

Better Bioassays:

Using Statistics to Design, Validate and Understand Bioassays

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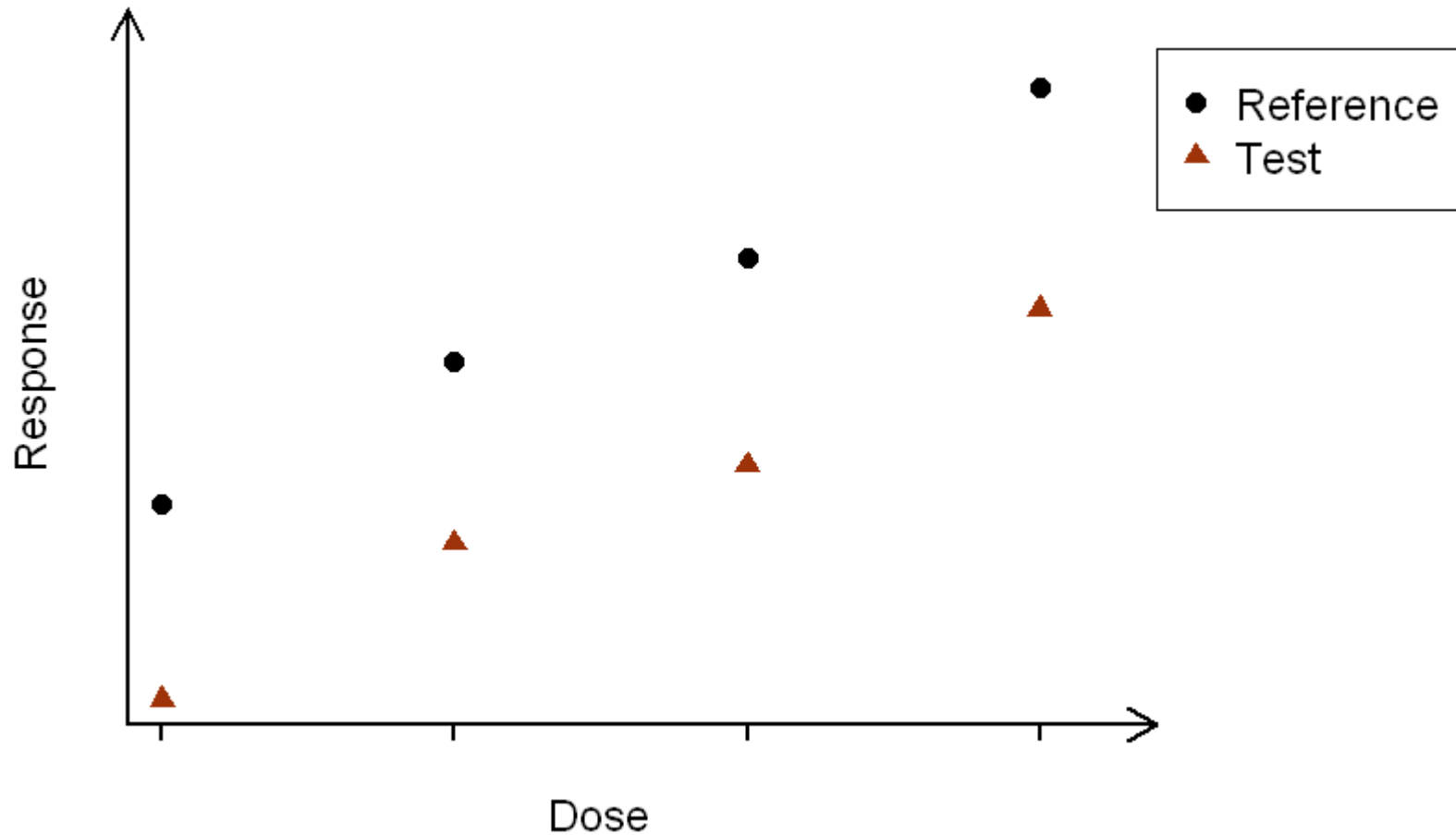
Contents

- Introduction
 - Bioassays
 - Analysis of bioassays
- Design
- Regulations
 - Validation

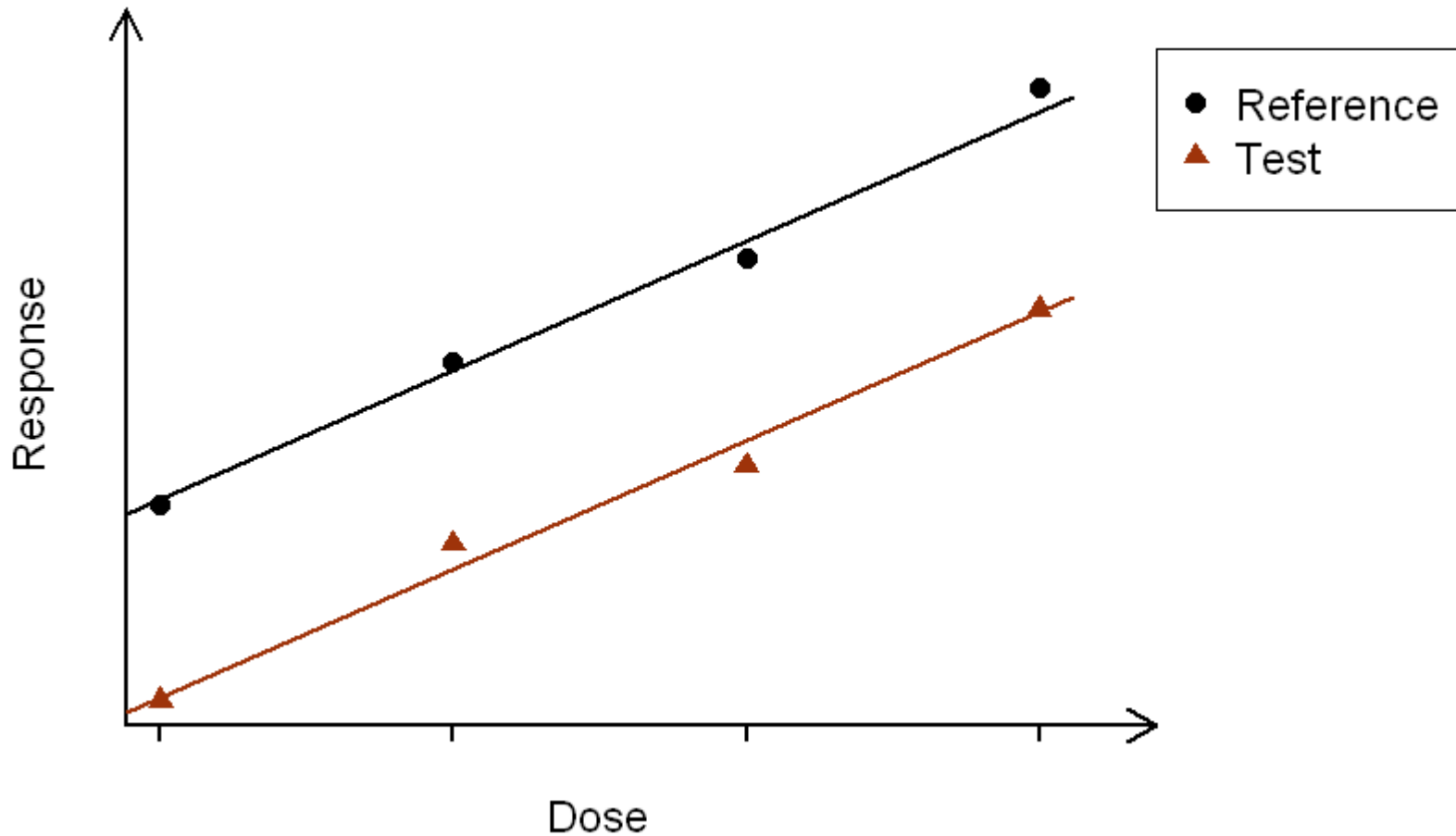
Intro to bioassays

- Measure the strength or activity of a test material
- By examining the response of living organisms or cells to the test and reference materials at different doses
- Usually relative to a reference (or standard) material (but not necessarily)

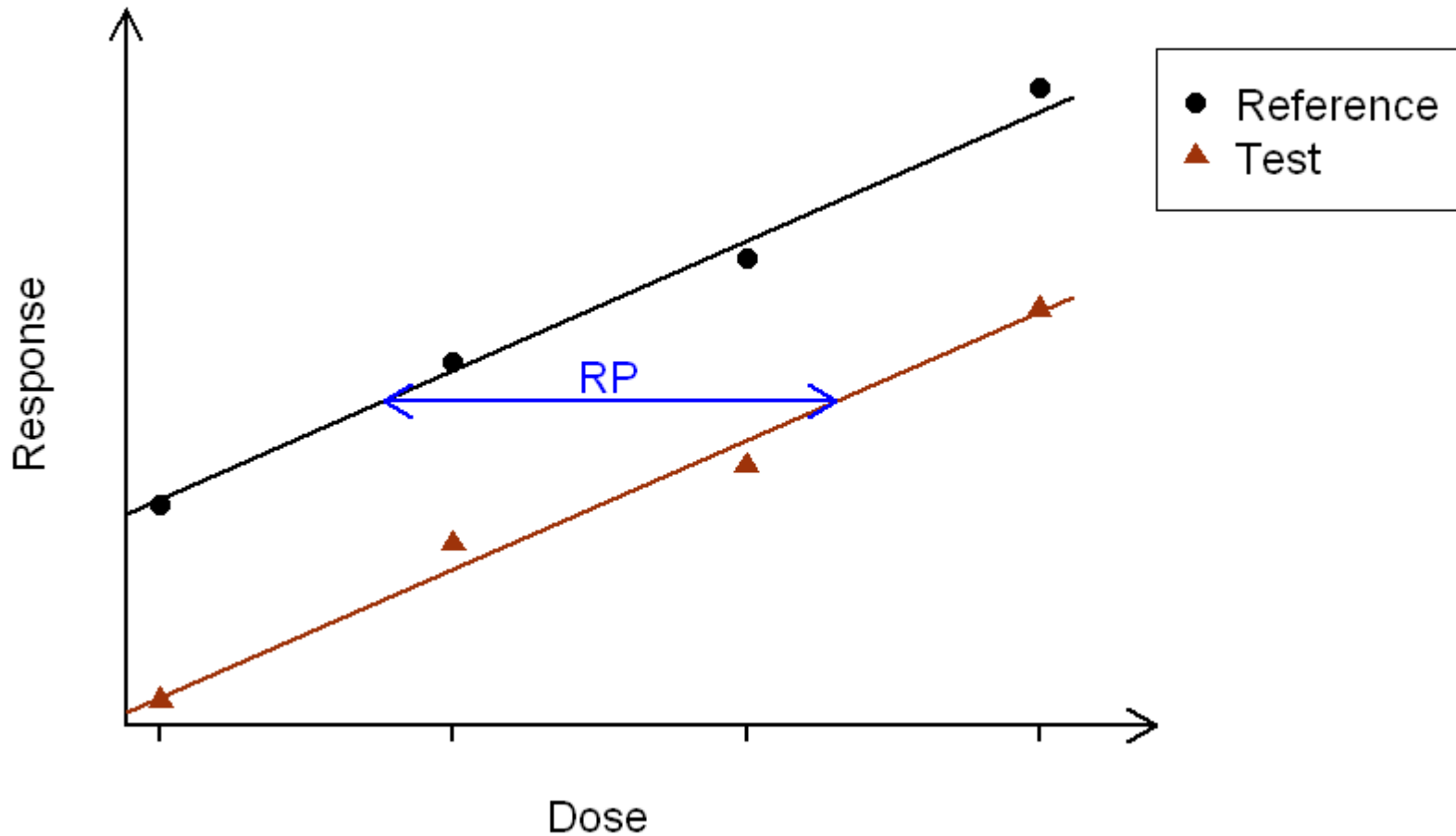
Intro to bioassays



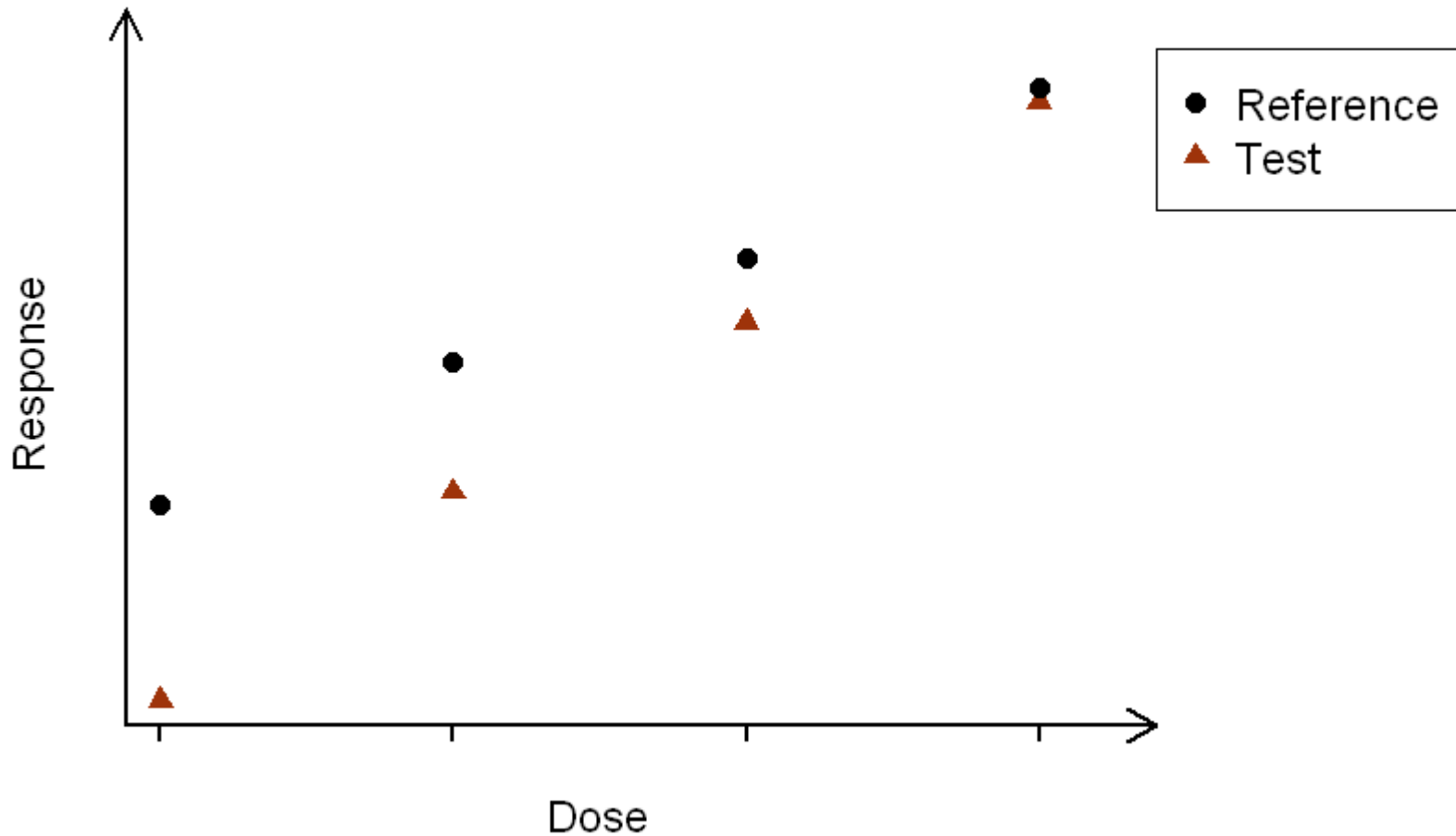
Intro to bioassays



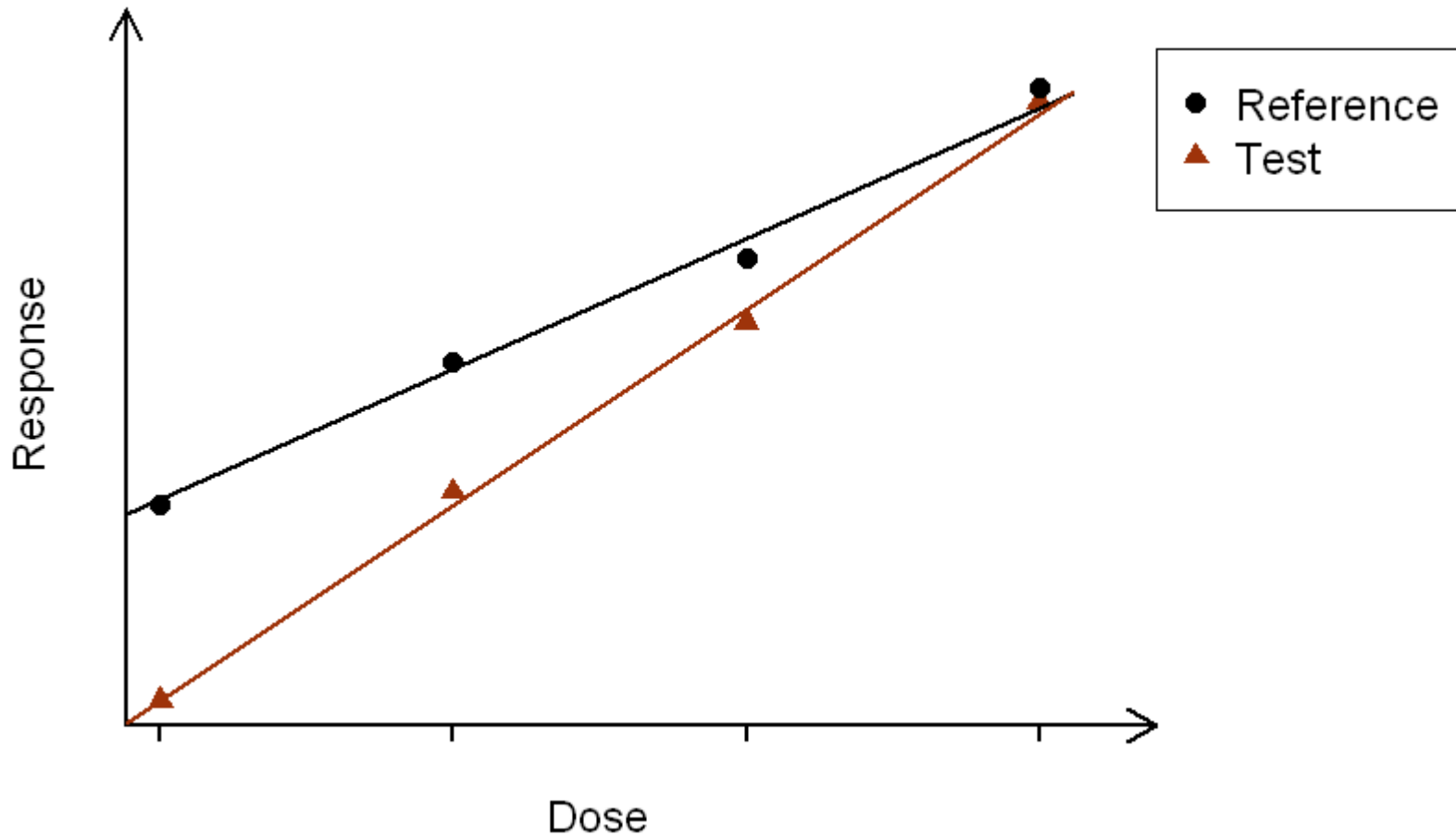
Intro to bioassays



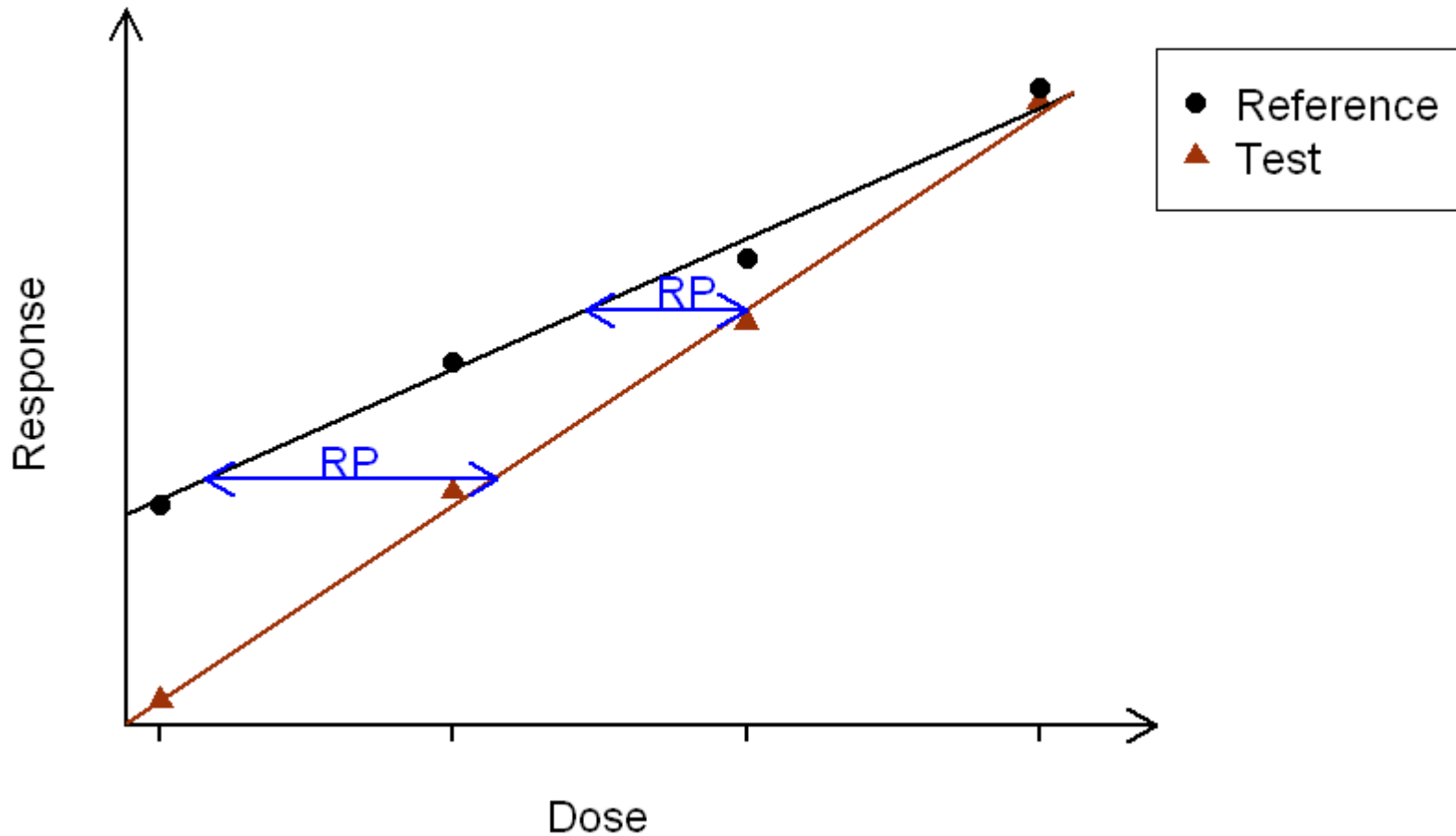
Intro to bioassays



Intro to bioassays



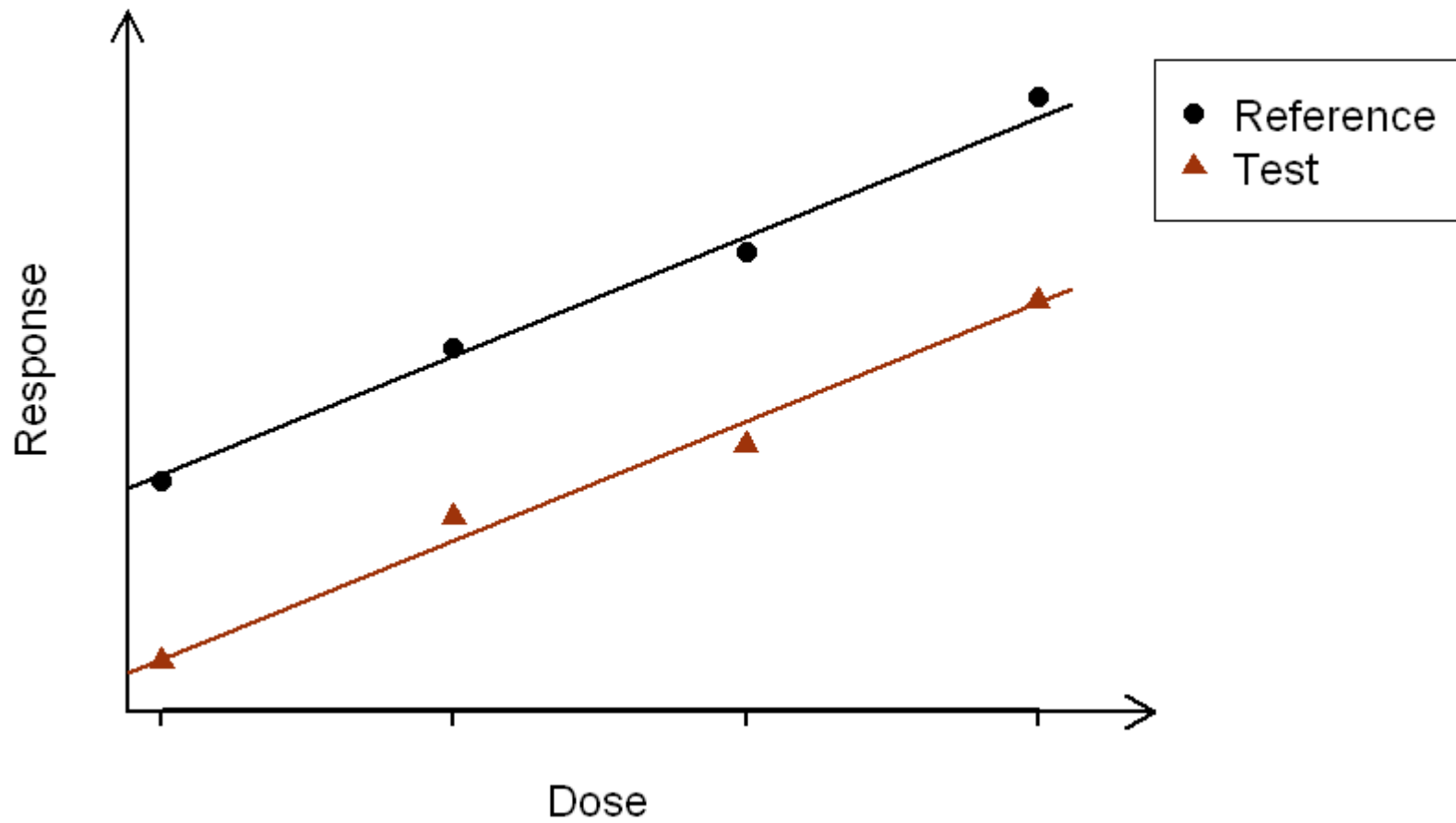
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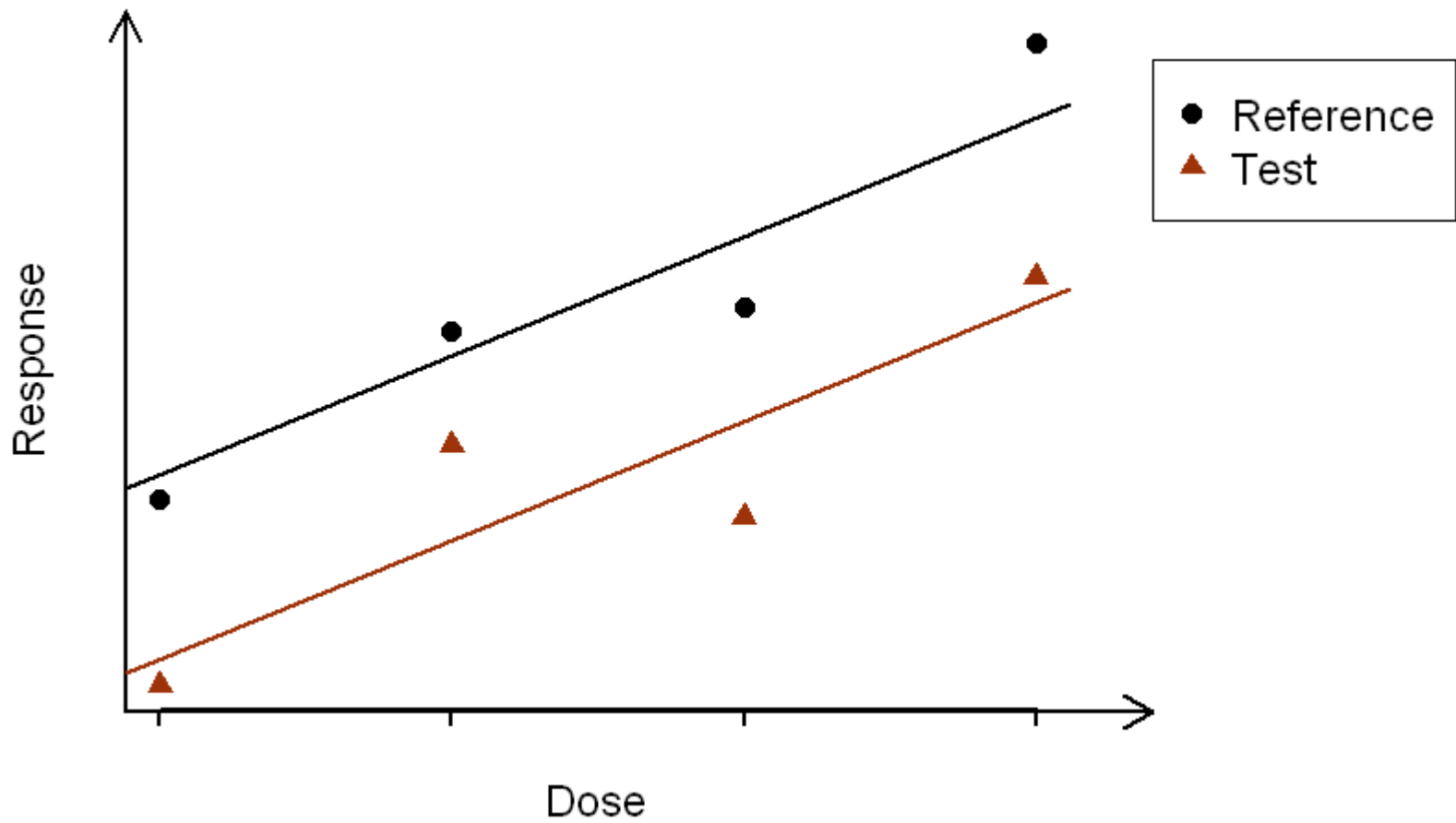
Reporting RP

- RP: often reported without precision
- More informative to report precision as well

Reporting RP



Reporting RP



Reporting RP

- RP: often reported without precision
- More informative to report precision as well

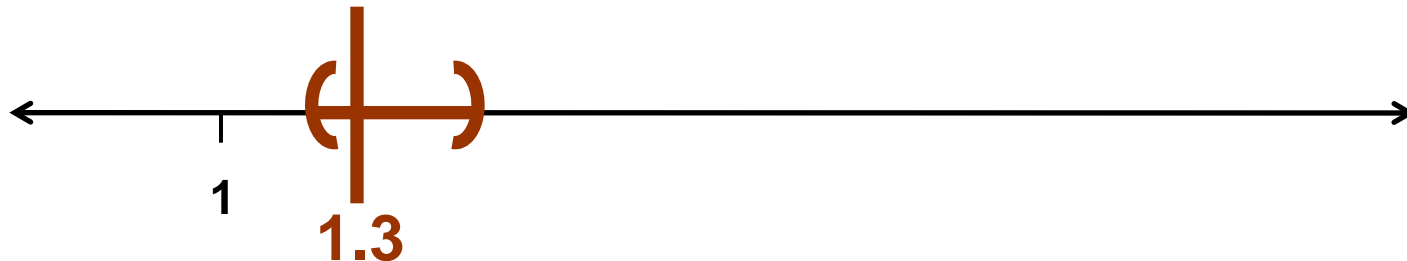
Reporting RP

- $RP = 1.3$

Reporting RP

- **RP = 1.3**

- **95% CI (1.18, 1.43) → New batch is more potent**



Reporting RP

- **RP = 1.3**

- 95% CI (1.18, 1.43) → New batch is more potent



- 95% CI (0.72, 2.34) → Inconclusive

Types of bioassays

- Animal based assays
 - For example, challenge assays
 - Measure dead/alive at a given time point
 - Or time to death
 - Other animal based assays
 - Measure weight gain
 - Measure antibody levels
- Cell based assays
 - ELISA assays

Design

- Aim: estimate RP efficiently
- Number of doses and the concentration at each dose
- Number of replicates per dose, e.g. number of animals or number of ELISA plate wells

Example 1: challenge assays

- Analyse the number of deaths per group
 - Standard approach to challenge assays

– OR –

- Analyse the time to death of each animal
 - Using a survival analysis model

→ Better precision of RP

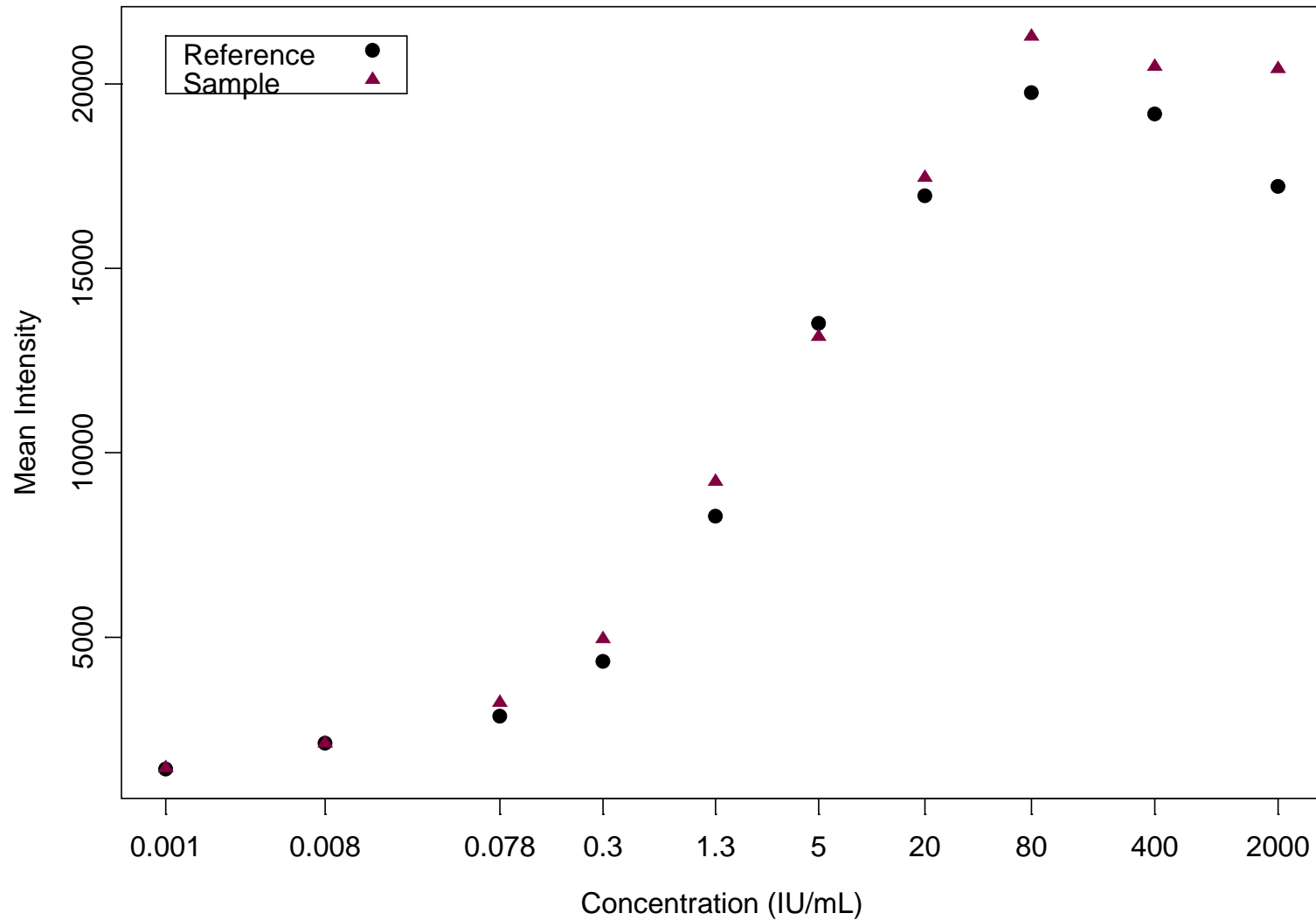
→ Same precision with fewer animals
(dataset we studied: 30% fewer)

Example 1: challenge assays

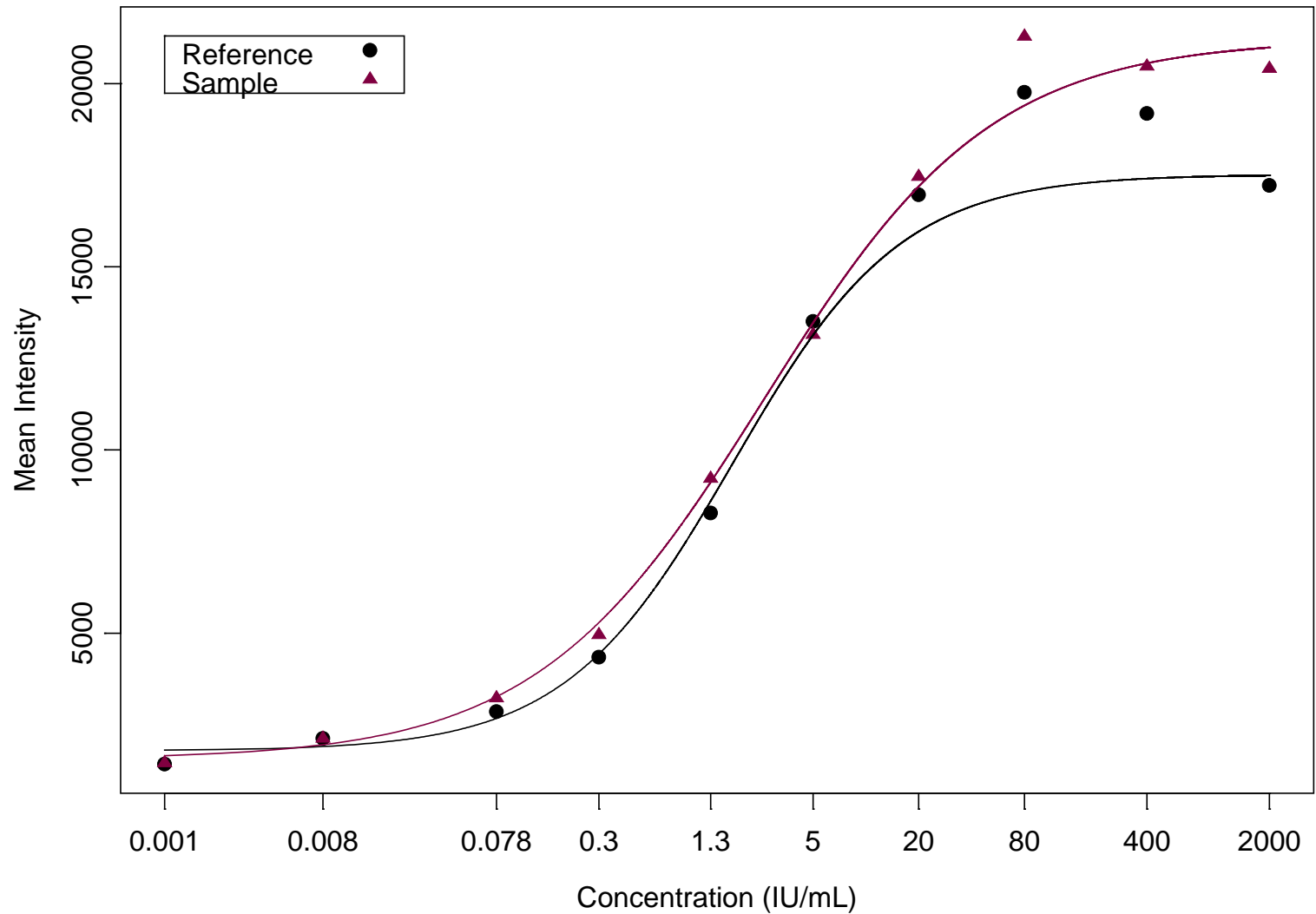
Same precision with fewer animals

- Reduce, refine and replace
- EU directive 2010/63/EU
 - Law from 2012
 - Will require scientists to provide evidence of experimental strategy and statistical design to minimize animal numbers

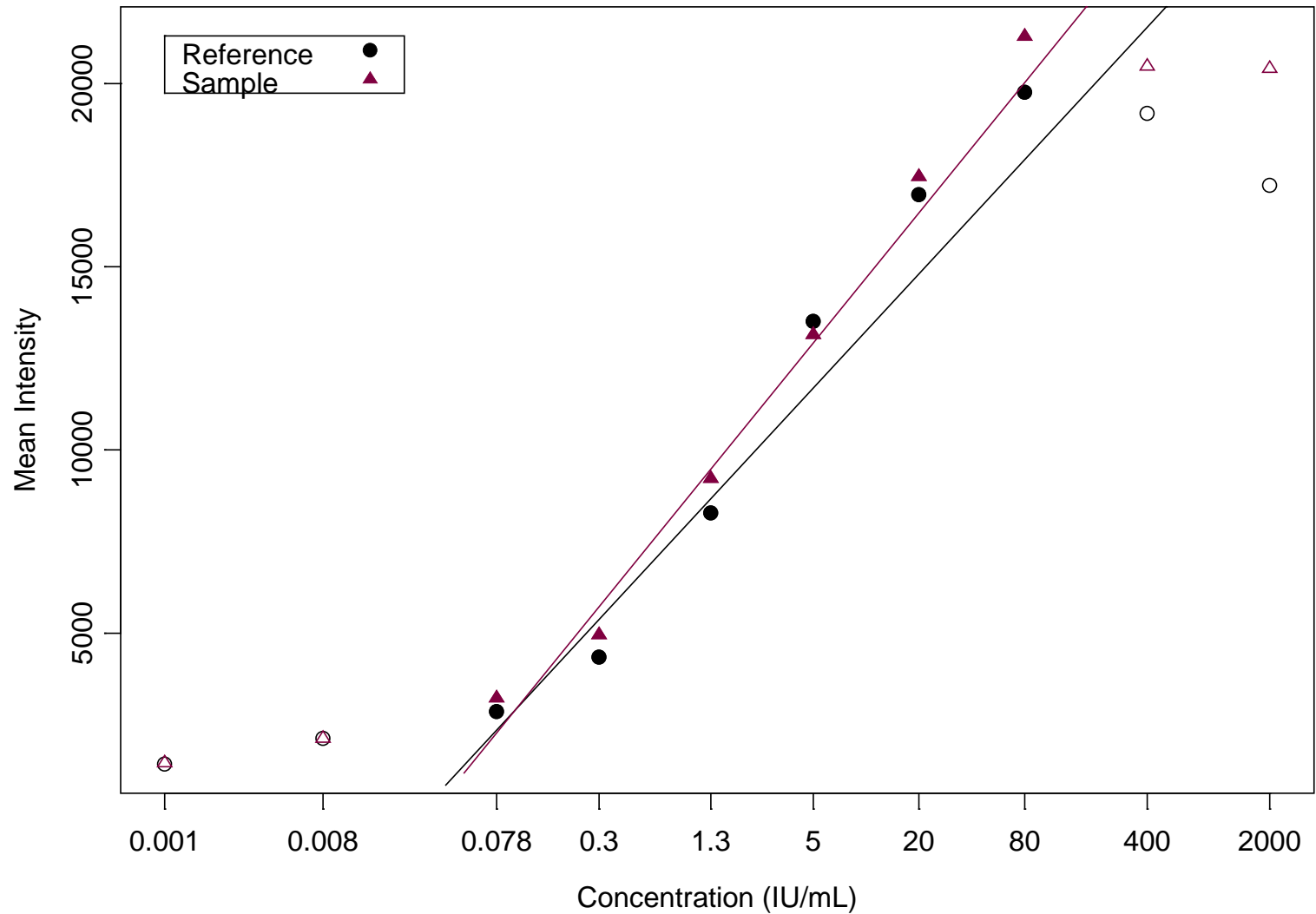
Example 2: choice of doses



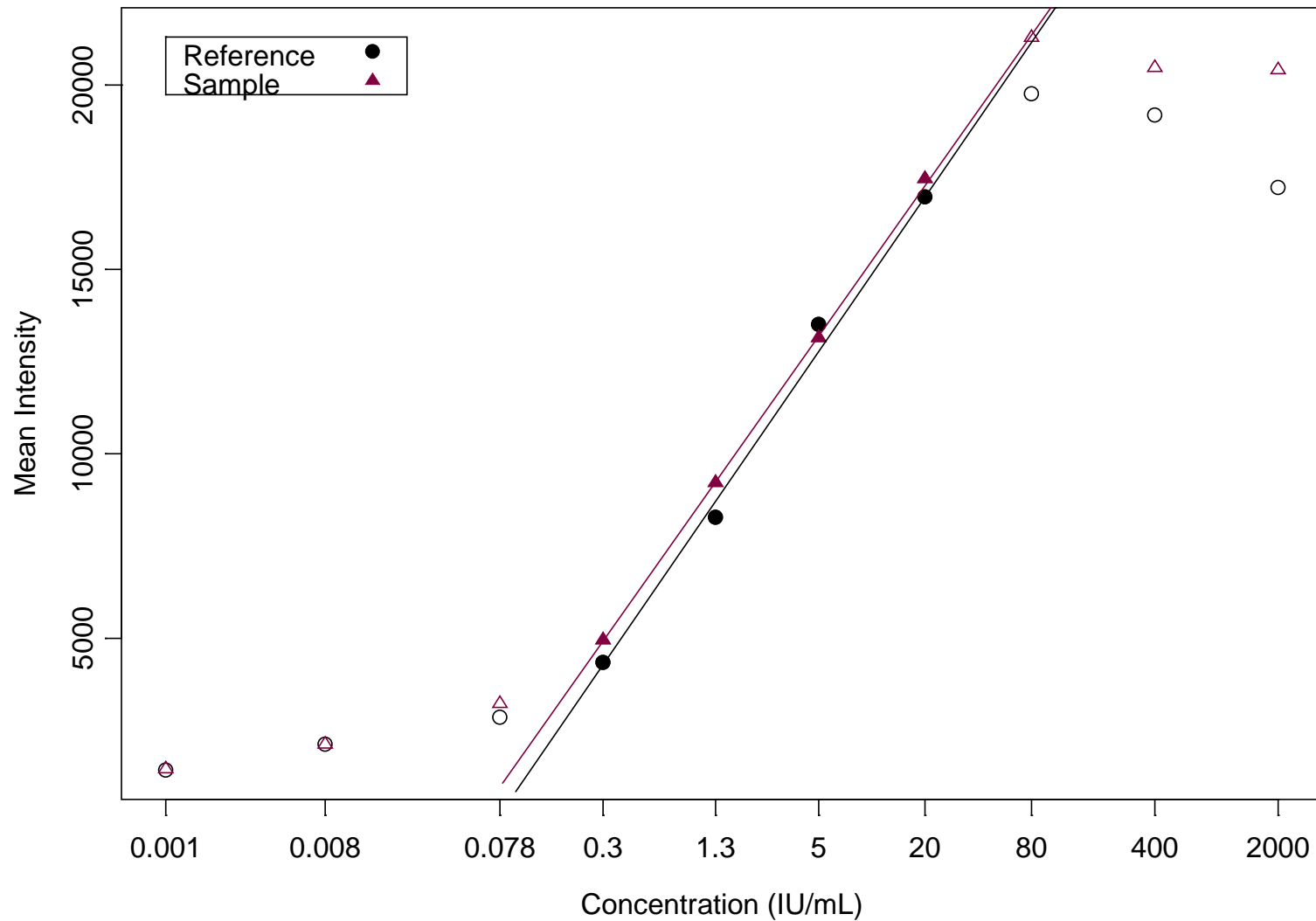
Example 2: choice of doses



Example 2: choice of doses



Example 2: choice of doses



Example 2: choice of doses

- Relative potency not biased
- Precision similar
- Sufficient to use **four** doses rather than **ten**

Regulations

- European Pharmacopoeia and current US Pharmacopeia
 - Design
 - Analysis
- Draft US Pharmacopeia (to be issued 2012)
 - Design
 - Analysis
 - Validation

Regulations

- Pharmacopoeias: generally similar
- Differ on parallelism testing:
 - European Pharmacopoeia:
Residual sum of squares (RSS) and the F-test
 - Draft US Pharmacopeia
Confidence intervals on differences between parameters

Regulations

- ICH

- **Validation**

- ICH Q2 (R1)* Validation of analytical procedures

- **Acceptance criteria**

- ICH Q6B* Test procedures and acceptance criteria for biotechnological/biological products

- **Stability testing**

- ICH Q1A (R2)* Stability testing

- ICH Q1E* Evaluation for stability data

Validation

- Necessary for registration applications
- Demonstrate the assay is fit for purpose
- Show that it has acceptable characteristics

Validation

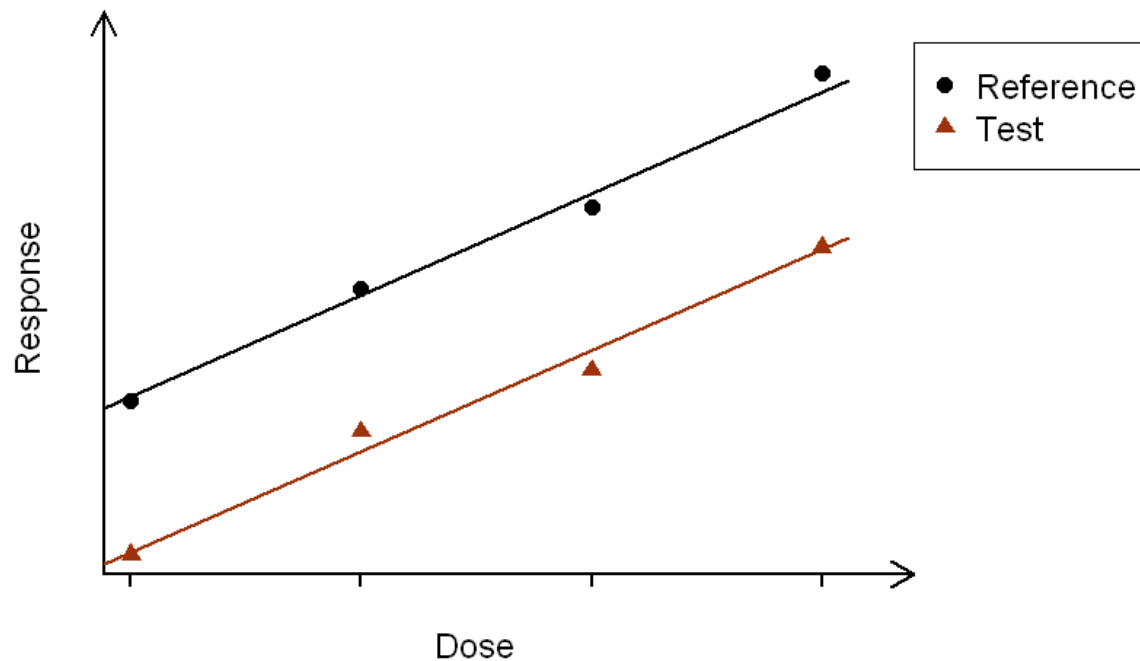
- Characteristics:
 - Accuracy
 - Precision: repeatability and intermediate precision
 - Linearity
 - Range
 - Specificity
- Define acceptance criteria
- Design an experiment to test whether assay meets acceptance criteria

Validation

- **Accuracy:**
 - The reported relative potency of a material must be acceptably close to true value
 - Can be demonstrated by comparing dilutions of a reference material to itself and calculating the relative bias
- **Precision:**
 - Repeatability (same operating conditions)
 - Intermediate precision (different days, operators, ...)
 - Can be demonstrated by a variance components analysis

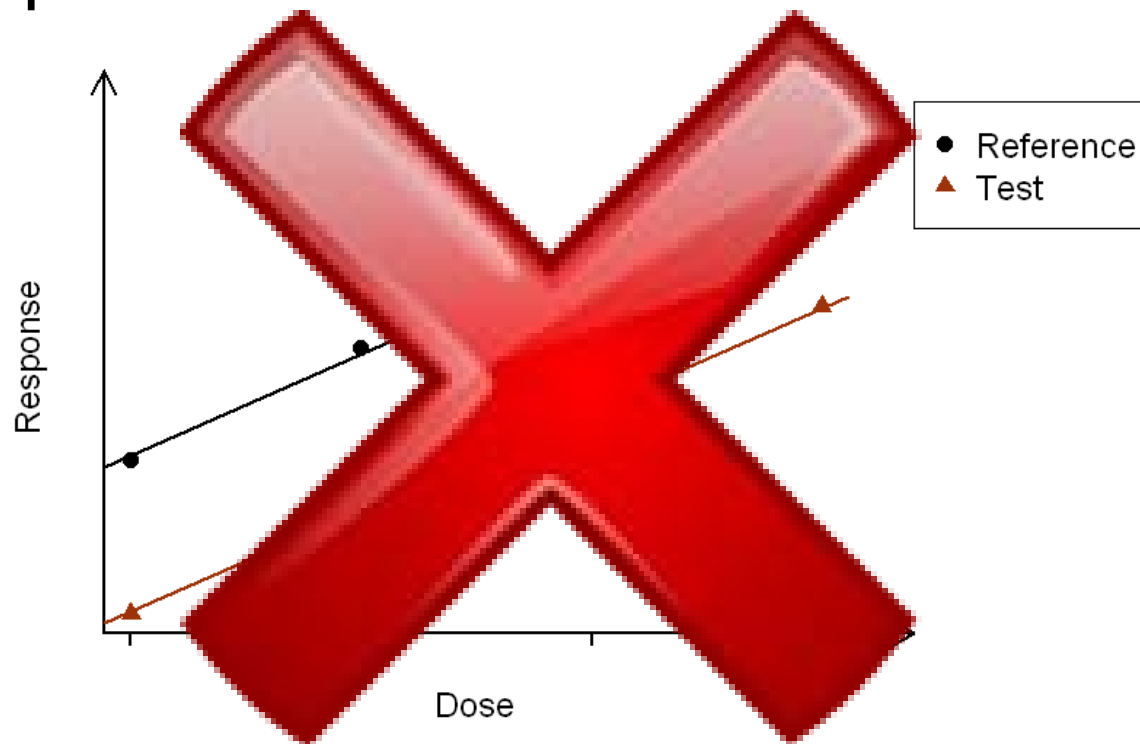
Validation

- Linearity:
 - The estimated relative potency must be proportional to the true value



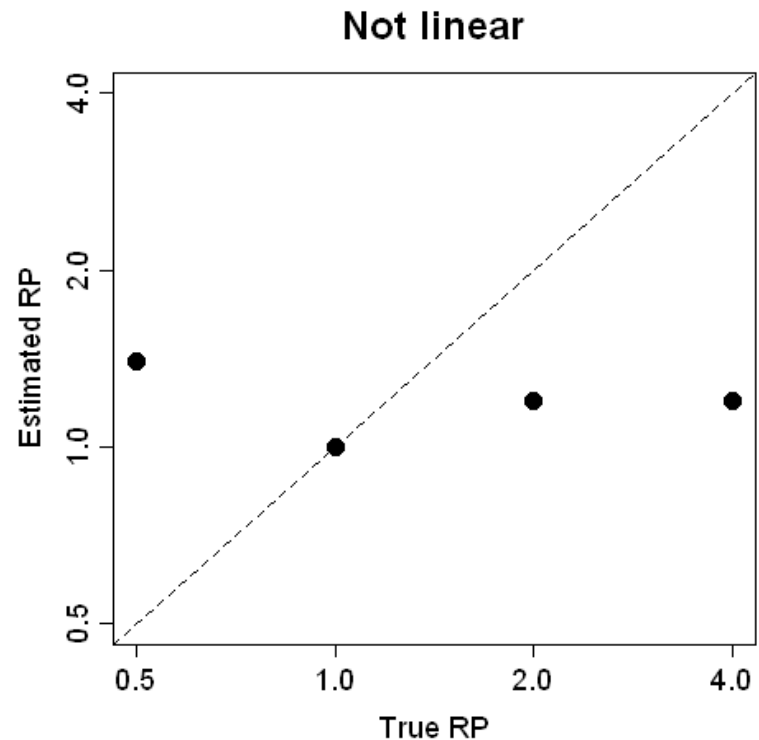
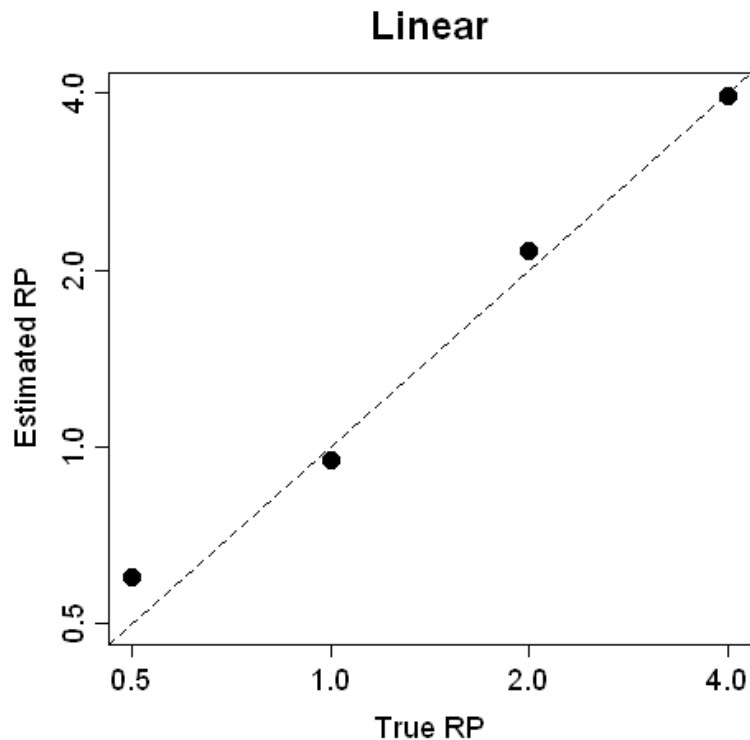
Validation

- Linearity:
 - The estimated relative potency must be proportional to the true value



Validation

- **Linearity:**
 - The estimated relative potency must be proportional to the true value



Validation

- Range
 - The range of relative potencies for which accuracy, precision and linearity have been demonstrated
- Through careful design all of these characteristics can be assessed in one experiment
- Most important to set achievable limits for each characteristic
- Can do this by using statistics to investigate any preliminary assay data that is available

Summary

- Design
 - Early work on design combined with statistical analysis => improvements in efficiency
- Reporting relative potency
 - Must estimate the precision of RP
- Regulations
 - European Pharmacopoeia
 - US Pharmacopeia: to be updated next year
 - ICH
- Validation

Further information

- Draft USP guidelines

<http://www.usp.org/meetings/workshops/bioassayGuidance.html>

- European Pharmacopeia 7th edition

<http://www.edqm.eu/en/European-Pharmacopoeia-1401.html>

- ICH guidelines

<http://www.ich.org>

- Quantics Consulting

<http://www.quantics.co.uk>

Further information

- ICH guidelines <http://www.ich.org>
 - *ICH Q1A (R2)* Stability testing
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2_Guideline.pdf
 - *ICH Q1E* Evaluation for stability data
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1E/Step4/Q1E_Guideline.pdf
 - *ICH Q2 (R1)* Validation of analytical procedures
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf
 - *ICH Q6B* Test procedures and acceptance criteria for biotechnological/biological products
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q6B/Step4/Q6B_Guideline.pdf