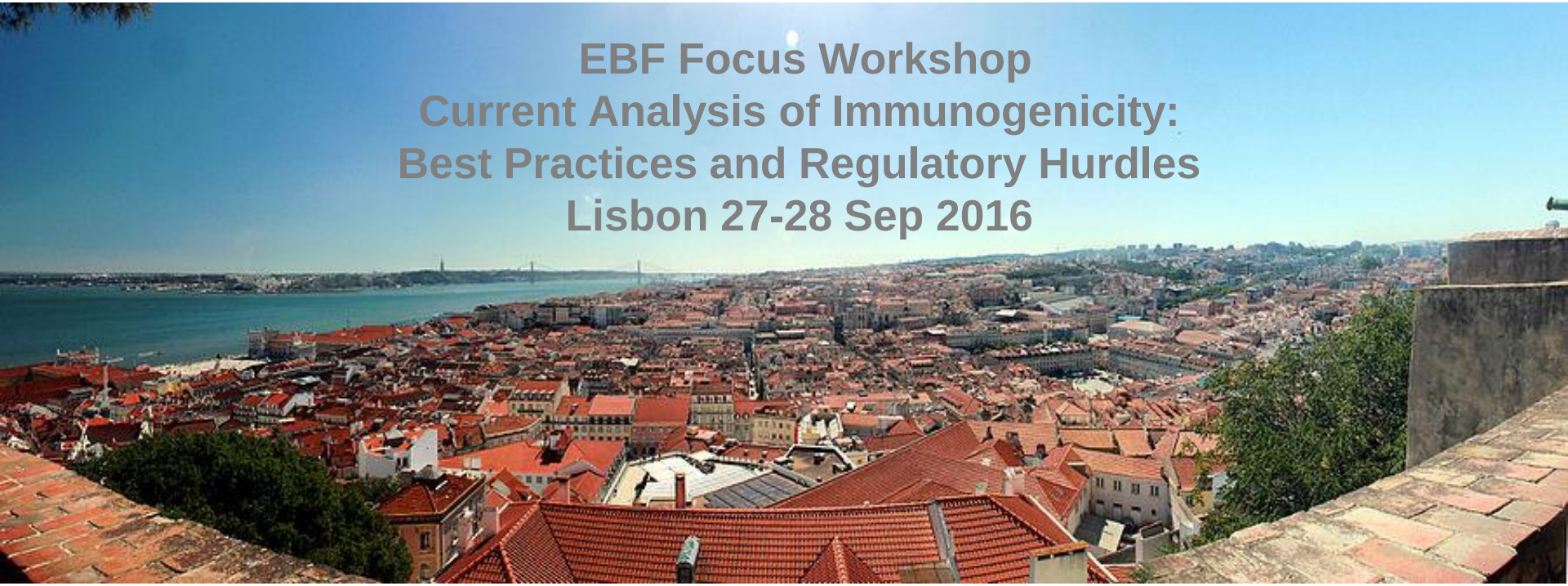
Pitfalls in cut point setting

Timo Piironen
Scientific Director
SYRINX Bioanalytics Oy



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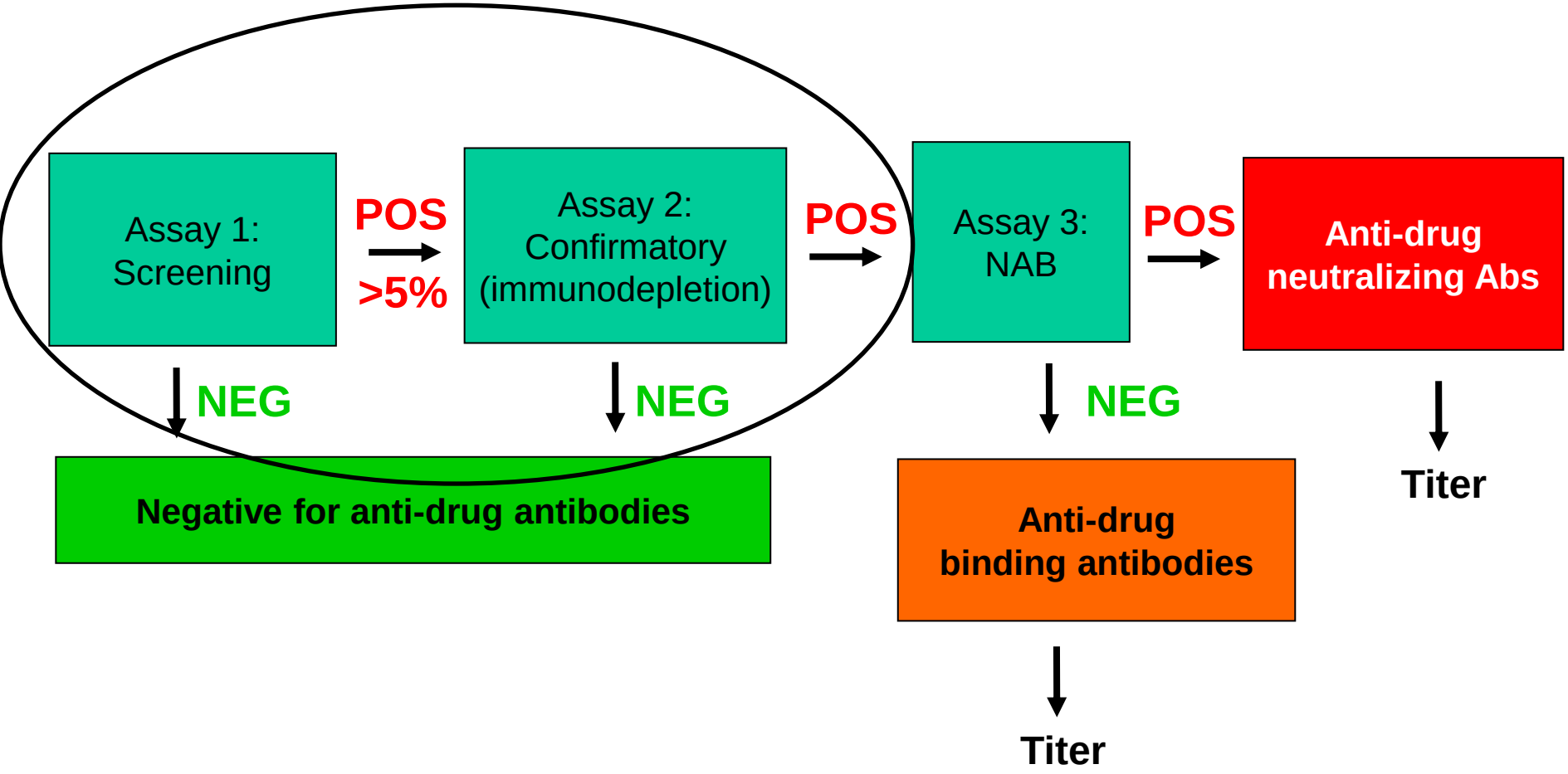
Tiered approach for immunogenicity testing



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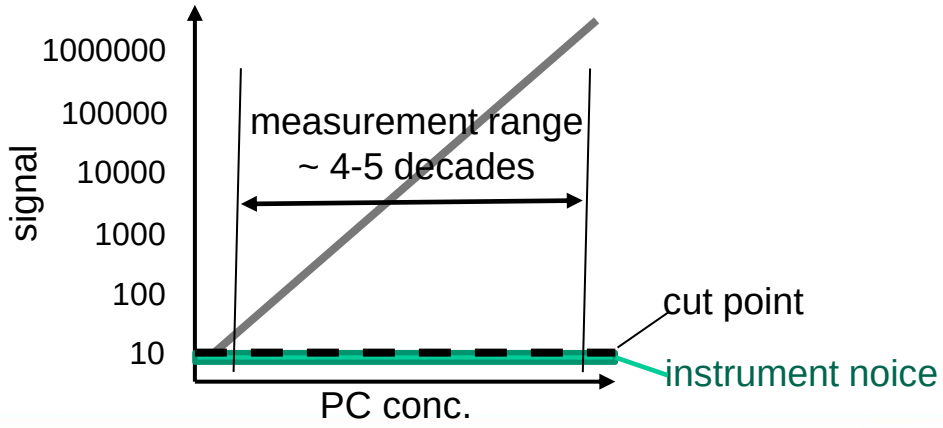
Background

- Up to date, Shankar et al. (*Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products, 2008*) has been the only guideline or white paper to provide specific instructions for the validation of assays for immunogenicity testing → STANDARD APPROACH
- The recent EMA and FDA draft guidelines follow the same principles
- While the recommendations may be suitable for some assay formats, they are problematic for others

Background

- The statistical approaches described are based on the assumption that the assay has significant background signal and considerable variation
- Very sensitive assays (< ng/mL) with a biological background signal close to the instrument background/noice and low variability often do not pass the statistical tests and recommendations of the article



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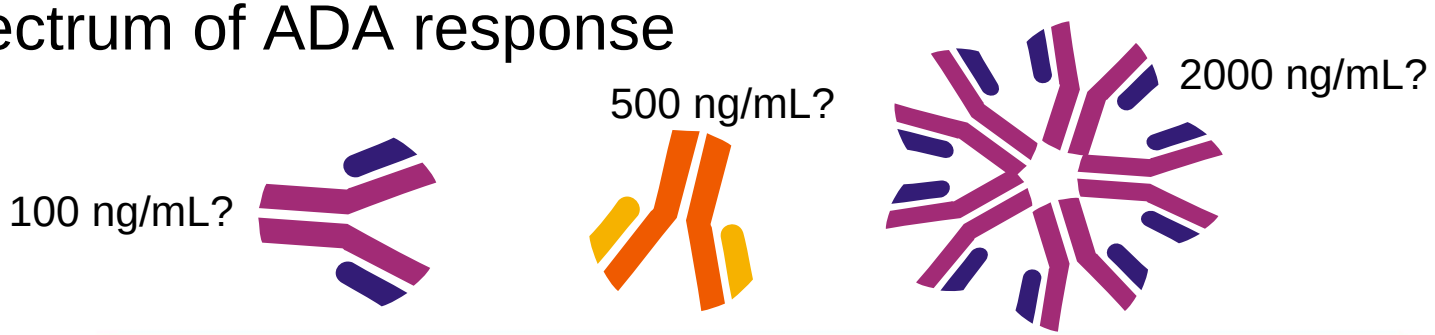
Background

- Although the requirements in the white paper are described as recommendations and examples, a CRO is most often (if not always) expected to follow the letter of the article

Positive control (PC) material

- Assays are developed based on a positive control Ab (except cut point)
- Assay optimized for a high affinity positive control antibody is not optimal for detecting the full spectrum of ADA response – including both high and low affinity antibodies
- Sensitivity and drug tolerance against a high affinity positive control antibody is not representative of the full spectrum of ADA response



Possible pitfalls

- Analytical and biological outlier removal
- Cut point evaluation (fixed, floating or dynamic)
- Sensitivity & selection of the LPC level
- Selectivity/matrix interference
- Run control acceptance
- Drug tolerance
- Titration
- Specific issues related to confirmation/specificity assays (inhibition of LPC/HPC and run control acceptance)

Analytical and biological outliers

- If there are several outliers, they do not separate as outliers in a limited population of 50-60 samples
- How to identify biological (e.g. pre-existing Abs) from analytical?
  - Presence of biological outliers can inhibit removal of analytical outliers by statistical tests
  - Robust alternatives for cut point calculation may be needed

Cut point evaluation (balanced design; 2 operators, 6 assay runs, 18 plates)

Means and Variances same = ***Fixed cut point***

Means different, Variances same = ***Floating cut point***

Means and Variances different = ***Dynamic cut point***

- In low background signal / low variability assays ANOVA and Levene's test often indicate unequal means and variances
- Still practically, a dynamic cut point is not a true option

Sensitivity & selection of the LPC level

LPC should be positive 99% of the time…

- In low signal / low variability assays long-term variability is difficult to simulate during limited validation tests
- LPC is often set very low (too low)
- Will pass statistical tests – but will eventually fail > 1 % of the time in-study
- High affinity positive control antibodies will be positive at very low concentrations (<ng/mL) → Sensitivity and LPC not representative of the whole ADA spectrum

Selectivity/matrix interference

Spike recovery in matrix should be same as in assay buffer…

- Matrix background is often higher than buffer background
- Equal (± 20%) signal is sometimes unreasonable
- Extremely low LPC may be consistently > cut point in blank matrix pool – but not in individuals
- Problem is not spike recovery, problem is too low LPC

Run control acceptance

PCs generally have low and high limits…

- High limit not necessary
- Higher incidence of false positives is OK (as they will be not confirmed as positive later on in the confirmation/specificity assay)

Drug tolerance

Assay should have good drug tolerance…

- LPC is set to be barely positive
- Unless free drug has no impact on signal (which it cannot, since it is required to inhibit signals) LPC drug tolerance is practically zero (as it should be)
- Is this meaningful for LPC?

Titration

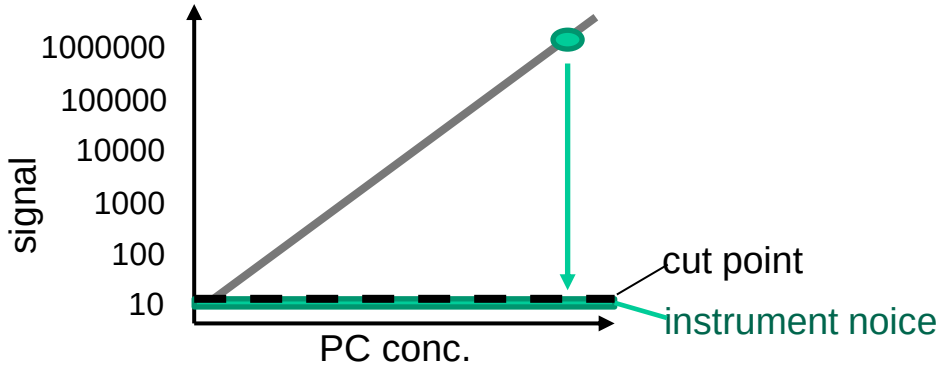
Samples are diluted in blank matrix until below cut point…

- Assays with a background signal close to instrument background and low variability, then LPC and cut point are close to the instrument background
- Signal of dilutions in blank matrix approaches background, but may never decrease below it, or are arbitrarily above/below

Specific issues related to confirmation/specificity assays

Amount of free drug added should inhibit the highest signal (HPC) below screening assay cut point…

- In wide range assays, the HPC is very high, it is then practically very difficult to inhibit



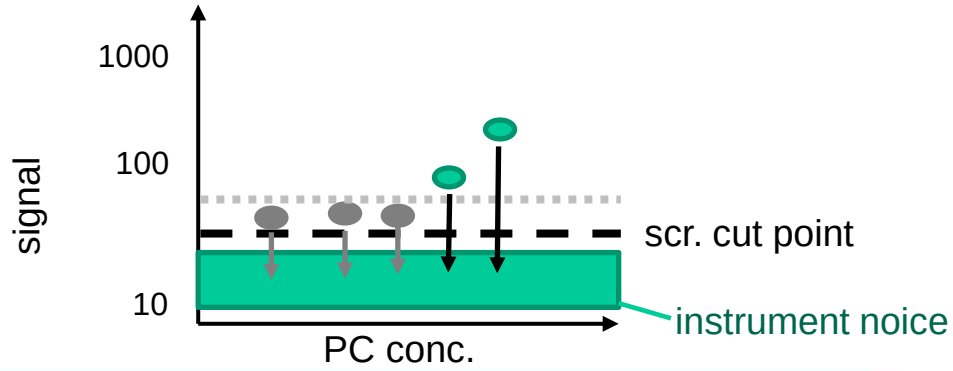
Amount of free drug should be 10-fold higher than determined above…

- Even the above is difficult to achieve, 10-fold more is often not possible due to drug stock concentrations

Specific issues related to confirmation/specificity assays

LPC should be positive…

- LPC concentration is selected only based on screening assay signal. LPC is often low – close to instrument background signal
- It may be inhibited by free drug less than the confirmation cut point (although it has approached the instrument background level)



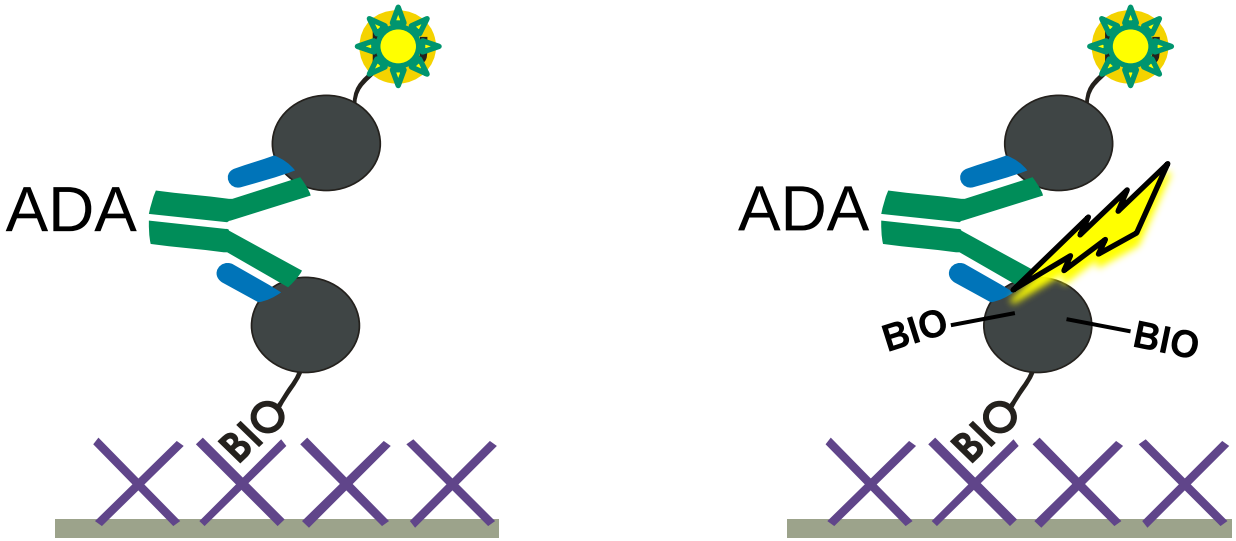
Specific issues related to confirmation/specificity assays

Run control acceptance: PCs generally have low and high limits…

- Are %-inhibition limits necessary for LPC / HPC?

Other pitfalls

- Epitope masking?



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