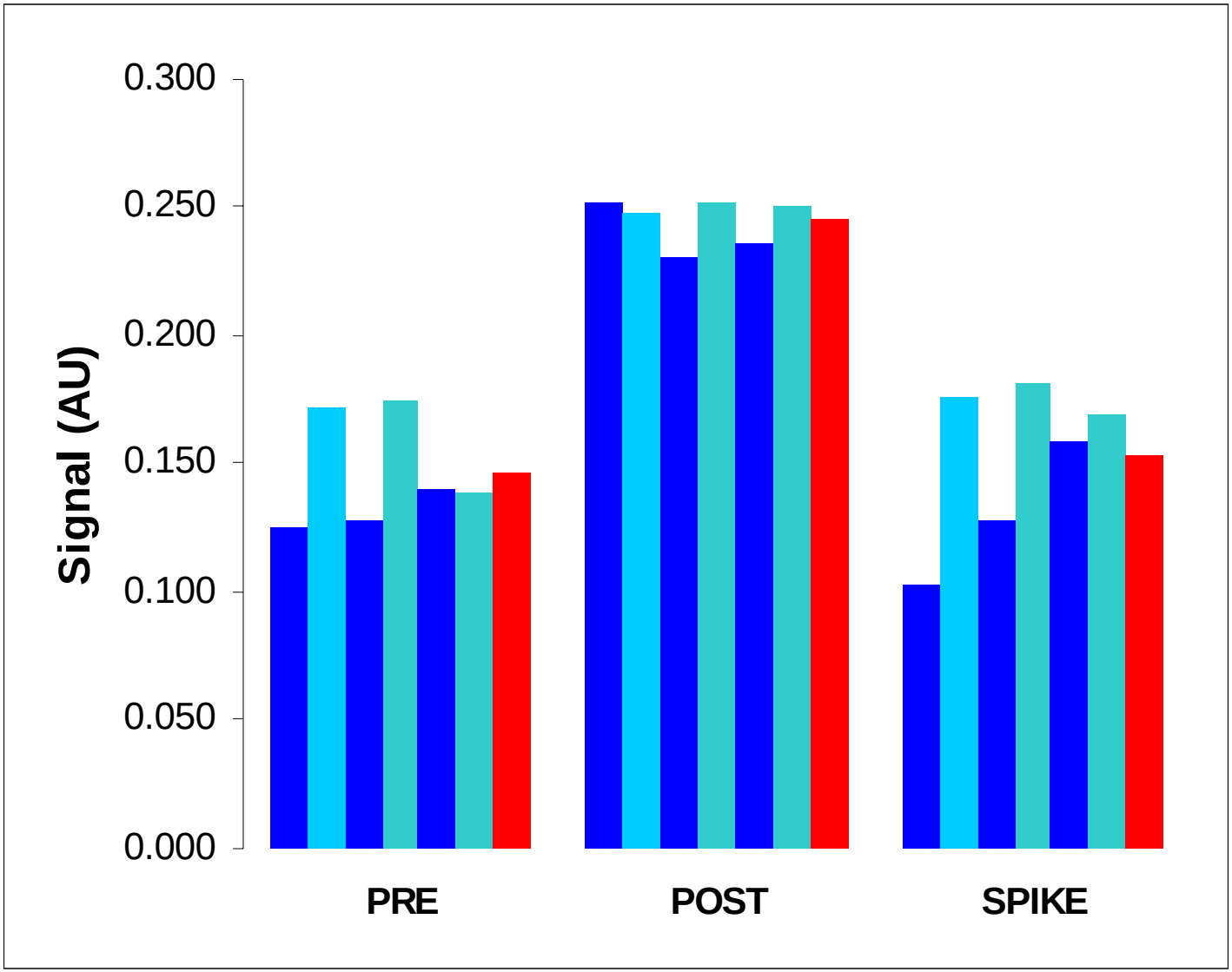
Same format?

- Compare pre and post-treatment
  - Treatment emergent response
- Drug inhibition (immunodepletion)
  - Drug-specific response

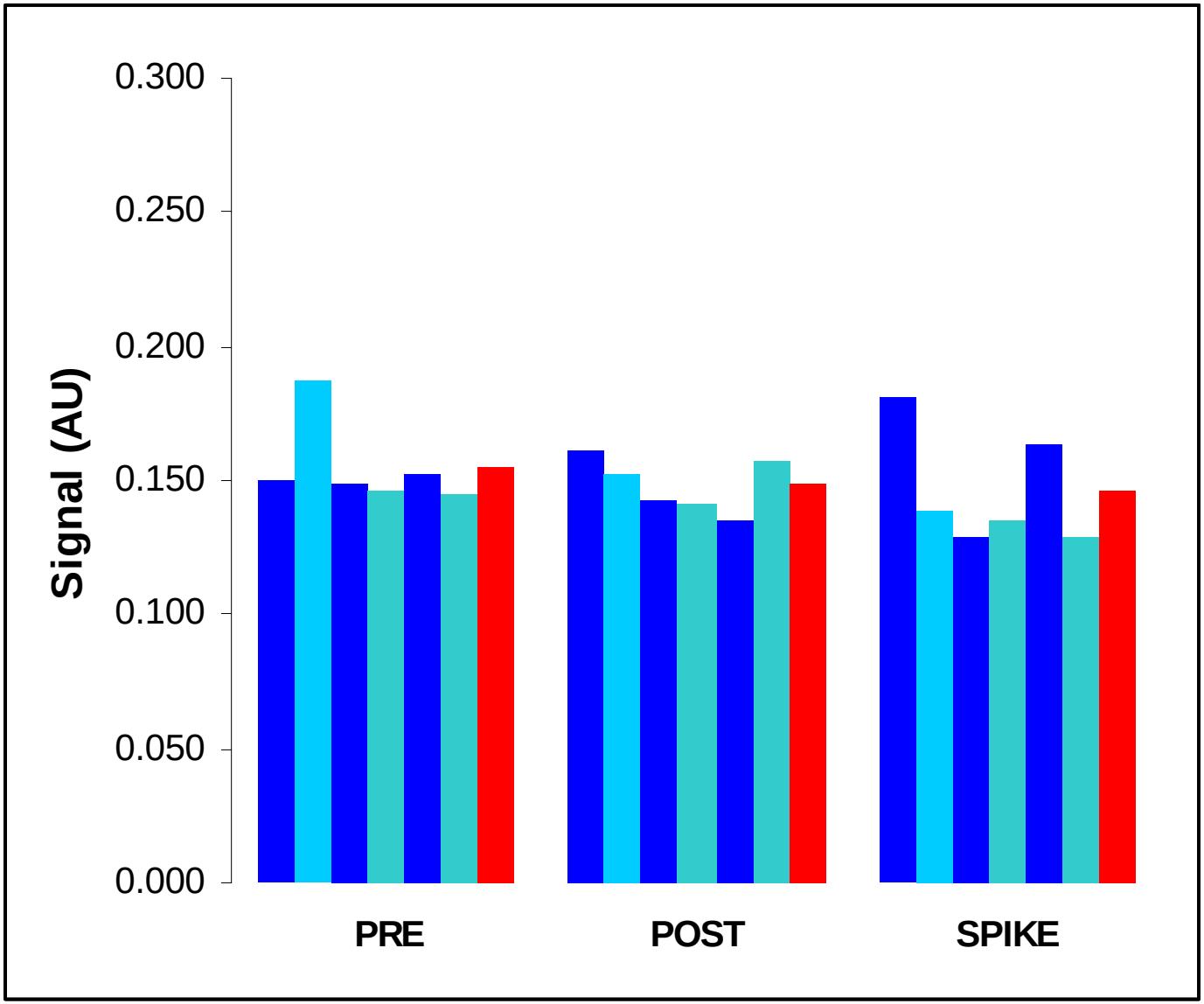
The following slides illustrate the effect of small sample size on the results of a confirmatory assay which is evaluated by a simple t-test.

There is little difficulty in confirming a strong positve response which Is fully inhibited by addition of drug.

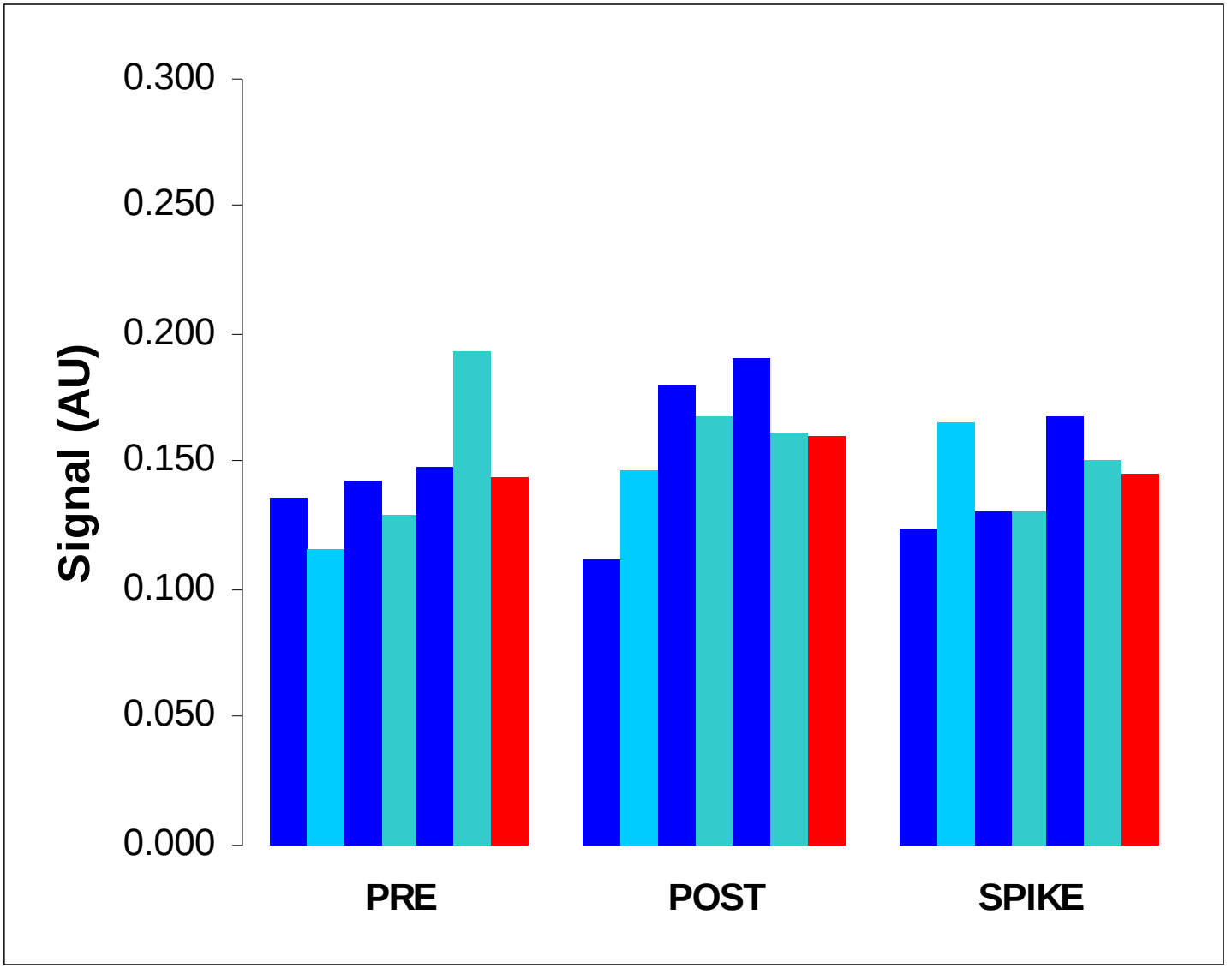
However, weak responses may sometimes be confirmed and sometimes not and the result of a particular assay is unpredictable. Improvements in assay precision can even be counter-productive, allowing small differences to falsely appear statistically significant.



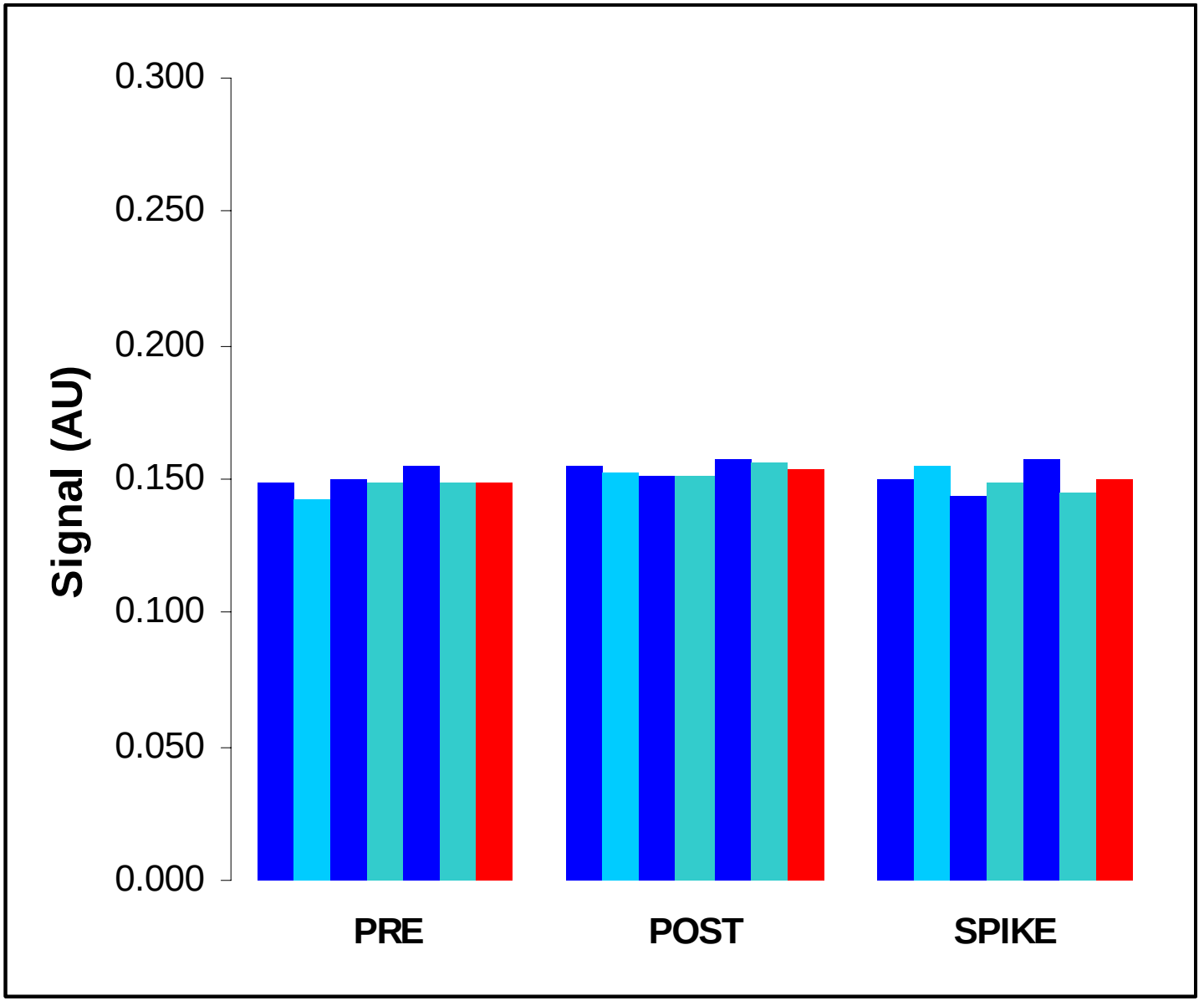
True positive, confirms treatment-emergent and drug specific



False positive, confirms negative



False positive, but confirms treatment-emergent and drug specific



False positive, but confirms treatment-emergent and drug specific

Validation

EMEA	Mire-Sluis	Clients
"… ensure all essential procedures are in place before commencement (of the clinical study). This includes … validation of all assays"	"The validation process will be discussed in a subsequent manuscript"	Validation to be completed before starting clinical trials, but guidance needed on how to do it.

Problems with pre-study validation

- Lack of suitable positive control
- Lack of suitable negative controls
- Lack of understanding of potential frequency and magnitude of responses

Pre-validation studies and early phase clinical trials

- Demonstrate analytical capabilities using surrogate reagents
- Determine potential analytical range and acceptance criteria
- Carry out in-study validation during Phase I clinical trials, eg local cut-point with local assessment of sensitivity
- Collect samples for positive controls