

# Method Validation

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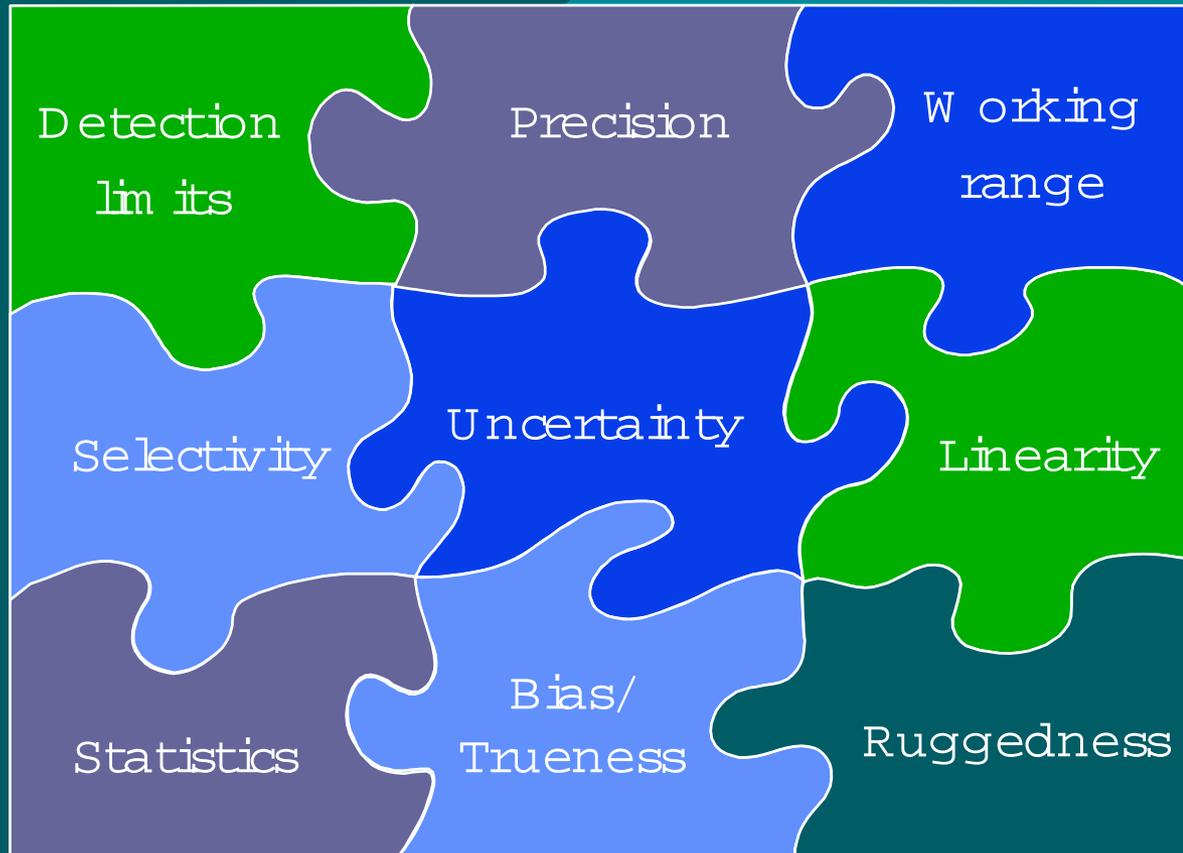


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in analytical science*

# Introduction

- What is method validation?
- Why is method validation useful?
- Validation for traceability
- Tools for validation

# A Puzzle ...



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# What is Method Validation?

"Demonstration of fitness for purpose" (ICH)

"..establishing, through documented evidence, a high degree of assurance that an analytical method will consistently yield results that accurately reflect the quality characteristics of the product tested"

(cGMP)

"Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled"

[ISO 8402:1994]



# Risk in production control

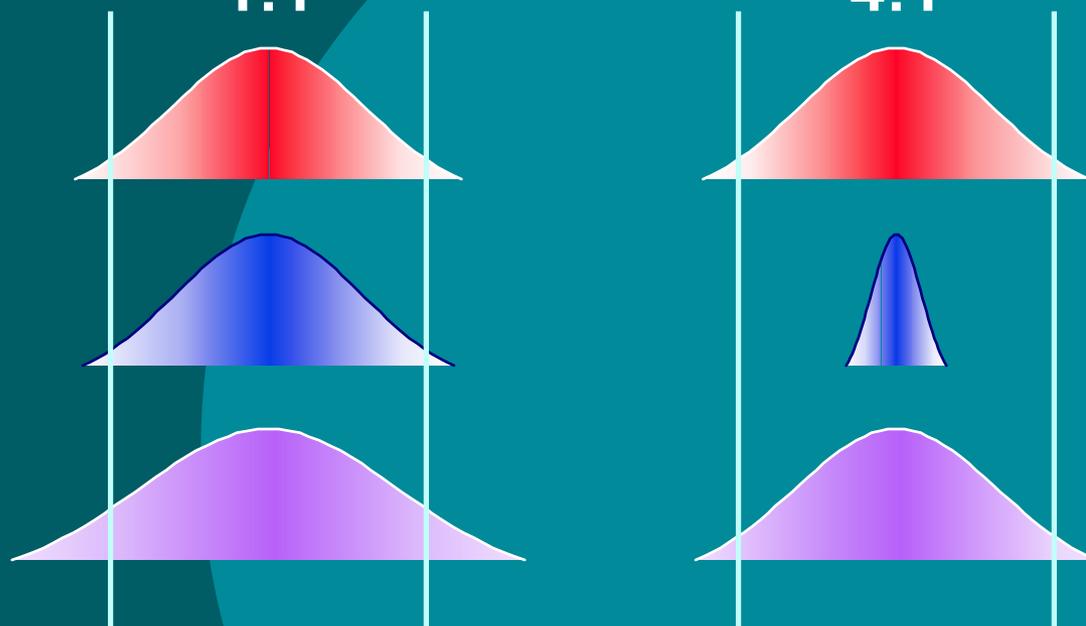
Variation  
from:  
Process

1:1

4:1

Method

Total



Rejects:

16%

5%



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## Traceability: Implications revisited

If we assume

$$y = f(x_1, x_2 \dots x_m) \Big|_{x_{m+1}, x_{m+2} \dots x_n}$$

- The assumption(s) involved must be tested and shown to hold

This is an essential part of method validation

Validation is crucial  
to practical traceability

# Method Validation

A decision on fitness for purpose supported by experimental evidence

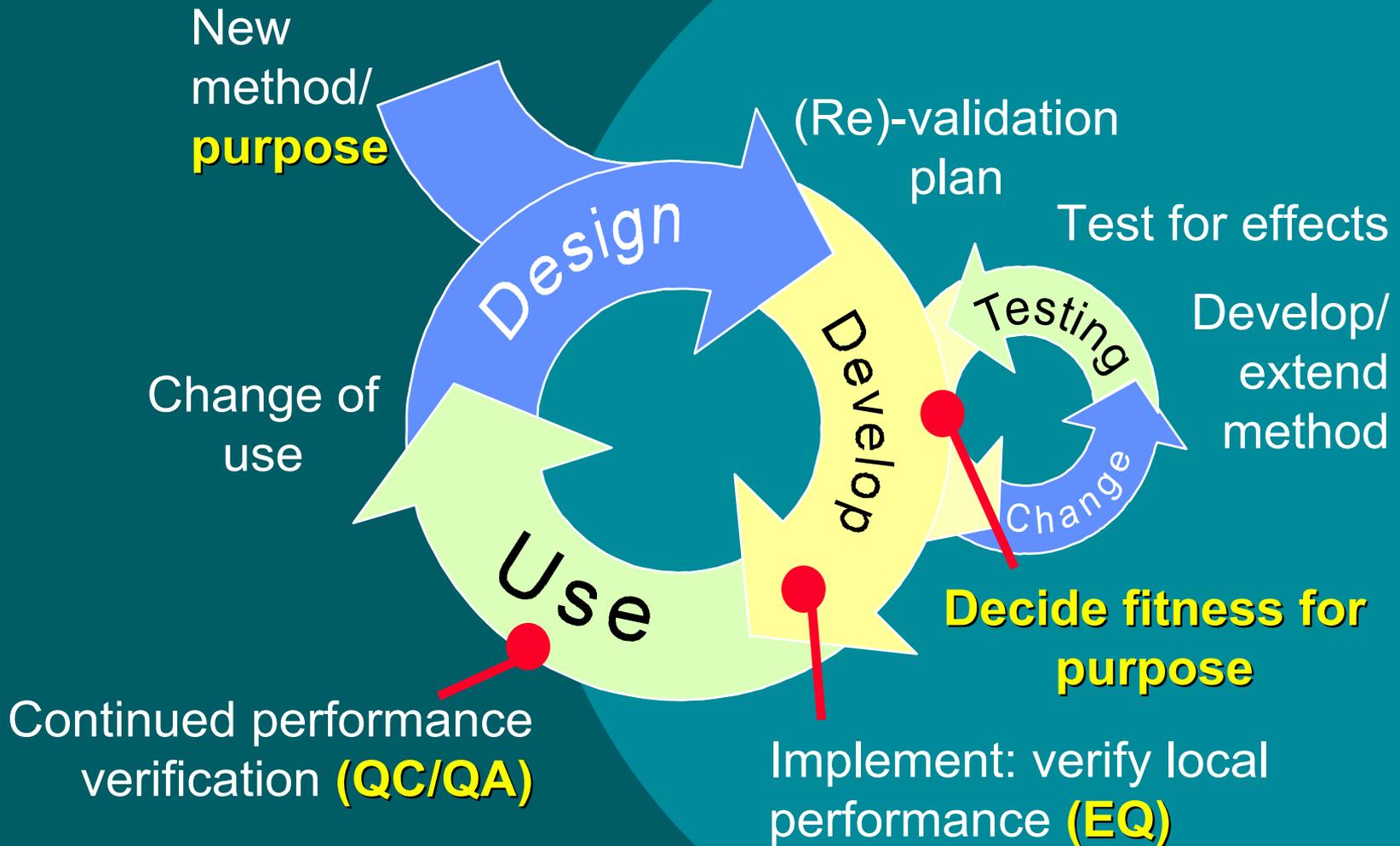
An experimental test of assumptions underlying the method

- Questions
  - what's 'fit'?
  - what experiments?

# Why validate?

- To make sure it will work
  - Establish control parameters
  - Decide calibration and traceability requirements
- To save money
  - Contingency costs; cost of error, knowledge of risk
  - Avoid method transfer failures

# Method development and validation



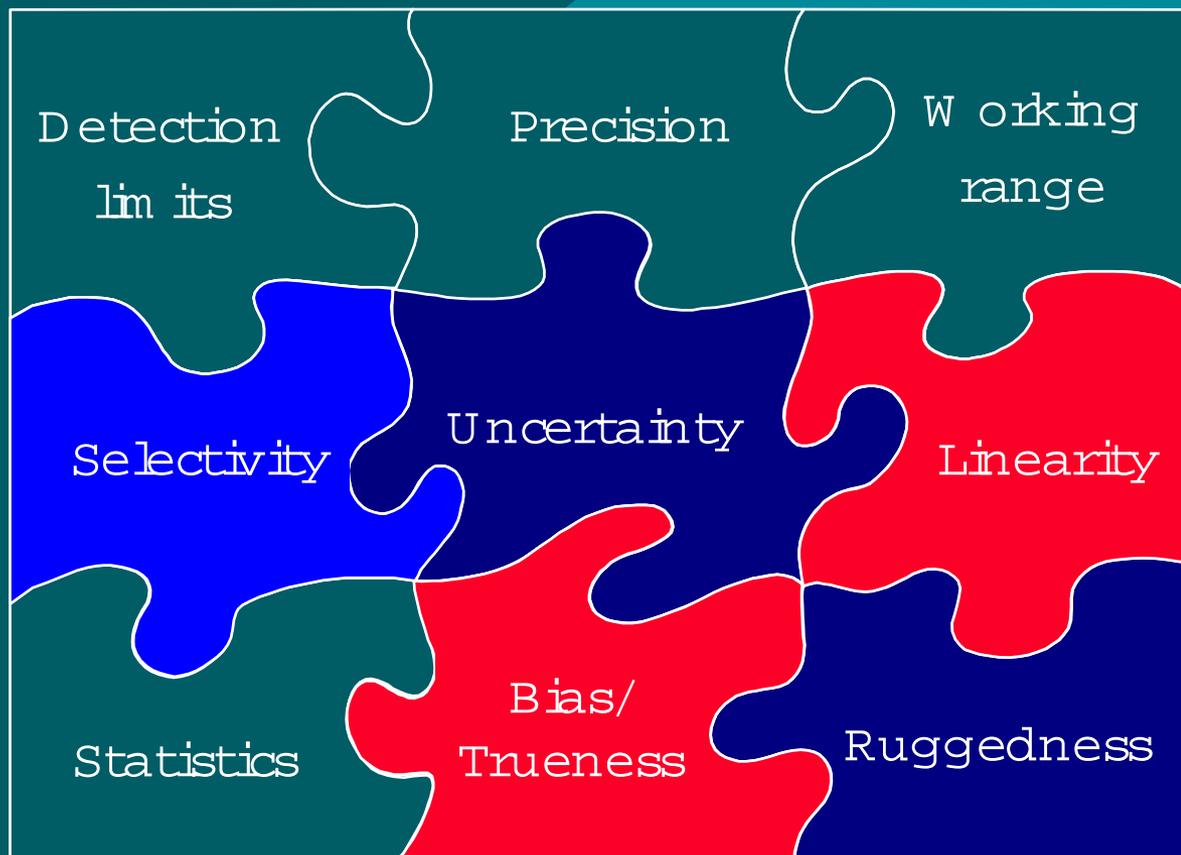
# Method development and validation

- Where does development stop and validation begin?
  - 'AOAC' approach (decide after development/testing)
  - Alternative; suite of tests after development
- Development lifecycle - when?
  - Testing methods; 'Approving' for use; extending, changing, re-validating.

# Fitness for purpose

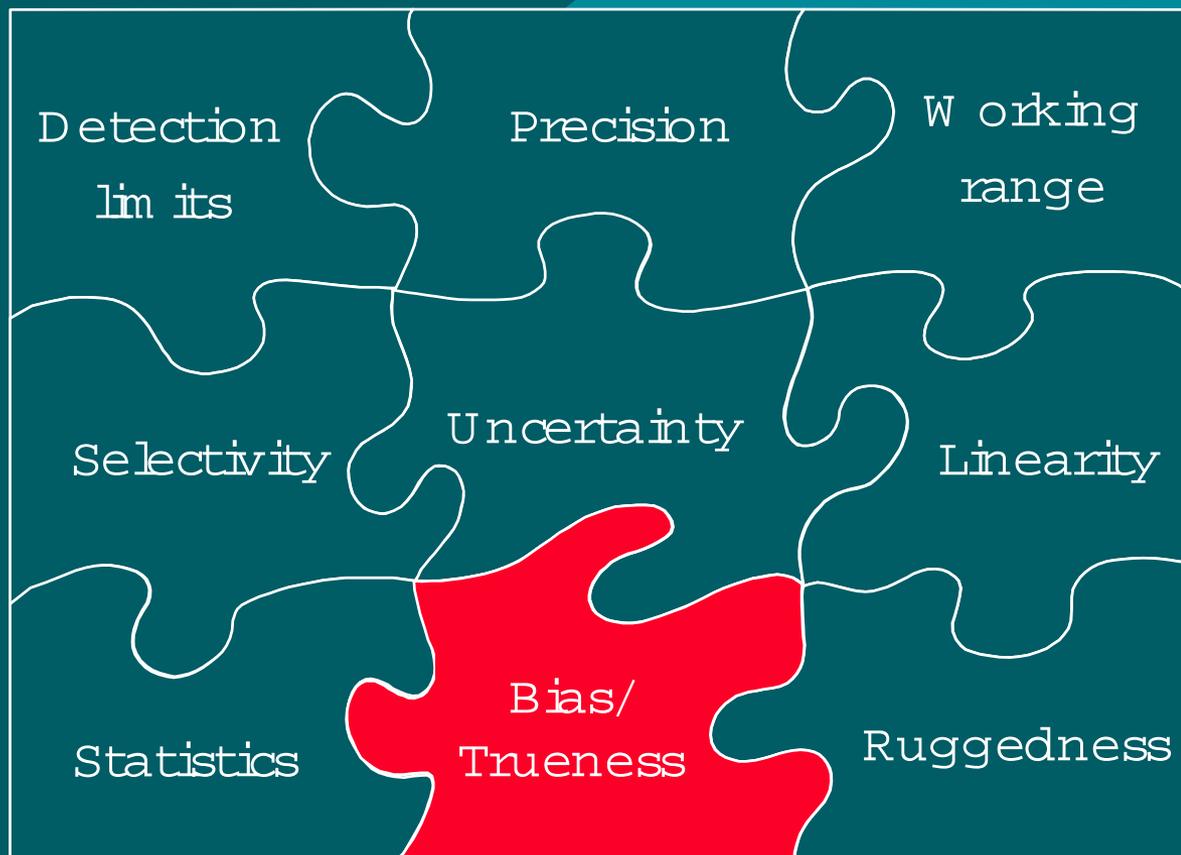
- What purpose?
  - Specify the purpose; analyte(s); matrix; levels; confidence required ...
- What's 'fit'?
  - criteria for testing lead to a 'validation plan'
  - subjective criteria include 'expert review'

# Validation for Traceability



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# Validation for Traceability



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# Bias

- Difference between observed mean value and reference value

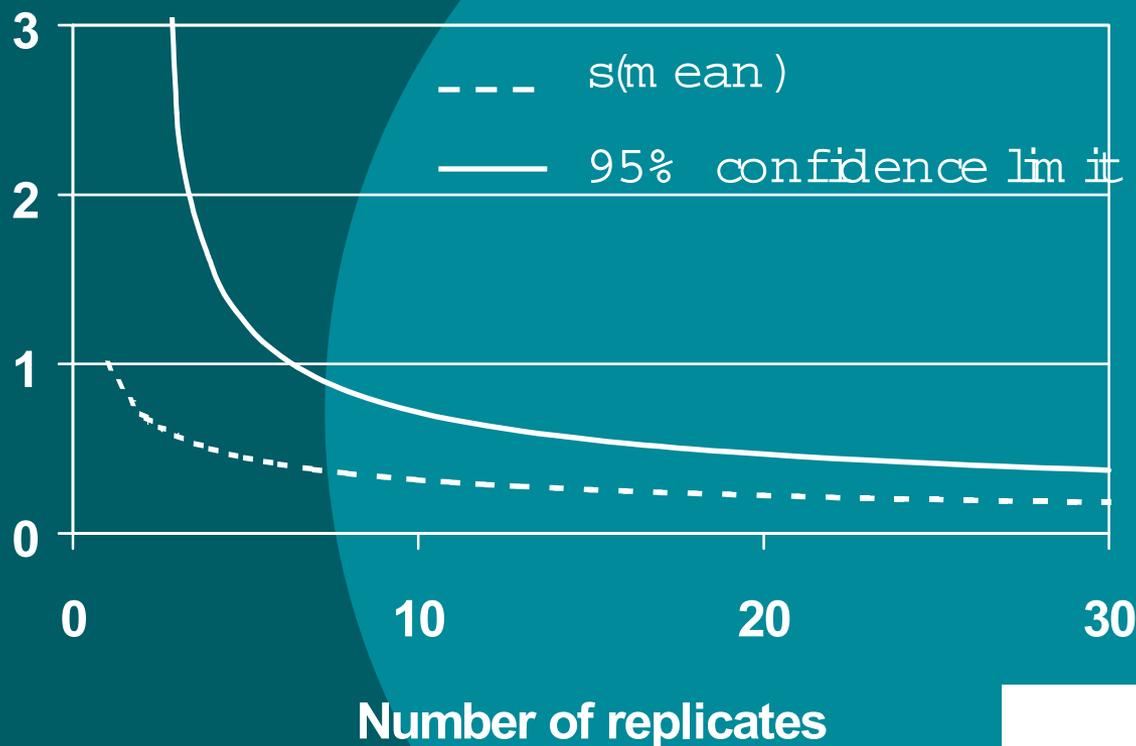


- Bias is a measure of **Trueness**

# Principles of Bias Assessment

- Sufficient precision to detect practically significant bias
- The most appropriate reference material and value available
- Tests covering the scope of the method adequately

# Sufficient Replication



Based on  
SD of 1.0

# Reference Materials

- Certified reference materials
  - essential for most regulatory work
  - recommended where available
  - natural matrix materials preferred
  - check certified uncertainty
- In-house or other established reference
  - useful alternative for checking consistency
  - uncertainties may be unavailable



**Ideal check  
on effective  
traceability**

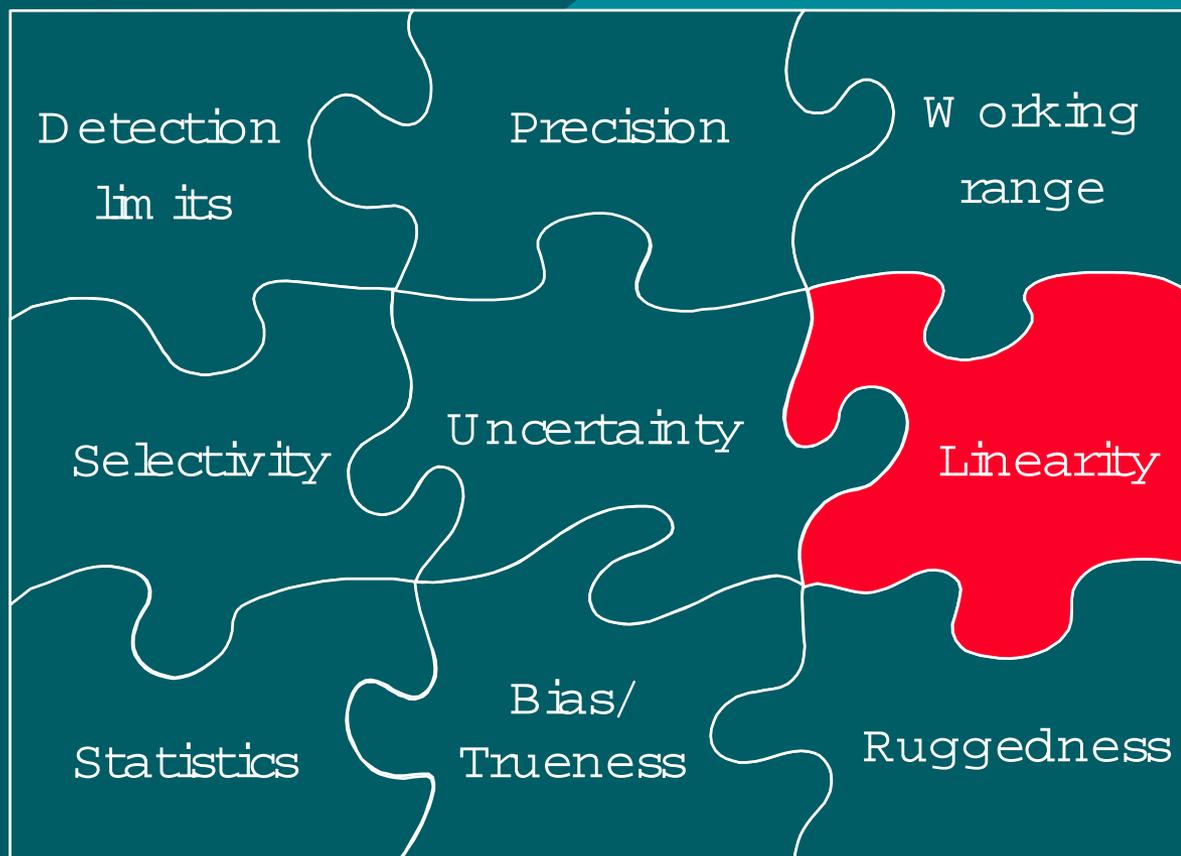
# Reference Methods

- Standard methods for the purpose
- Comparisons on a range of materials
- Paired comparisons
- Future comparability depends on execution

# Spiking Studies

- 'Spiked' test samples
  - add pure materials to previously measured test samples
  - add spike before processing if possible
- Valuable check on some types of interference
  - signal suppression, quenching
- Added and naturally incurred materials behave differently
  - added materials bind weakly to surface
  - allow for equilibration if possible

# Validation for traceability



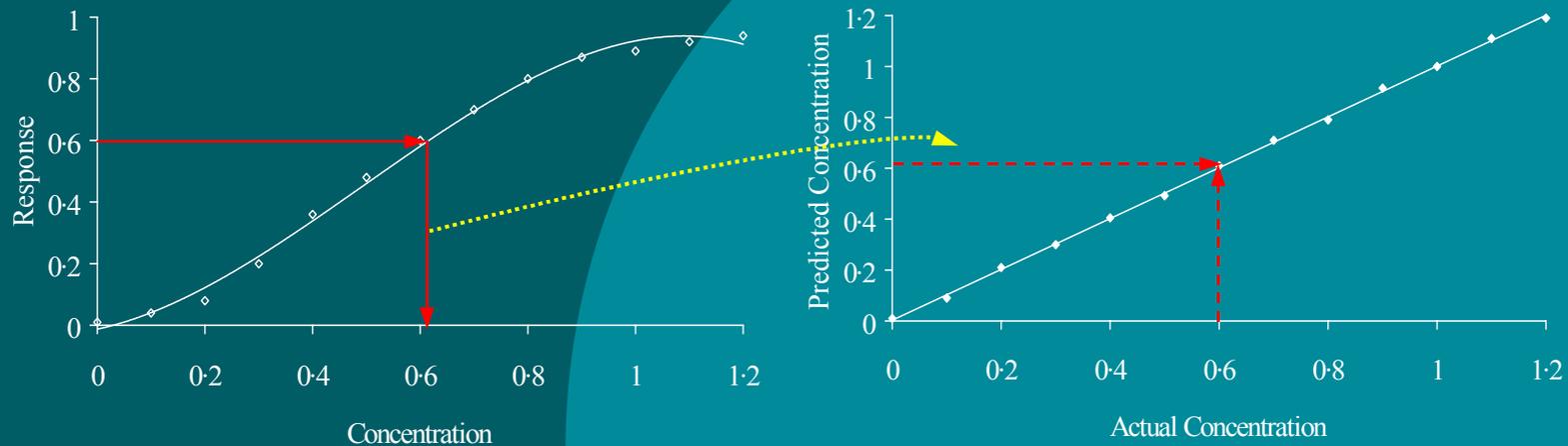
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# Linearity

- “Defines the ability of the method to obtain test results proportional to the concentration of analyte.” (AOAC-PVM C)

Note: The linear range is the range of analyte concentrations over which the method gives test results proportional to the concentration of the analyte.

# Calibration and Prediction Linearity



- Calibration can be non-linear but follow a known mathematical function
- When predicted results are calculated via the function a plot of predicted versus actual concentration can be linear

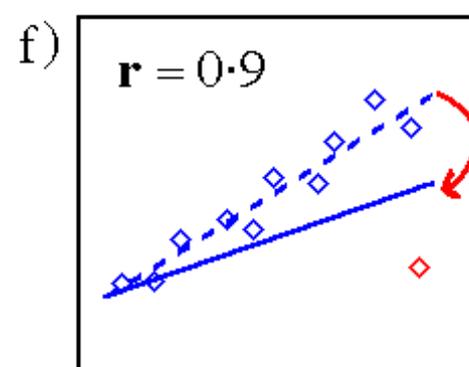
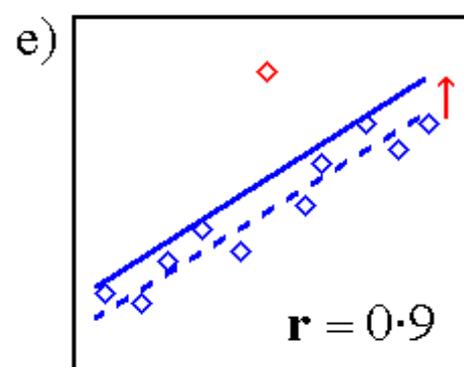
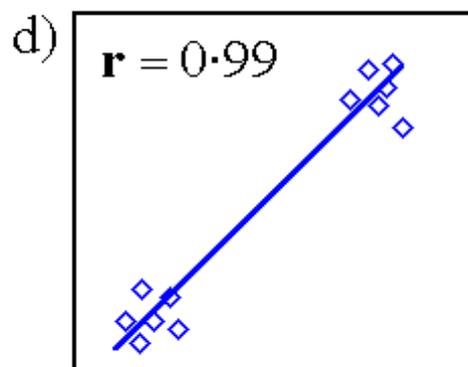
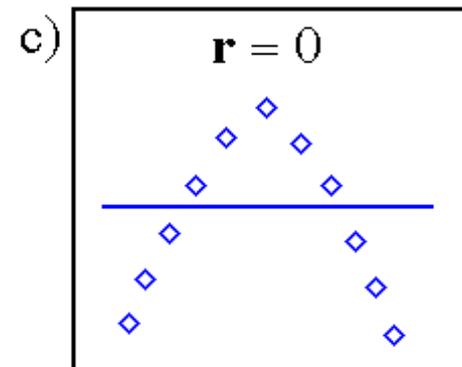
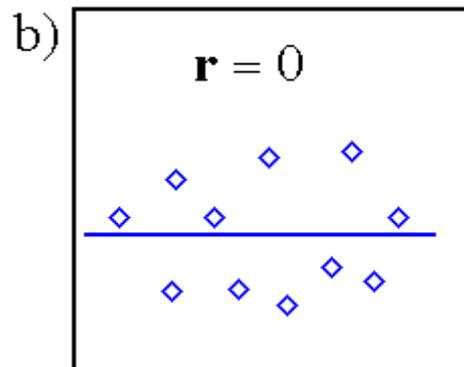
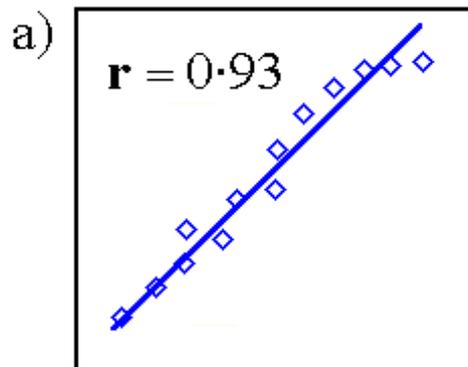


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# Some Supporting Statistical Measures

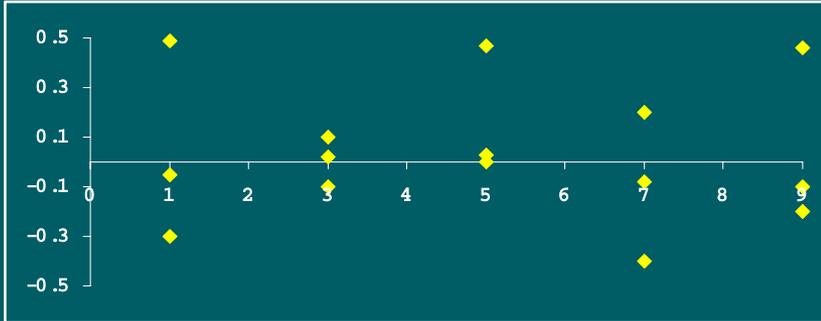
- Correlation coefficient ( $r$ )
- Residual standard deviation
- Slope and intercept
  - standard deviation
- Residual plot

# Correlation coefficient: Misuses

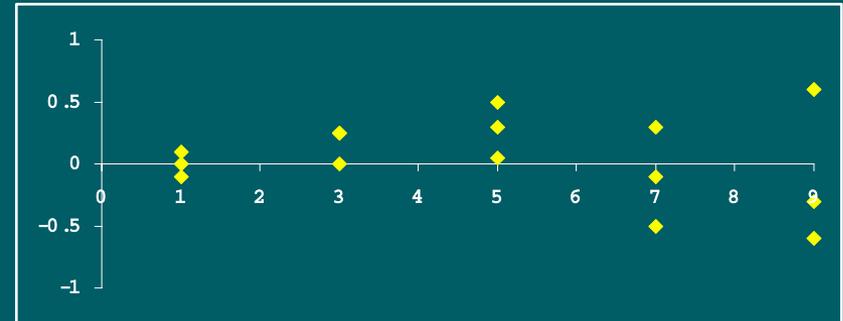


# Residual Plots

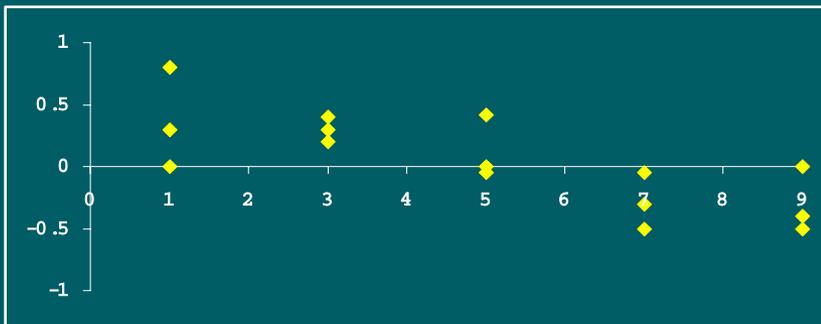
Typical



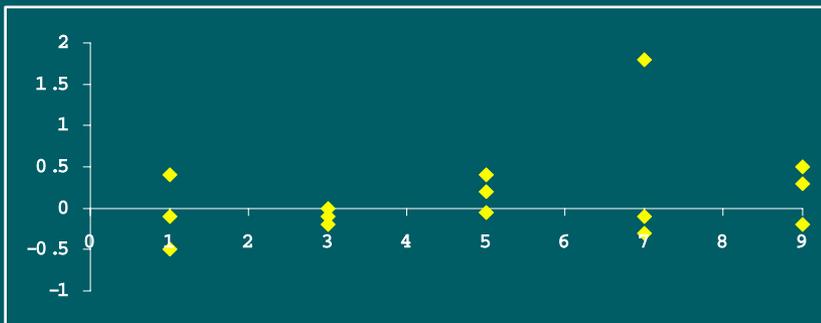
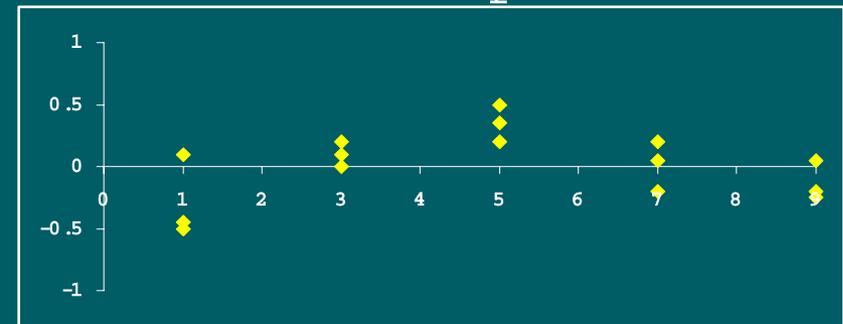
Error depends on Y



Incorrect zero intercept



Curved response

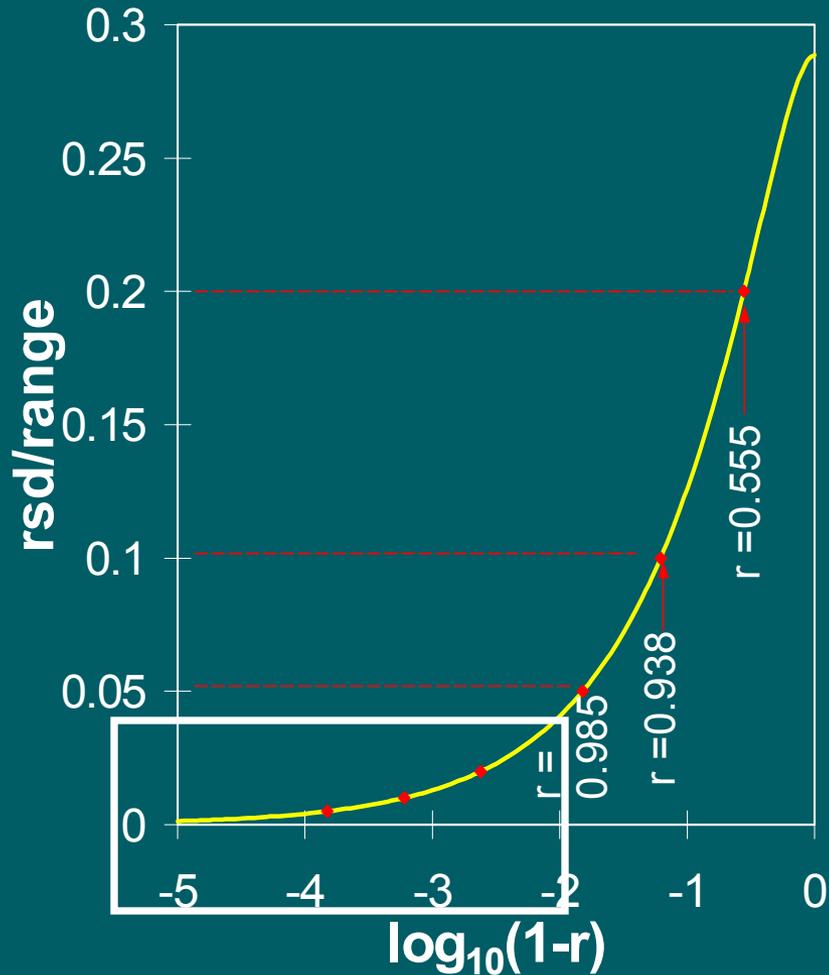


Single outlier

# Lack-of-Fit Measures

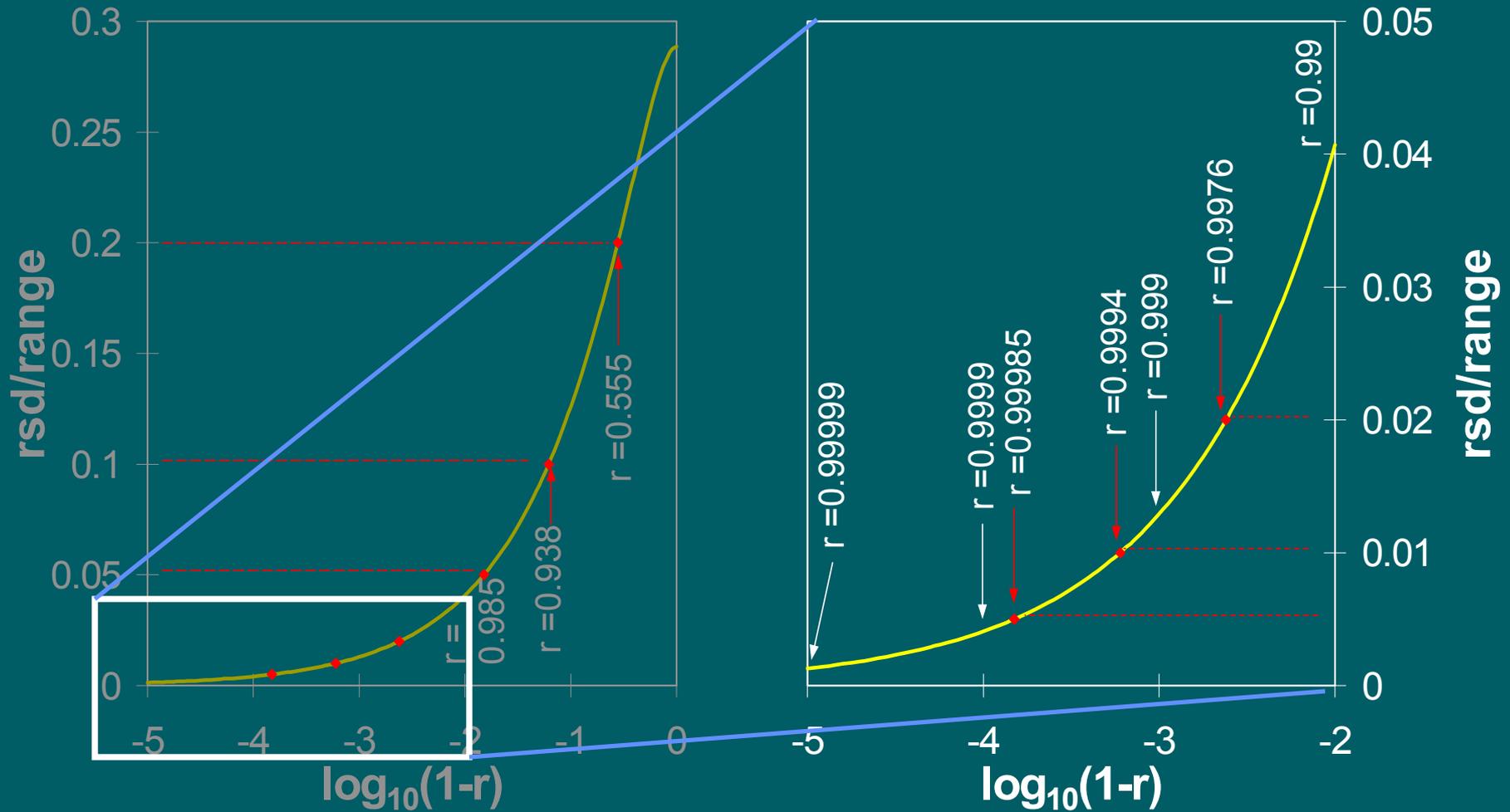
- Correlation coefficient ( $r$ )
- Residual Standard Deviation
  - F test to compare against repeatability sd
- Comparison with a quadratic fit
  - test for significant difference between linear and quadratic rstds
  - test for significance of higher order terms

# Correlation Coefficient: Prediction and $r$



**Correlation coefficient can be related to residual sd**

# Correlation Coefficient: Prediction and $r$



# Linearity: best practice

- Space points out well
  - Correlation coefficient then meaningful
- Use tests for NON-linearity
- Inspect residual plots

# Tools for validation

- Brain – *THINK!*
- Chemical knowledge and literature
  - Understanding the chemistry
- Statistics
- Protocols and guidelines
- Reference materials
  - Vital for accuracy estimation and stability

# Statistics

- Experimental design
  - Economic design
- Exploratory statistics
  - Data quality; unexpected behaviour
- Performance parameter calculation
- Significance testing
  - Objective testing against specification

# Extent of validation

More validation effort = greater confidence

Wider method scope = greater effort required

- Validation effort depends on :
  - Scope and **available prior data**
  - Criticality and purpose
  - Value to the business

# Method validation : Summary

- Validation and Traceability mean reliable results
  - ..and *reliability saves money!*
- Neither purely statistical nor mechanical –  
*Think!*
- Confidence is proportional to effort
  - Aim for a 'Reasonable test'
- Plan validation with purpose and prior knowledge in mind