ADA Testing in Repeated Dose Toxicity Studies - Strategies

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Immunogenicity in Repeated Dose Tox Studies

Scope

- **Toxicities of biologics are mainly due to exaggerated pharmacology rather than off-target effects**
- **The validity of toxicity studies for biologics relies upon the demonstration of active drug exposure throughout the study**
- **Most biologics induce the formation of anti-drug antibodies (ADAs) in animals**
    - Clearing or sustaining antibodies impairing PK (and consequently PD)
    - Neutralizing antibodies reducing PD with or without impaired PK
- **It is crucial for the interpretation of safety data to assess whether and to what extent ADA-bound biologics retain pharmacological activity**
    - PK assay format is utmost important (“total”; “active”)

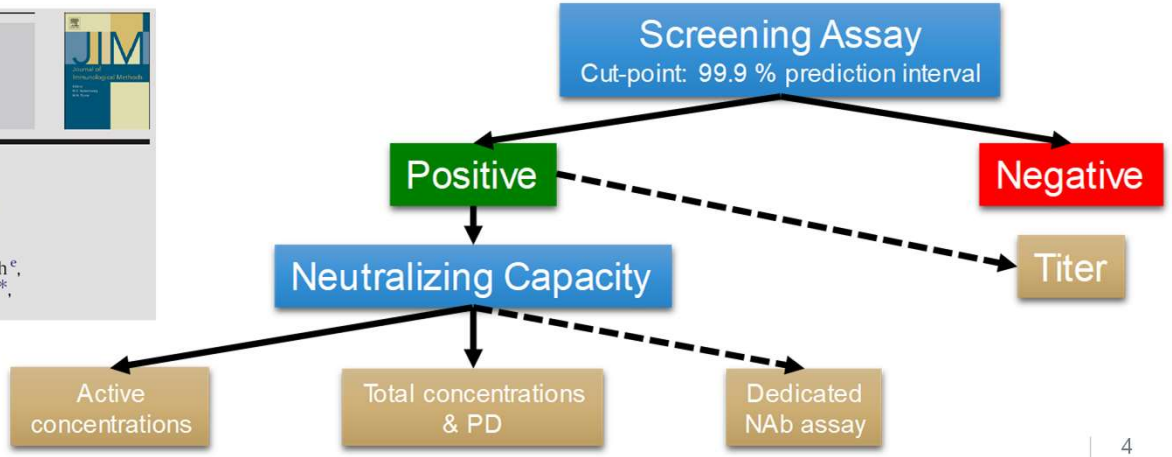
Regulatory Framework

- **ICH S6(R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals (2011)**
  - The induction of antibody formation in animals is not predictive of a potential for antibody formation in humans
    - Measurement of anti-drug antibodies (ADAs) should be performed in order to aid in the interpretation of **repeated dose** toxicity studies
  - ADA samples in repeated dose toxicity studies should be drawn but only analyzed in the following cases:
    - Evidence of altered PD activity
    - Unexpected changes in exposure in the absence of a PD marker
    - Evidence of immune-mediated reactions (e.g. immune complex disease, vasculitis, anaphylaxis)
- **EMA Guideline on Immunogenicity assessment of therapeutic proteins (2017)**
  - There is no need for immunogenicity assessment in single dose toxicity studies
  - The assays for ADA assessment in nonclinical toxicity studies should be **validated**
  - Drug interference in the ADA assay needs to be considered (due to high doses administered)

ADA Testing – Repeated Dose Tox Studies

Testing Strategy

- **Primary aim of non-clinical immunogenicity testing is to aid in the interpretation of toxicity results but not to ensure safety of the tox species**
- **Striving for the highest sensitivity is not necessary (FDA: Expected sensitivity 1 µg/mL)**
  - Screening cut point at the 99.9 % prediction interval
  - No confirmatory assay (as no false-positive results are expected)
  - Drug tolerant screening assay might be needed (acid dissociation)
- **Neutralizing capacity of ADAs can be assessed indirectly with an "active" PK assay (preferred), a combination of total PK assay and PD (if available) or directly in a dedicated neutralizing antibody assay (backup solution)**
  - The assessment of the neutralizing capacity of ADAs might also be warranted in cases where the consequences of neutralizing antibodies in humans could be anticipated from nonclinical studies

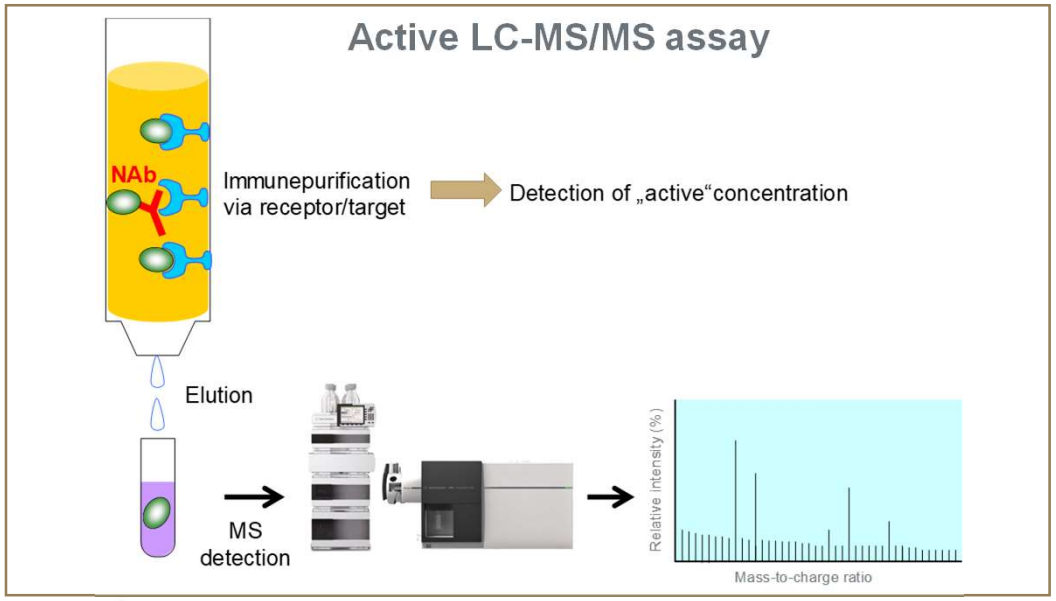


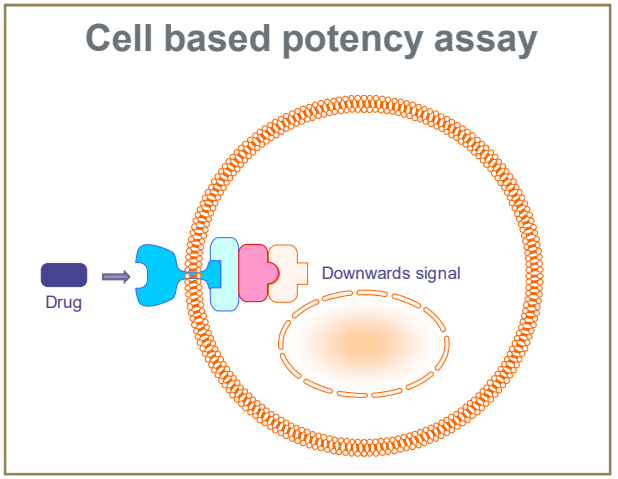
PK Assay Format for TK Assessment

- **The use of a PK assay detecting "active drug" is highly recommended to assess TK in repeated dose toxicity studies for biologics**
  - No additional PD read-out needed to proof active exposure of the animals
  - No dedicated NAb assays necessary

- **Active PK assays can employ**
  - LC-MS/MS assay with previous immunopurification
  - Ligand binding assays
  - Cell based potency assays (backup solution)
    - Only applicable to rather potent drugs (sensitivity issue)
    - Low throughput / high costs

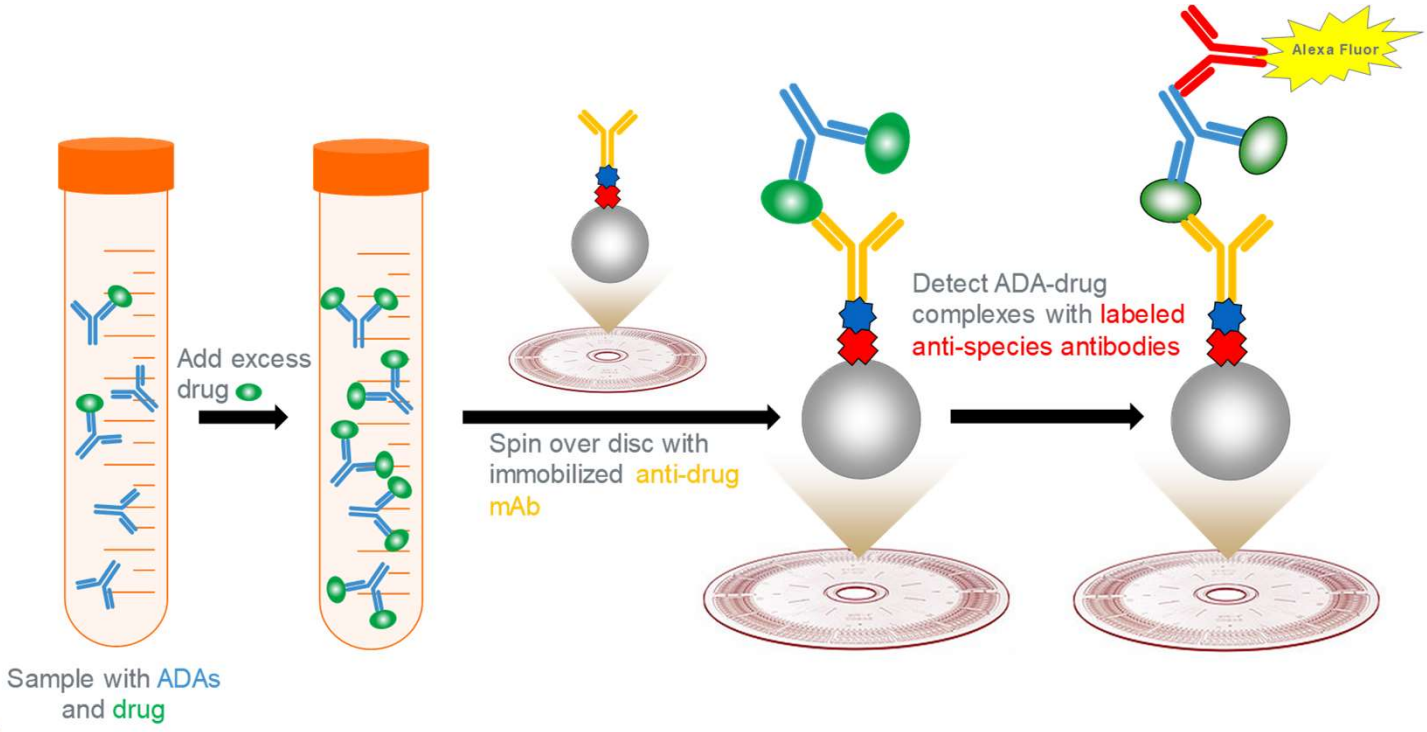
} Using the receptor/target for drug capture (preferred)





ADA Assay Format – Repeated Dose Tox Studies

- **Due to limited blood volume, rodent tox studies usually employ dedicated subgroups for TK and ADAs**
  - Assessment of active exposure in main group animals rather difficult/impossible
  - The low sample consumption of the Gyros platform might allow to assess TK and ADAs from main group animals
- **Tox trials utilize high doses which calls for a rather drug tolerant ADA assay**
  - Detecting ADA/drug complexes (instead of “free” ADAs) is an alternative to cumbersome acid dissociation assays



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ADA Assay Validation - Repeated Dose Tox Studies

Validation Parameter

Validation Parameter (Pivotal) Clinical Trials	Validation Parameter Repeated Dose Tox Studies
Screening Cut Point • At least 50 samples tested in 6 assay runs by 2 analysts in a balanced design • 95 % confidence interval	**Assay Cut Point** • At least 25 samples tested in 3 assay runs by one analyst • 99.9 % confidence interval
Confirmatory Cut Point • 99 % confidence interval	-
Sensitivity • Six independent serial dilution series of the positive control antibody spanning the assay cut-point (3 per analyst)	**Sensitivity** • Three different low positive controls (LPCs) will be tested during the precision runs (500 ng/mL; 750 ng/mL; 1000 ng/mL). The lowest LPC that tests positive in alll precision runs will be defined as assay sensitivity

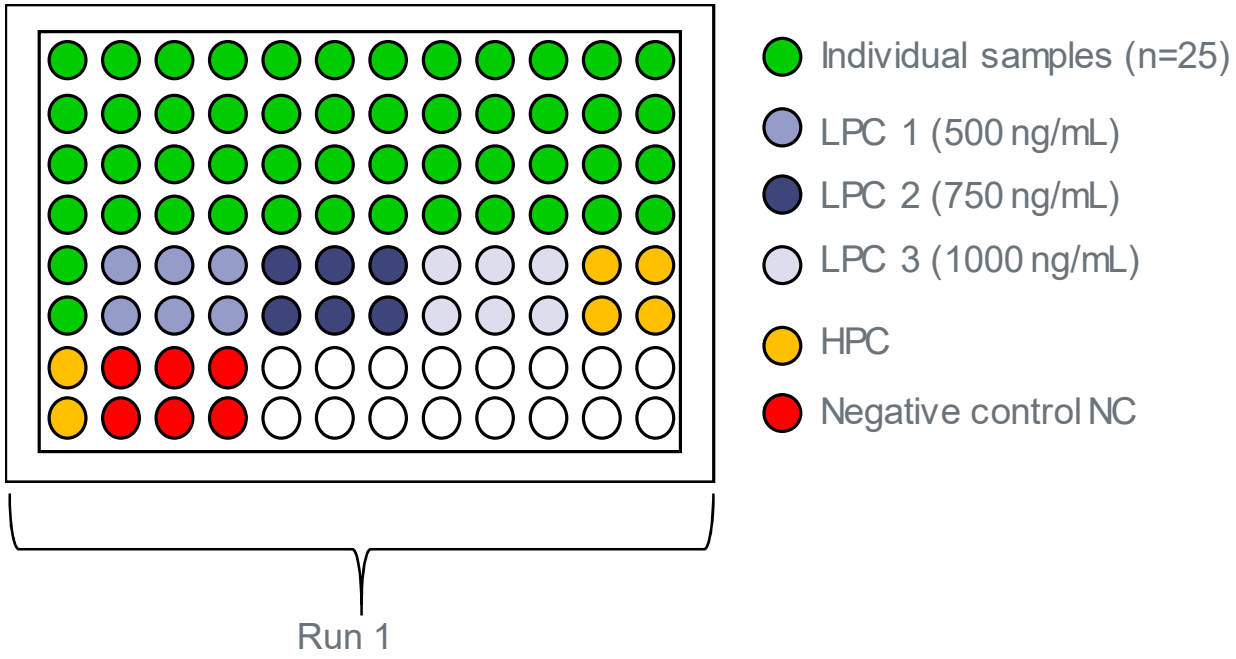
Precision (intra-assay and inter-assay)
• Intra-assay precision
  • Six independent aliquots of NC, LPC, MidPC and HPC on one plate by one analyst
• Inter-assay precision
  • One aliquot of NC, LPC, MidPC and HPC on three different days (two plates per day) by two analysts (i.e. 12 plates in total)
	Precision (intra-assay and inter-assay)
	• Three independent aliquots of NC, LPC1 (500), LPC2 (750), LPC 3 (1000 ng/mL) and HPC on 3 different days (one plate per day), by one analyst (i.e. 3 plates in total)
	• Will be performed during cut-point runs

Selectivity • Recovery of LPC & HPC in matrix vs. assay buffer	-
Specificity • Blocking of binding of LPC and HPC with unlabeled drug	-
Free drug tolerance • Response of different concentrations of the positive control are evaluated in presence of increasing amounts of drug in a “checker board” layout	**Free drug tolerance** • Response of the selected LPC and HPC are evaluated in presence of at least two concentrations of drug
Stability • Bench top • Freeze/thaw	**Stability** • Bench top • Freeze/thaw

ADA Assay Validation - Repeated Dose Tox Studies

Cut-Point / Precision / Sensitivity

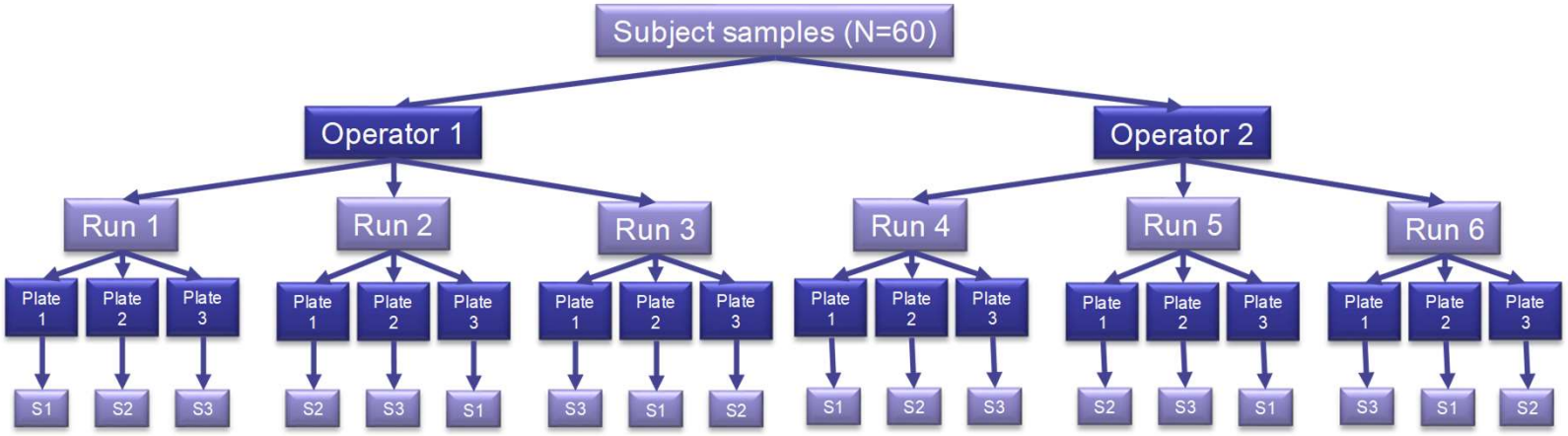
- **Assay cut-point; sensitivity, intra-assay and inter-assay precision will be evaluated in the same validation runs**
  - Only 3 runs necessary in total
  - Additional runs only needed for “free drug tolerance” and “stability”



Assay Cut-Point

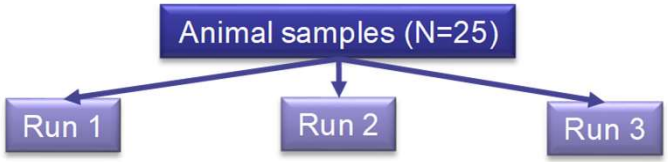
Analytical Design

- **Balanced design ADA validation for pivotal clinical trials:**



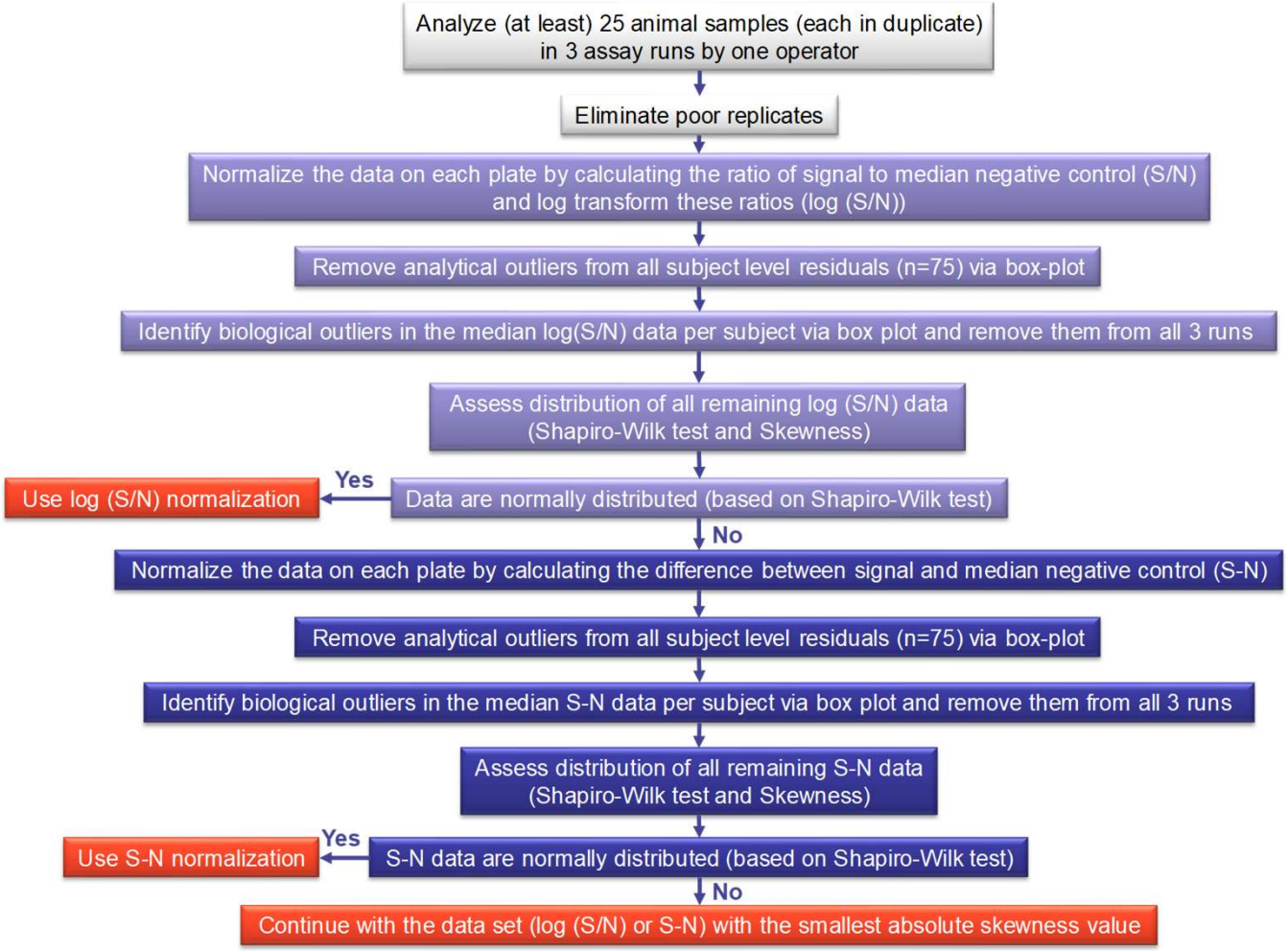
S1: Subject samples 1-20
S2: Subject samples 21-40
S3: Subject samples 41-60

- **Design ADA validation for repeated dose tox studies:**



Assay Cut-Point

Outlier Removal



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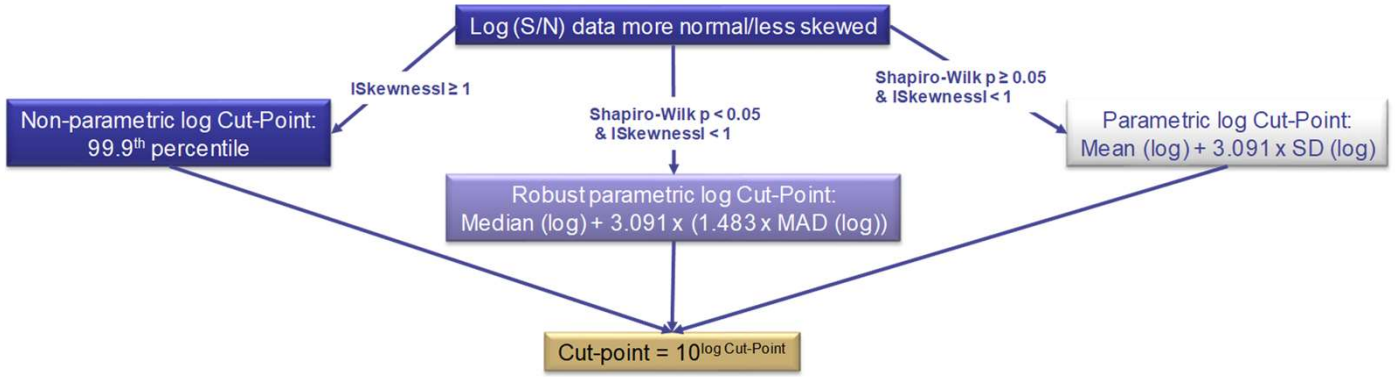
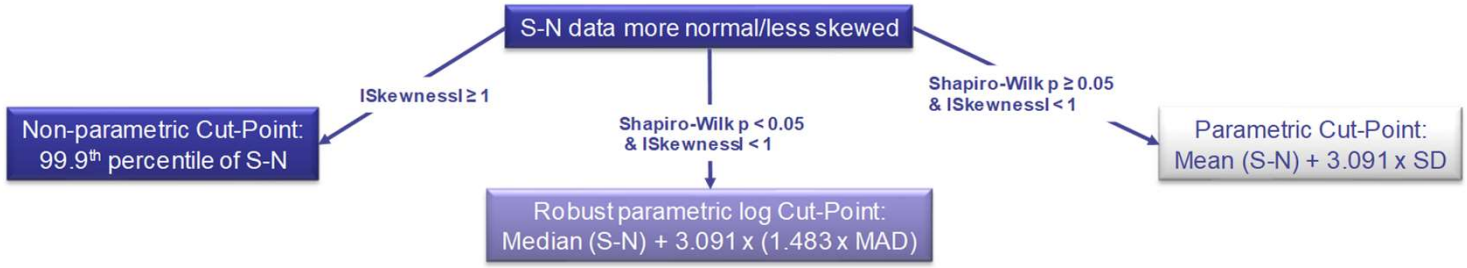
Assay Cut-Point

Calculation of the Assay Cut-Point

- **If log(S/N) data are closer to a normal distribution / less skewed**



- **If S-N data are closer to a normal distribution / less skewed**



Summary

- **A major prerequisite for the validity of toxicity studies for biologics is exposure of animals to active drug**
- **Assessment of immunogenicity should only be performed in order to aid in the interpretation of repeated dose toxicity studies**
  - Microsampling (e.g. Gyros) might allow to assess TK and ADAs from main group animals in rodent tox studies
  - Detection of ADA/drug complexes (instead of “free” ADAs) seems to be a good alternative to cumbersome acid dissociation assays
  - A dedicated neutralizing antibody assay should be the last resort (assessment of “active PK” is the method of choice)
- **The immunogenicity assays for repeated dose toxicity studies need to be validated**
  - The scope of the validation can be limited compared to a pivotal clinical setting

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