Multi-tiered versus semi-quantitative single-tiered immunogenicity testing in real-life datasets

Floris Loeff

Sanquin Research
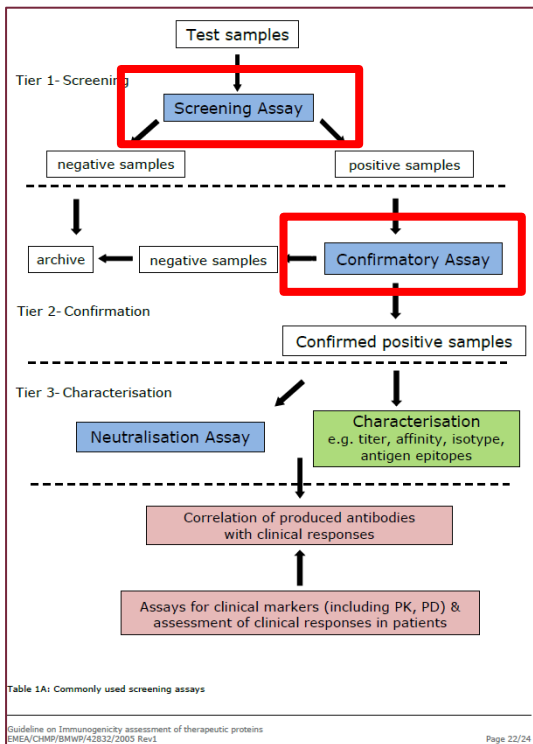
21th of October 2019
EBF Open Symposium

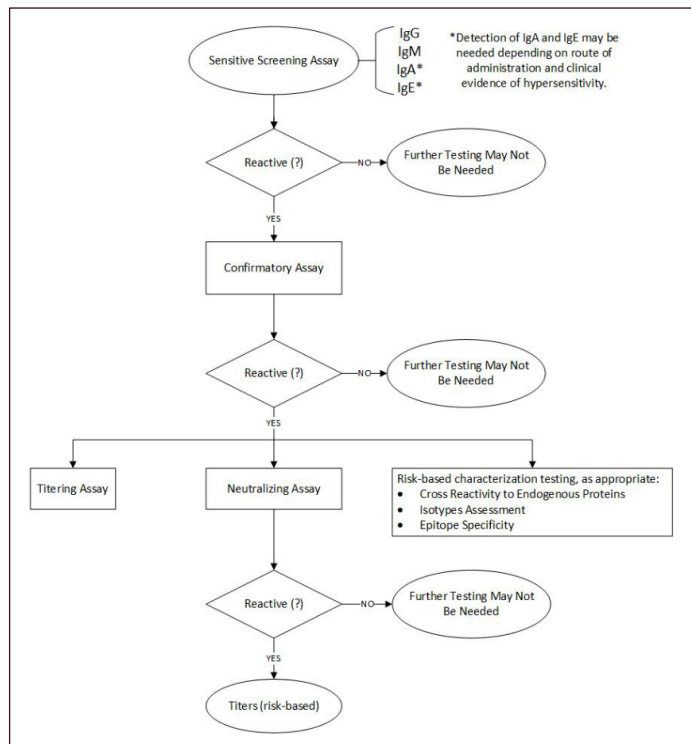**Blood and Beyond**

Our perspective

- Dutch Blood Supply Foundation
- Not for profit

- Hospital diagnostics
- Post approval studies
- CRO activities

- Historical starting point: clinical observations
- Clinical relevance of TDM and ADA
- Translation to physicians

Strict multi tiered approach for anti-drug antibody testing
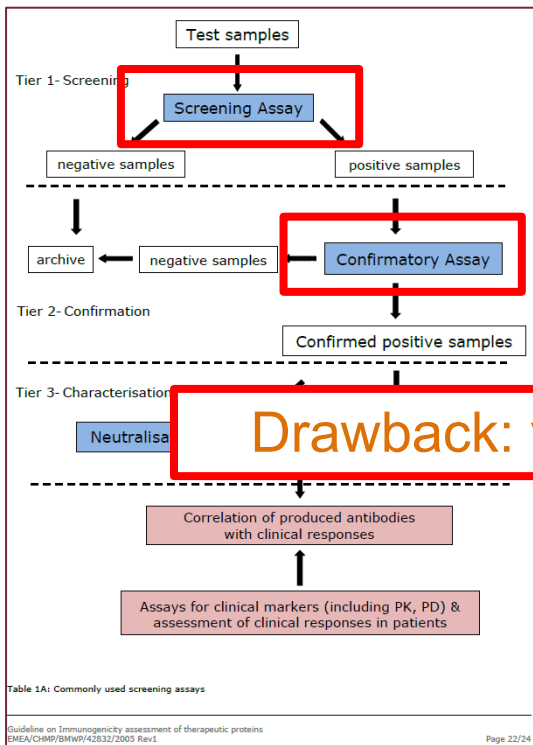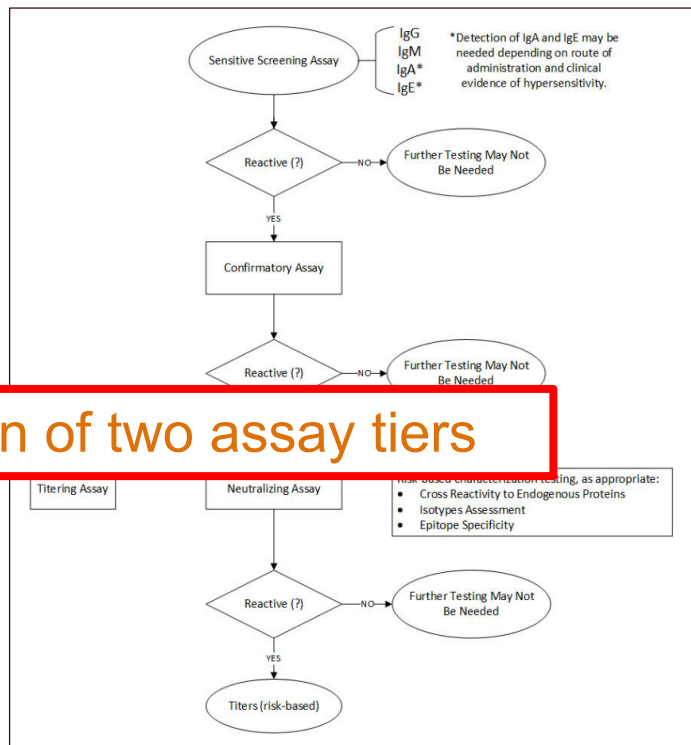
EMA

2017, Guideline on Immunogenicity assessment of therapeutic proteins

FDA

2019, Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Strict multi tiered approach for anti-drug antibody testing

EMA

Tier 1- Screening

Test samples → Screening Assay → negative samples / positive samples

Tier 2- Confirmation

archive ← negative samples | Confirmatory Assay

Confirmed positive samples

Tier 3- Characterisation

Neutralisa...

Correlation of produced antibodies with clinical responses

Assays for clinical markers (including PK, PD) & assessment of clinical responses in patients

Table 1A: Commonly used screening assays

Guideline on Immunogenicity assessment of therapeutic proteins
EMEA/CHMP/BMWP/42832/2005 Rev1 — Page 22/24

FDA

Sensitive Screening Assay — IgG, IgM, IgA*, IgE*

*Detection of IgA and IgE may be needed depending on route of administration and clinical evidence of hypersensitivity.

Reactive (?) — NO → Further Testing May Not Be Needed
YES ↓

Confirmatory Assay

Reactive (?) — NO → Further Testing May Not Be Needed

Titering Assay | Neutralizing Assay

Risk-based characterization testing, as appropriate:
- Cross Reactivity to Endogenous Proteins
- Isotypes Assessment
- Epitope Specificity

Reactive (?) — NO → Further Testing May Not Be Needed
YES ↓

Titers (risk-based)

Drawback: validation of two assay tiers

2017, Guideline on Immunogenicity assessment of therapeutic proteins

2019, Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection
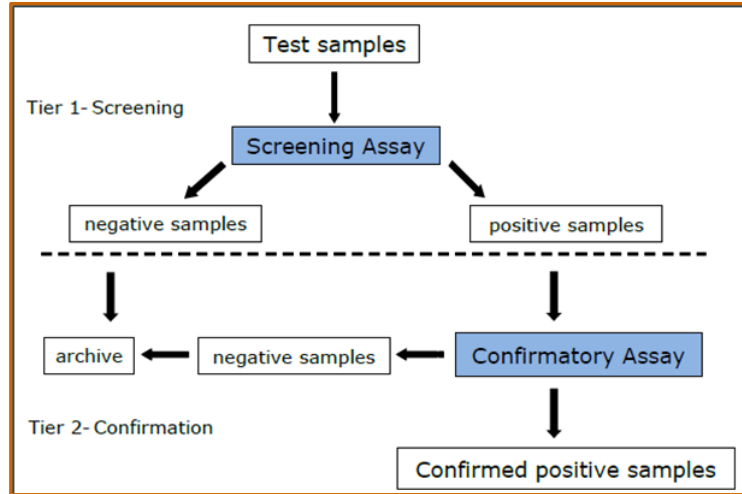
| 4

5% FPR for screening cut point, assuming independence of confirmation tier to prevent false positive results

G. Shankar et al. / Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1267–1281

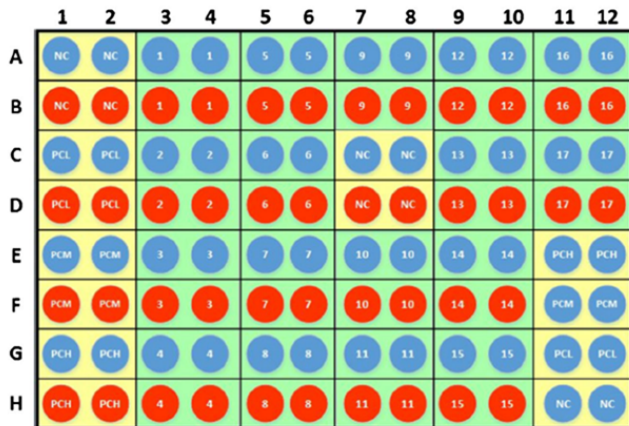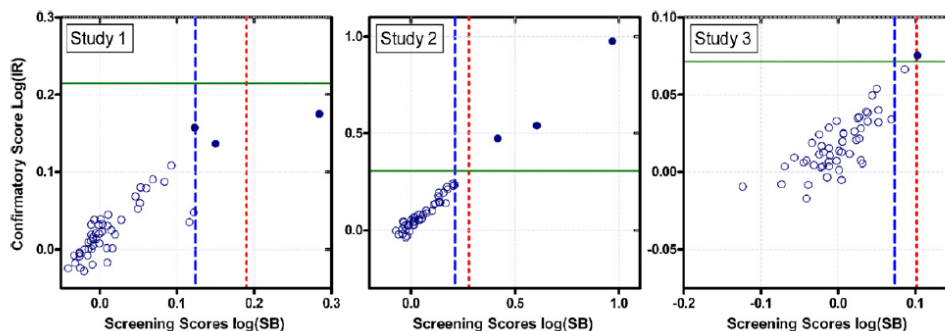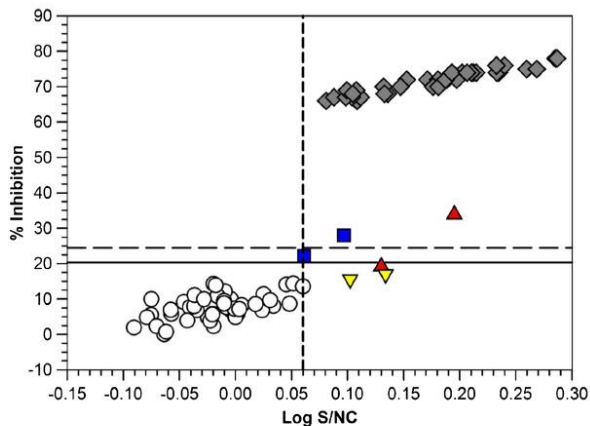Recommendations for Systematic Statistical Computation of Immunogenicity Cut Points

Fig. 2. Suggested plate layout for evaluation of test samples and controls. This figure offers a suggested layout that accommodates evaluation of 17 test samples with (*red*) and without (*blue*) added excess biotherapeutic. The *yellow areas* denote suggested placement of controls, while the *green areas* represent placement of the test samples. *NC* negative control, *PCL* low positive control, *PCH* high positive control
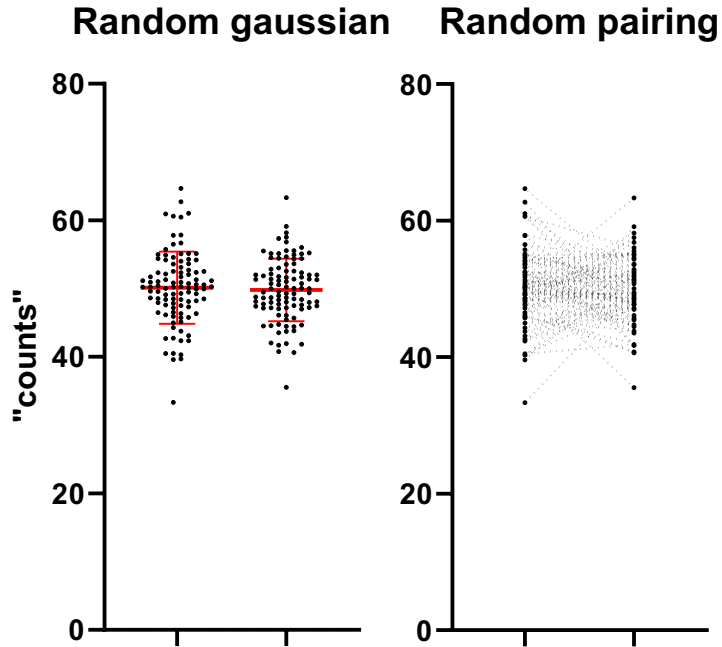
Our discussion has been focused on screening cut point analysis. The same flowchart can be used for confirmatory cut point analysis. Typically the confirmatory assay is validated together with the screening assay. On the same plate, a sample without drug and spiked with drug is tested on the same plate side by side.

Devanarayan et al. AAPS J. 2017
Zhang et al. J. Immunol Methods. 2013

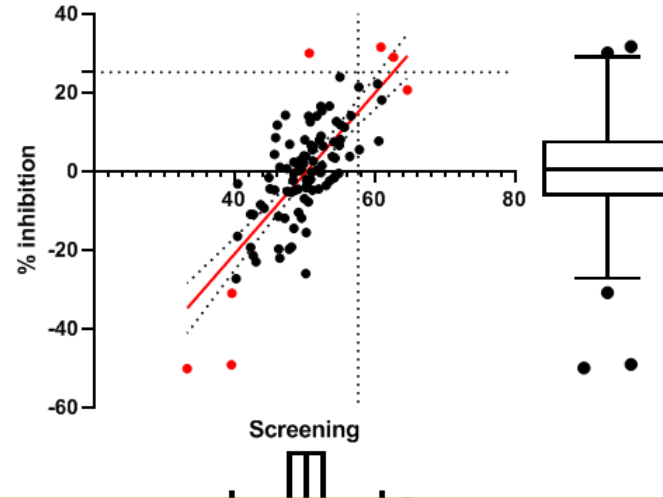# In sample evaluation, assessing screening and confirmation on the same plate results in non-orthogonal results

Outliers labeled "pre-existing ADA", which might be correct for biological outliers, but what about statistical outliers?

Devanarayan et al. AAPS J. 2017
Kubiak et al. J Pharm Biomed Anal. 2013

Assessing screening and confirmation on the same plate can only lead to non-orthogonal results

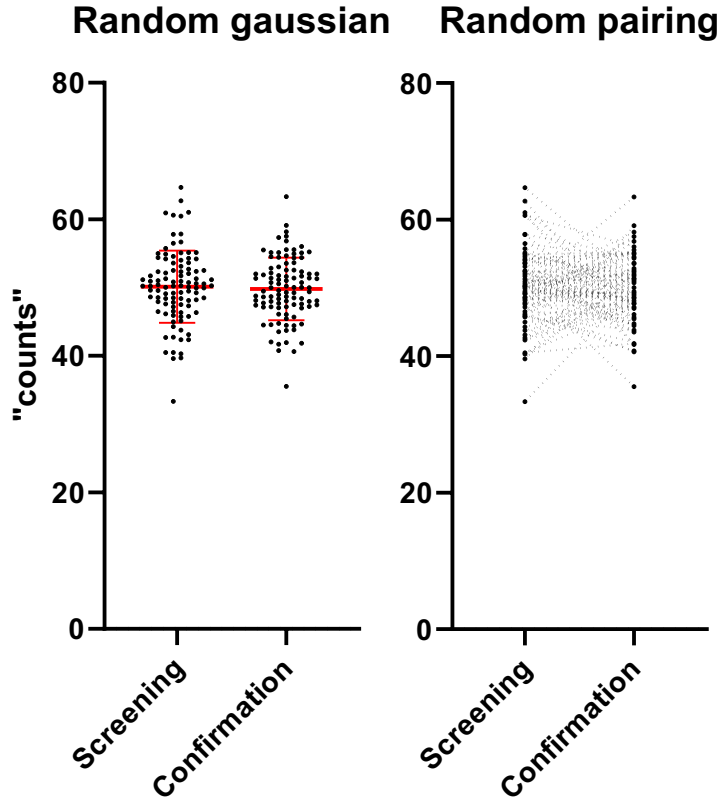**Random gaussian**   **Random pairing**

Screening value is used for screening score and as denominator for the confirmation score
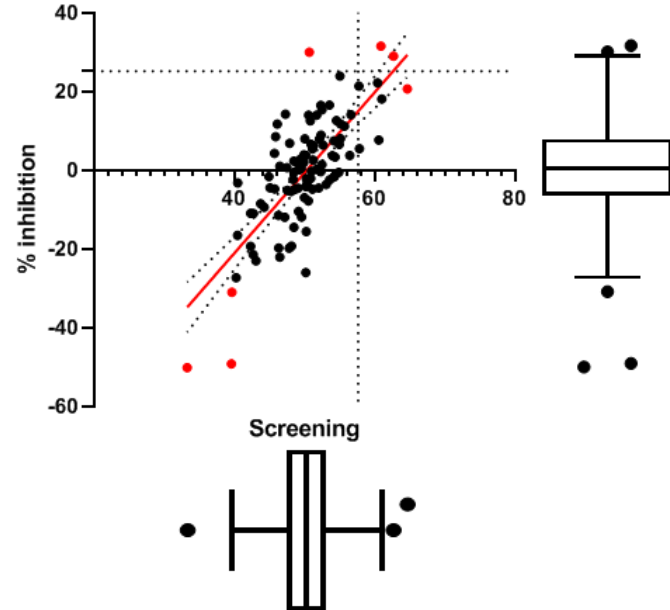
Non-orthogonal assay results may in part explain the high incidence of "false positives"

Assessing screening and confirmation on the same plate can only lead to non-orthogonal results
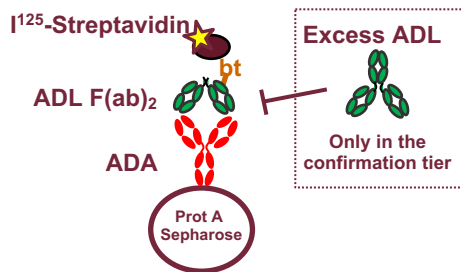

Random gaussian   **Random pairing**

Screening value is used for screening score and as denominator for the confirmation score
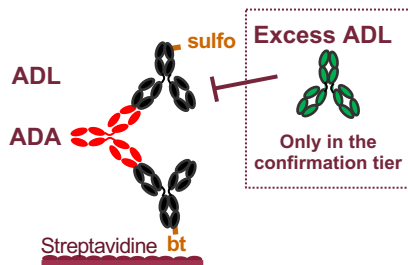
random.org/gaussian-distributions/

Real-life sample sets with diverse ADA levels for evaluating multi-tiered vs single-tiered immunogenicity testing

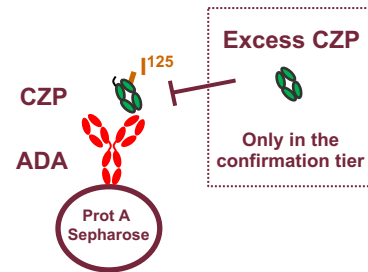## Acid-dissociation Radioimmunoassay (ARIA) Adalimumab

I[125]-Streptavidin
ADL F(ab)₂
ADA

Excess ADL
Only in the confirmation tier

Prot A Sepharose

Drug-tolerance:
Acid pretreatment

Bridging ECL Adalimumab

sulfo
ADL
ADA

Excess ADL
Only in the confirmation tier

Streptavidine bt

Drug-tolerance:
Acid pretreatment

Radioimmunoassay (RIA) Certolizumab

I[125]
CZP
ADA

Excess CZP
Only in the confirmation tier

Prot A Sepharose

Drug-tolerance:
Removal of non ADA-bound drug before detection

Dataset	Assay platform	Disease	Drug	# Baseline samples	# Treatment samples
1	ARIA	Rheumatoid arthritis	Adalimumab	40	122
2	ECL	Rheumatoid arthritis	Adalimumab	40	81
3	RIA	Rheumatoid arthritis	Certolizumab	41	83

What does the confirmation tier normally protect against?

- Not drug target-mediated false positivity
- Not rheumatoid factor-mediated false positivity

- Only false positivity mediated by the modifications that were introduced to the drug detection reagent, tagging (biotinylation / sulfo) or fragmentation $(F(ab)_2$-fragment, anti-hinge)
  - Can (partially) be negated using specific buffer components

Solution

Clinical trials: look at baseline samples

Hospital diagnostics: drug level should be decisive factor

Conclusions

- Non-orthogonal results are obtained when samples are evaluated using the screening sample for the screening result as well as denominator for the confirmation result

- Almost identical results to the multi-tiered approach were obtained when samples were assessed only in a screening assay in two independent duplicates and with a less stringent cutpoint

- Discrepant results were predominantly observed in samples with assay signals just above the cut points. Clinical relevance of these low titers is likely negligible.

- Single-tiered immunogenicity testing ablates need for validation of the confirmation assay and saves taking the confirmation condition along in testing

Acknowledgements

- **Sanquin Research, Department of Immunopathology**
  - Lisanne Dijk
  - Simone Kruithof
  - Pleuni de Heer-Ooijevaar
  - Lea Berkhout
  - Gertjan Wolbink
  - **Theo Rispens**

- **Sanquin Diagnostics, Biologics Laboratory**
  - Karien Bloem
  - Astrid van Leeuwen
  - Annick de Vries

- **Reade**
  - **Gertjan Wolbink**