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Development of generic goal posts for equivalence testing of potency assay methods

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Disclosure of potential conflicts of interest

Thorsten Pflanzner is an employee of AbbVie Deutschland GmbH & Co. KG and may own AbbVie stock or stock options.

The presentation reflects the view of the author and not necessarily by any means the view of AbbVie Deutschland GmbH & Co. KG.

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Outline

- Problem statement
- Guideline and challenges
- Equivalence testing approach

Background: potency testing and F-test

Test for parallelism (similarity) is required to report potency

F-test cannot prove similarity

H0: $\mu 1 = \mu 2$ is

Decision		True	False
	Reject (prove)	***Type I error* α *(false pos.)***	***Correct decision***
	Fail to reject (‚accept')	***Correct decision***	***Type II error* β *(false neg.)***

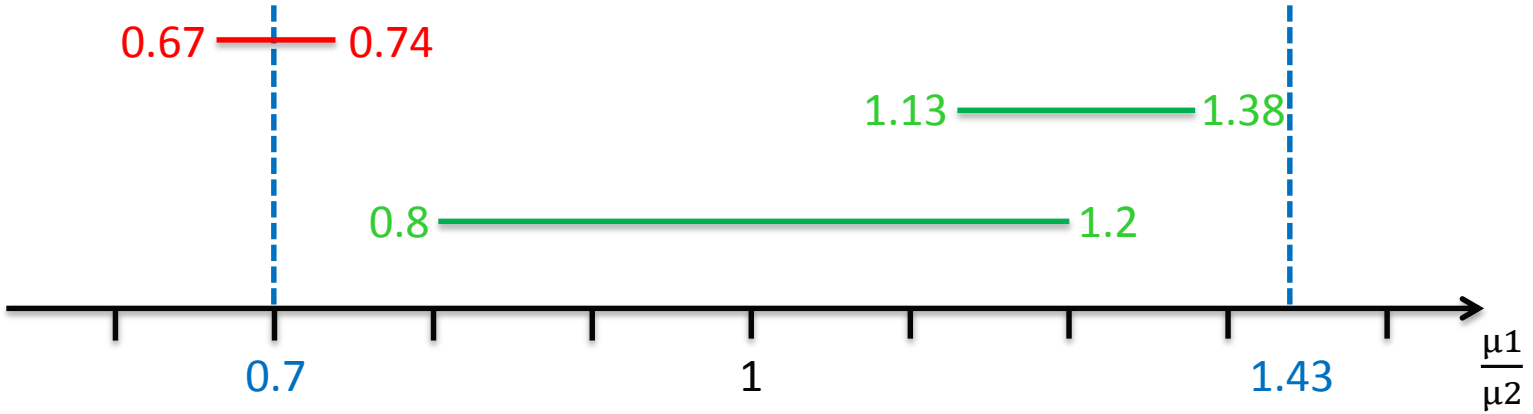
F-test generates false positives: high precision

Bioassays / **ELISAs**

$$F = \frac{SSE_{constrained} - SSE_{unconstrained}}{SSE_{unconstrained}}$$

F-test tolerates lack of precision: potential false negatives

Background: equivalence testing can prove that two parameters are similar



H0: $\left|\frac{\mu 1}{\mu 2}\right| >$ upper goal post or $\left|\frac{\mu 1}{\mu 2}\right| <$ lower goal post

H1: lower goal post $\leq \left|\frac{\mu 1}{\mu 2}\right| \leq$ upper goal post

Goal posts must be determined

USP <1032> Design and development of biological assays: provides guidance but does not define goal posts for NBEs

Identify relevant parameters to assess similarity

Specify a range of acceptable values (goal posts)

reference vs reference / reference vs sample / reference vs degraded sample

Calculate 95% confidence intervals (CIs) for each parameter and tolerance interval (TI) for CIs

Bioequivalence goal posts for generic products (0.8, 1.25) and/or ratio of slopes (0.77, 1.3)

Four challenges: data set, approach, goal posts and life cycle

Identify suitable **data sets**

490 assays evaluated

Identify **statistic approach** that meets AbbVie‘s needs

Ratio (sample/reference)

vs

Absolute Difference (sample – reference)

vs

Relative Difference (1 – sample/reference)

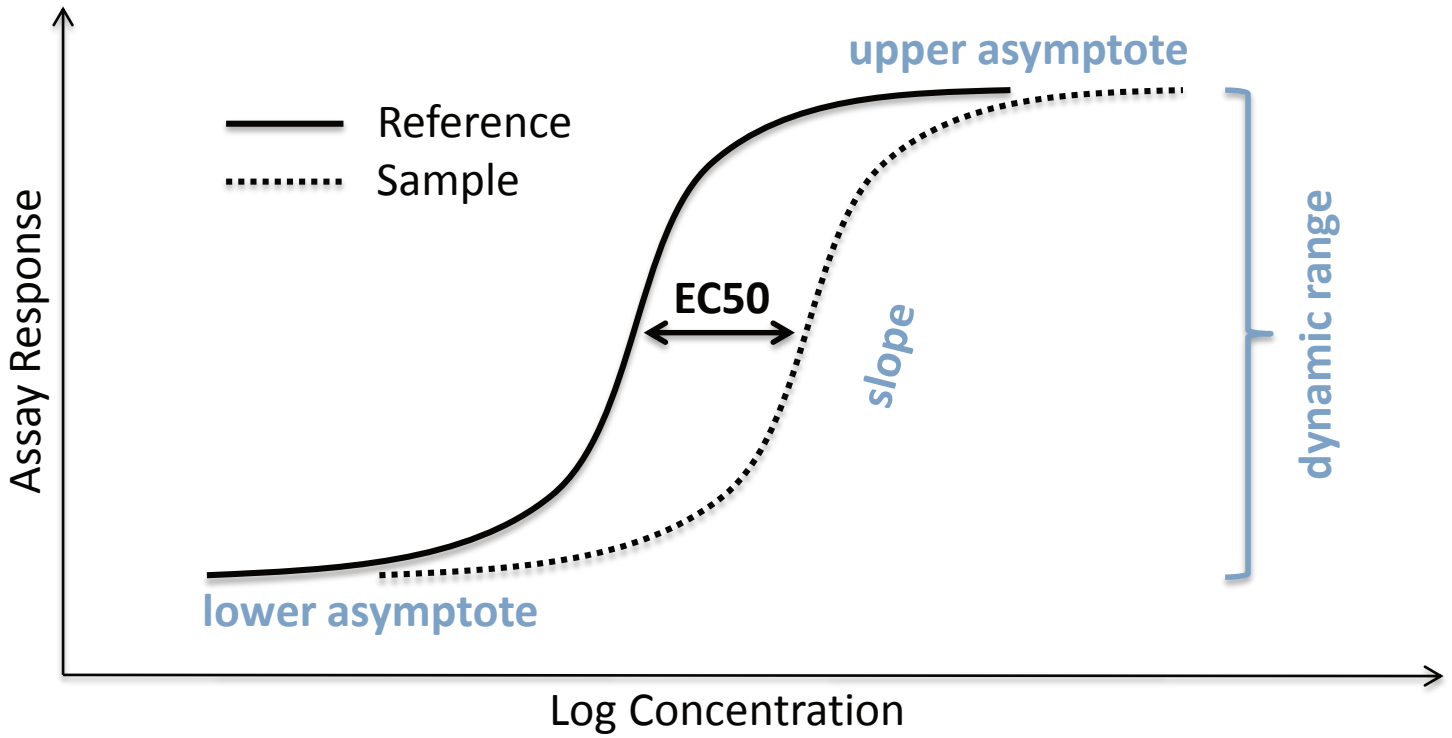
Determine **generic (?) goal posts**

Process for **life cycle management**

Data set: heterogeneity

Method	Assay set-up	Read-out	n
Bioassay A	Protein secretion	Fluorometric	41
Bioassay B	Metabolic activity	Luminometric	72
Bioassay C	Metabolic activity	Luminometric	67
Bioassay D	Metabolic activity	Colorimetric	44
Bioassay E	Reporter gene	Luminometric	38
ELISA A/B	Indirect	Colorimetric	59
ELISA C	Indirect	Colorimetric	10
ELISA D	Sandwich	Colorimetric	63
ELISA E	Sandwich	Colorimetric	39
ELISA F	Sandwich	Colorimetric	43
AlphaScreen A	Not applicable	Luminometric	14

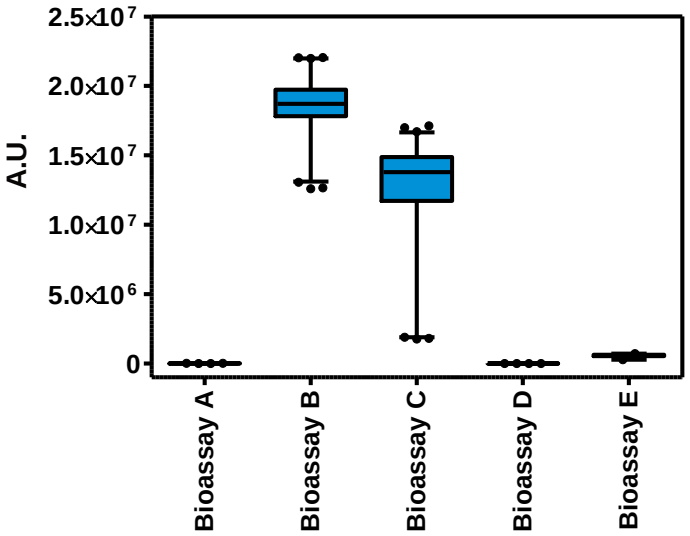
Data set: asymptotes, dynamic range and slope are relevant parameters



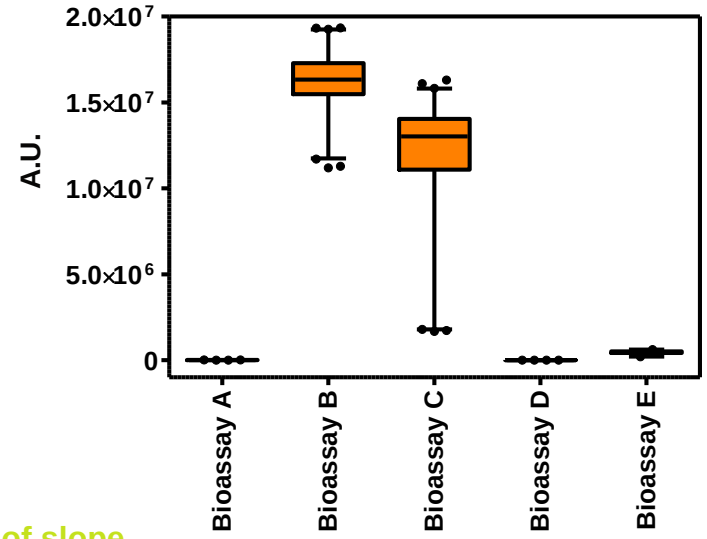
Dynamic range = upper asymptote – lower asymptote

Statistic approach: absolute values vary across bioassays

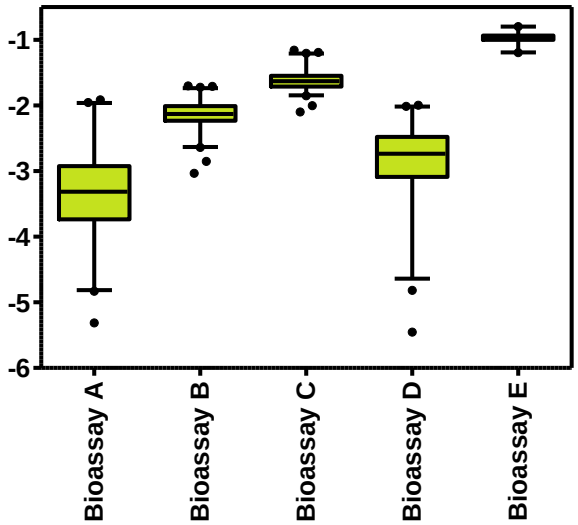
Parameter estimate of upper asymptote



Parameter estimate of dynamic range

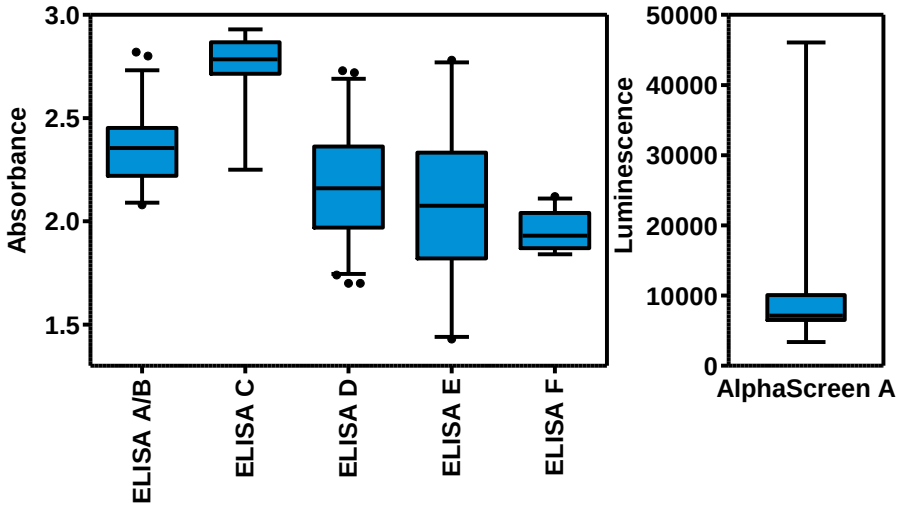


Parameter estimate of slope

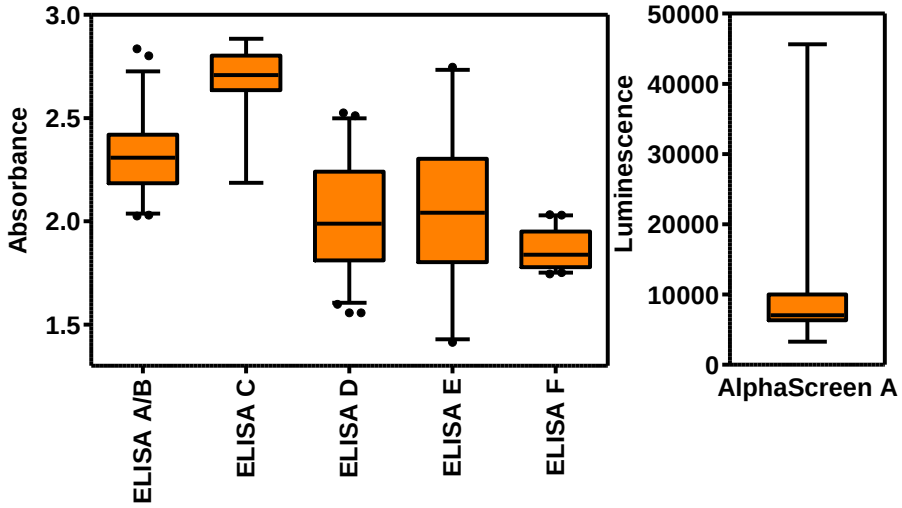


Statistic approach: absolute values vary across binding assays

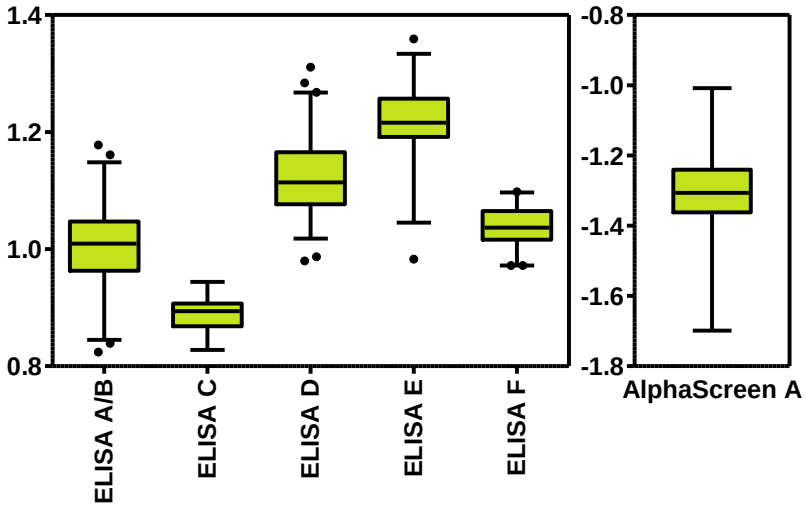
Parameter estimate of upper asymptote



Parameter estimate of dynamic range

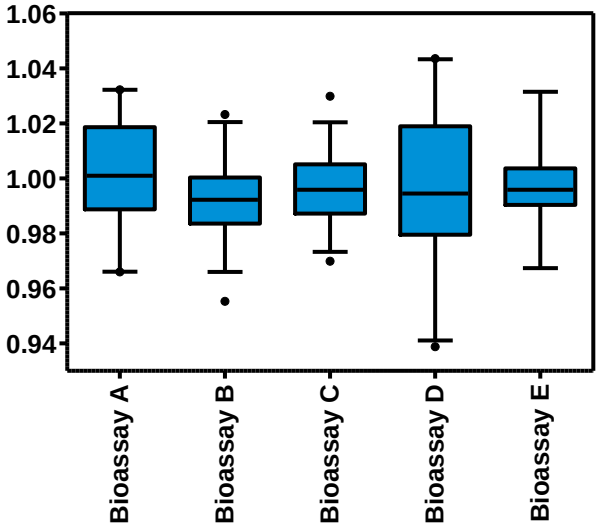


Parameter estimate of slope

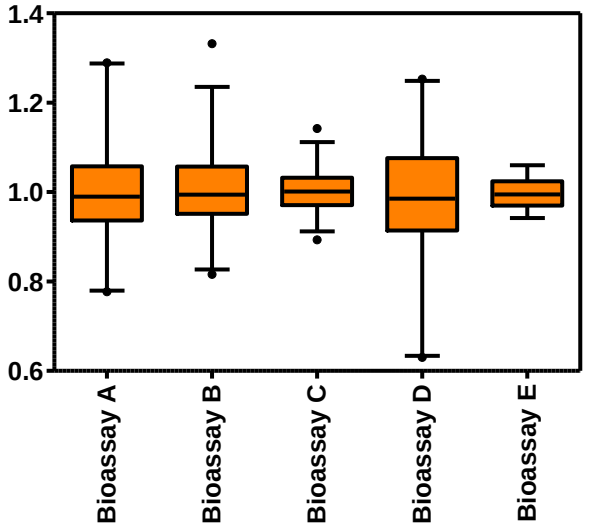


Statistic approach: ratios are comparable across bioassays

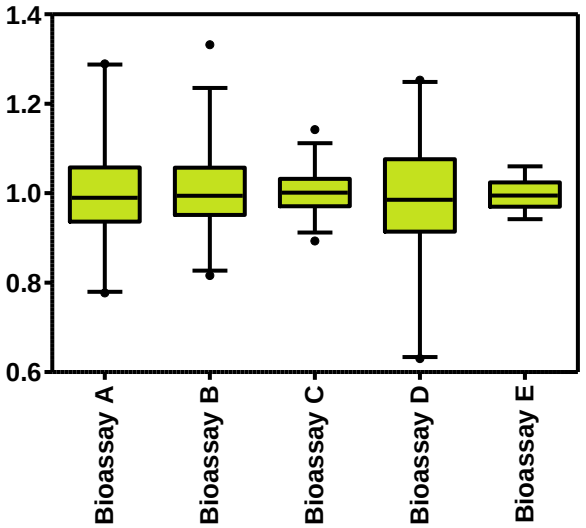
Parameter estimate ratio of upper asymptote



Parameter estimate ratio of dynamic range

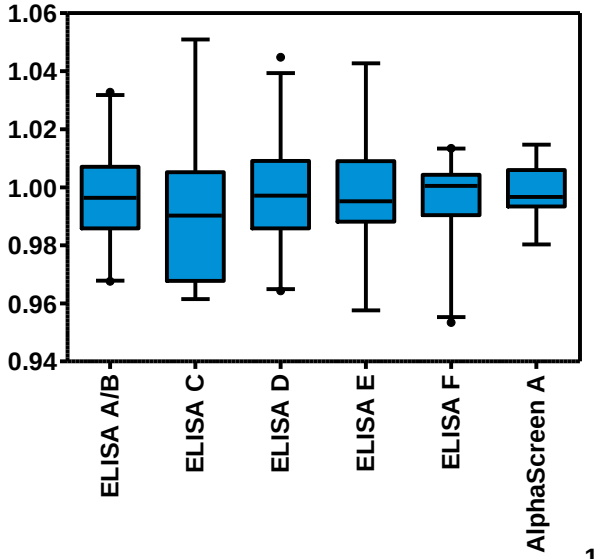


Parameter estimate ratio of slope

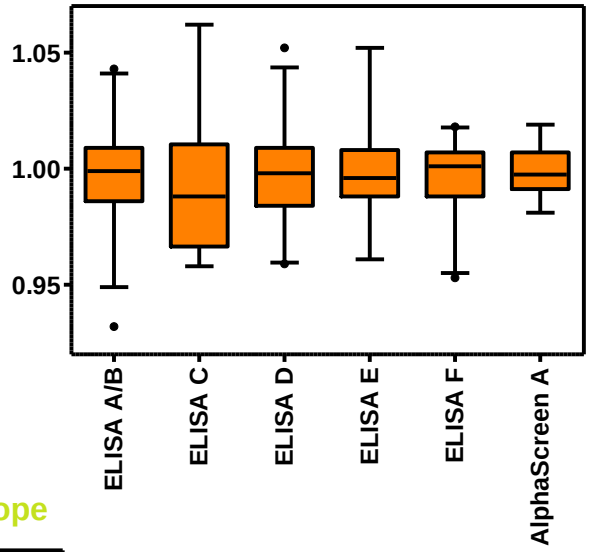


Statistic approach: ratios are comparable across binding assays

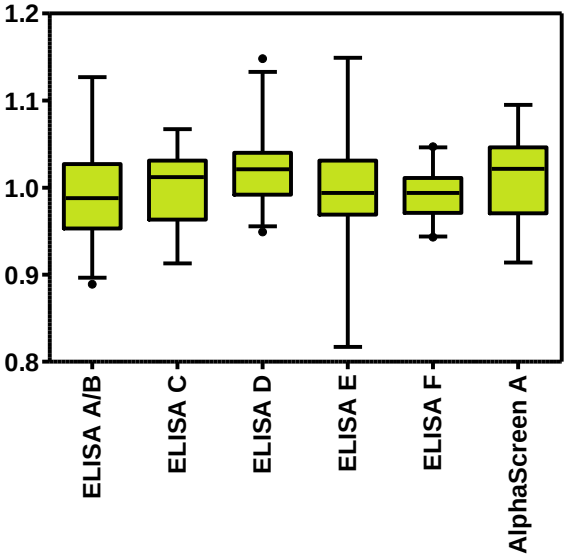
Parameter estimate ratio of upper asymptote



Parameter estimate ratio of dynamic range



Parameter estimate ratio of slope



Statistic approach: confidence interval of ratio is constructed according to Fieller's theorem

$$95\%\ Confidence\ Interval\ of\ \left(\frac{\mu_1}{\mu_2}\right) = \frac{\hat{\mu}_1\hat{\mu}_2 \pm t_{df,0.975}\sqrt{\hat{\mu}_1^2\hat{\sigma}_{22}^2 + \hat{\mu}_2^2\hat{\sigma}_{11}^2 - t_{df,0.975}^2\hat{\sigma}_{11}^2\hat{\sigma}_{22}^2}}{\hat{\mu}_2^2 - t_{df,0.975}^2\,\hat{\sigma}_{22}^2}$$

μ_1	=	parameter estimate reference
μ_2	=	parameter estimate sample
t	=	t-distribution
df	=	degrees of freedom
σ_{11}^2	=	variance reference
σ_{22}^2	=	variance sample

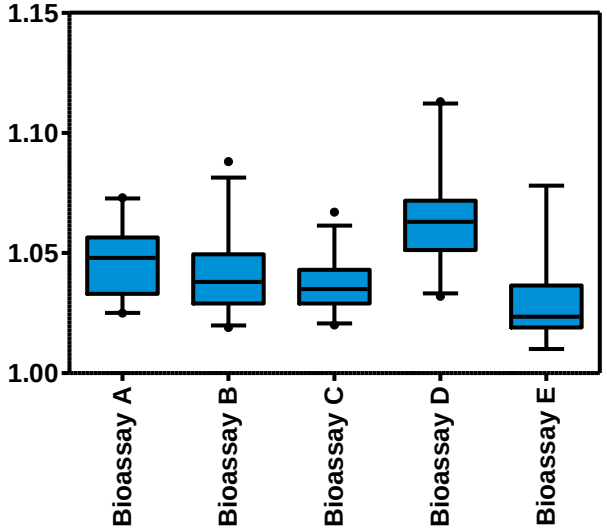
Covariance σ_{12}^2 was set to 0.

e.g. 95% CI = (0.7, 1.1)

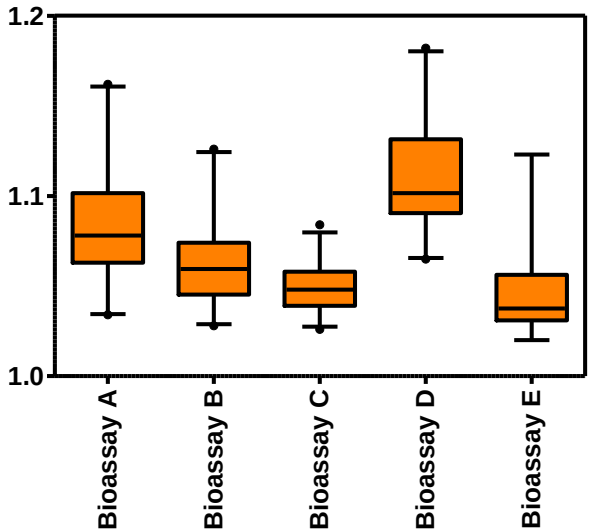
→ CI_{max} = MAX (1/0.7; 1.1) = MAX (1.4; 1.1) = 1.4

Statistic approach: ratio CIs are comparable across bioassays, except for bioassay D

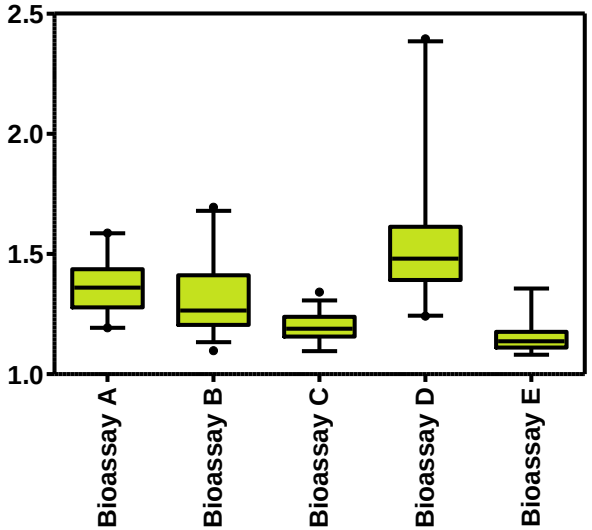
Parameter estimate ratio CI_{max} of upper asymptote



Parameter estimate ratio CI_{max} of dynamic range



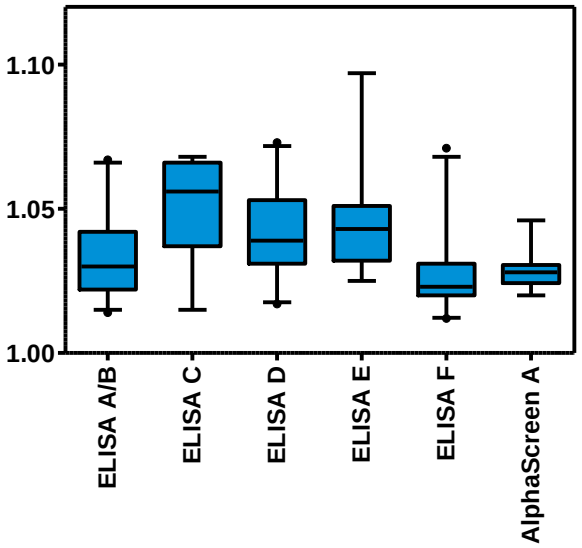
Parameter estimate ratio CI_{max} of slope



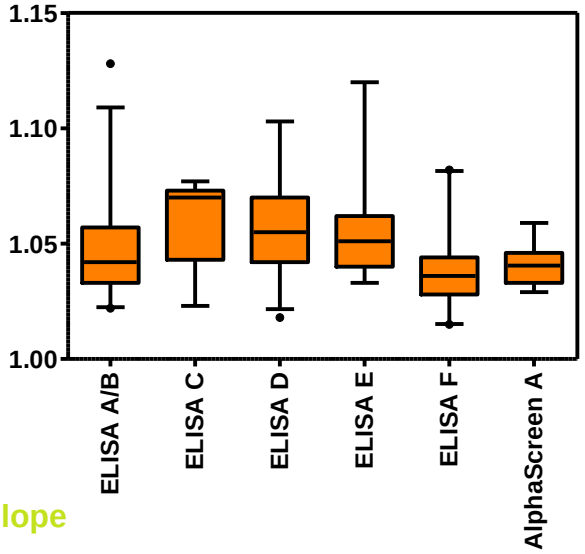
Bioassay D excluded for further steps

Statistic approach: ratio CIs are comparable across binding assays

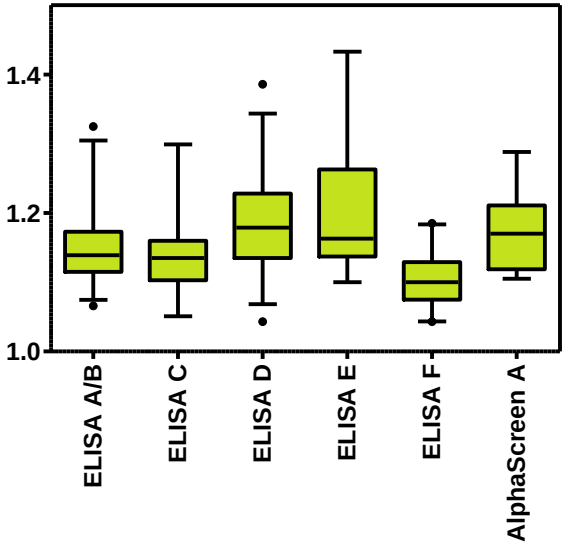
Parameter estimate ratio CI_{max} of upper asymptote



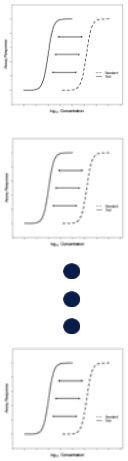
Parameter estimate ratio CI_{max} of dynamic range



Parameter estimate ratio CI_{max} of slope



Generic goal posts: based on tolerance intervals



$95\%\ CI\colon\ (L_1, U_1)$ $\quad CI_{max}\ (1/L_1, U_1)$

$95\%\ CI\colon\ (L_2, U_2)$ $\quad CI_{max}\ (1/L_2, U_2)$

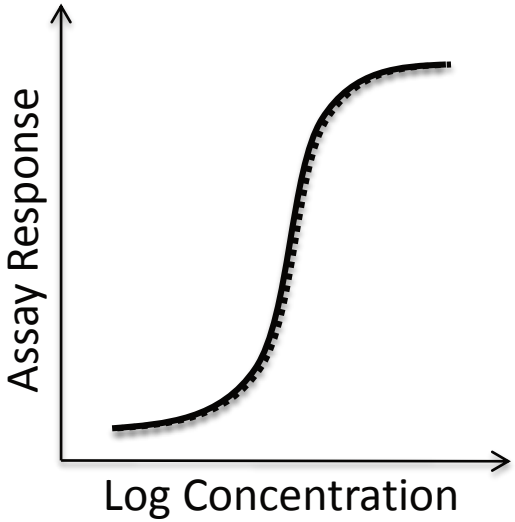
$95\%\ CI\colon\ (L_N, U_N)$ $\quad CI_{max}\ (1/L_N, U_N)$

Generic goal posts	Binding assay	Bioassay
Upper asymptote	0.91 – 1.10	0.92 – 1.09
Dynamic range	0.89 – 1.13	0.86 – 1.16
Slope	0.70 – 1.43	0.59 – 1.69

Generic goal posts: validation

Reference
Sample

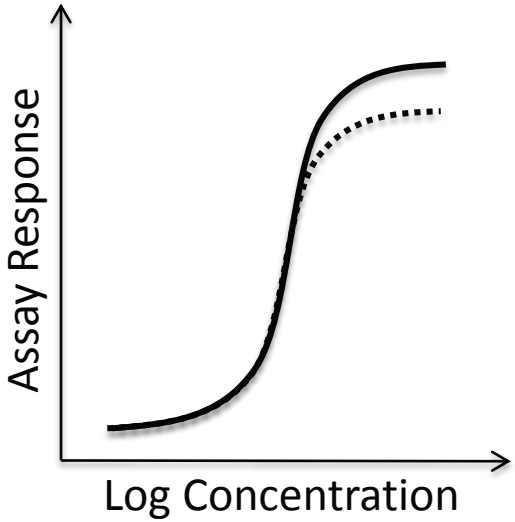
IgG:
High precision



F-test result **FAIL**

Equivalence test result **PASS**

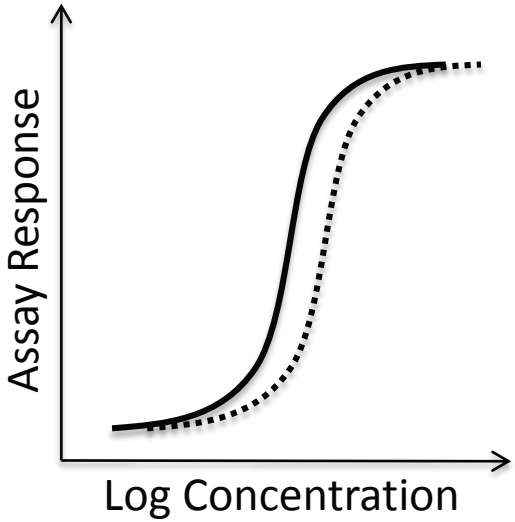
Bi-specific Ig:
SEC main peak ↓
SEC HMW ↑



F-test result **FAIL**

Equivalence test result **FAIL**

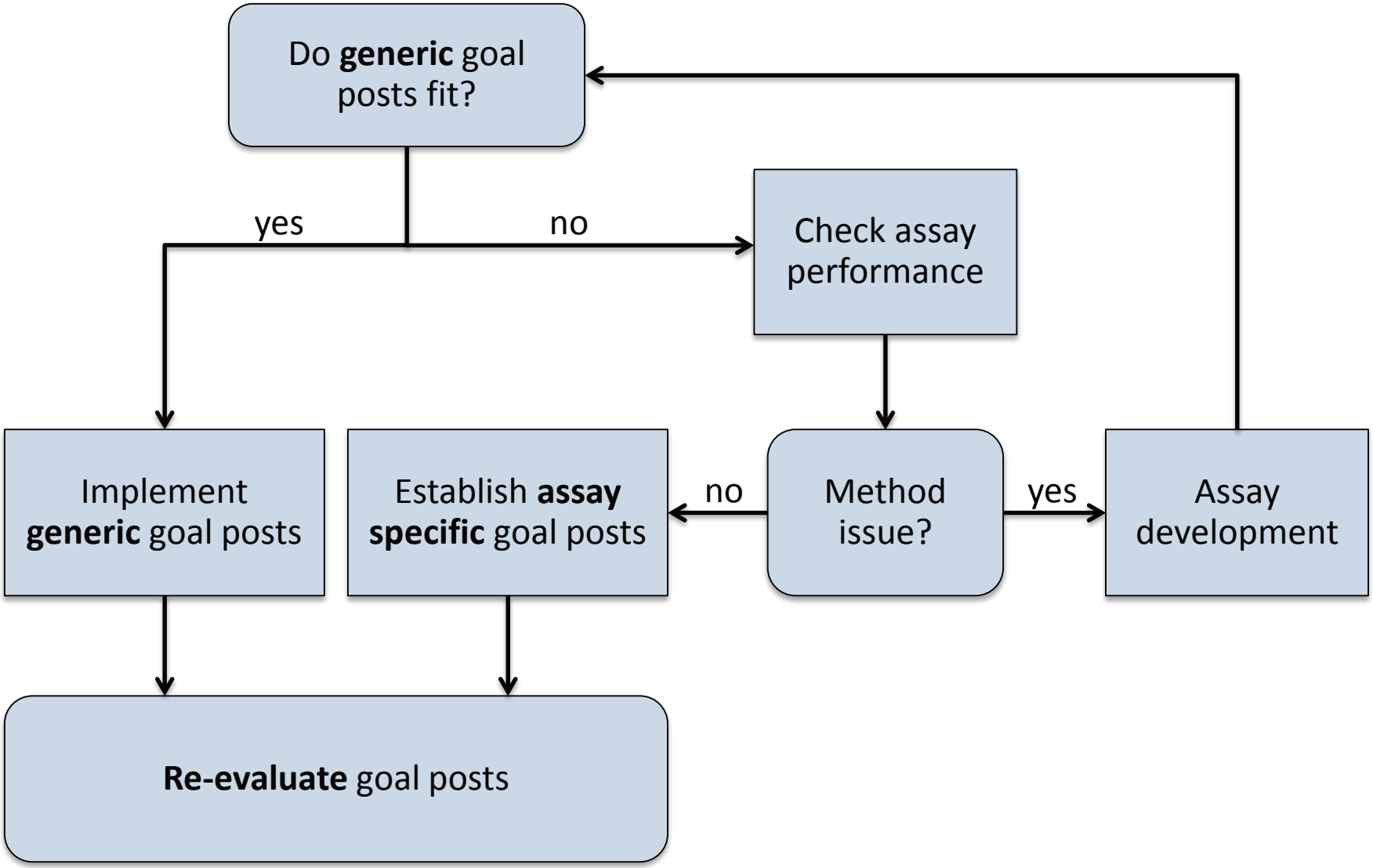
Antibody drug conjugate:
High precision
SEC main peak ↓
SEC HMW ↑
DAR ↓
Unconjugated IgG ↑



F-test result **FAIL**

Equivalence test result **PASS**

Life cycle management: process to cover all assay phases



Life cycle management: goal posts can be based on parametric and non-parametric tolerance intervals

Re-evaluate goal posts

Establish **assay specific** goal posts

When is the right time? How much data do I need?

At least two options:

- Non-parametric TI based on existing data

- Parametric TI (Beal *et al.* 2012) based on existing data and correction factor

→ Sample size depends on approach and, desired confidence and coverage

Milestones

Harmonized approach with generic goal posts across AbbVie sites

Customized commercial GMP software solution

Process covering all clinical phases

Trained AbbVie staff

Implemented approach

The core team

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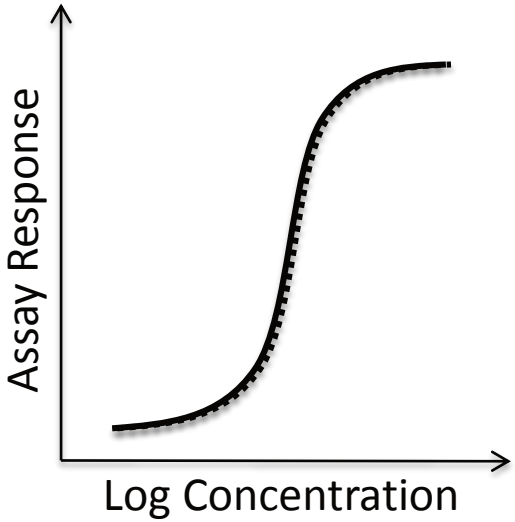
Thank You

Example: F-test type I error

Reference
Sample

IgG:
High precision



F-test result **FAIL**