การตรวจสอบความถูกต้องทางชีววิธี
Bioassay Validation

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14 ตุลาคม 2558

What is a bioassay?
ICH definition and requirements

• Bioassay (Biological Assay) – ICH Q6B

Definition: The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay),based on the attribute of the product which is linked to the relevant biological properties.
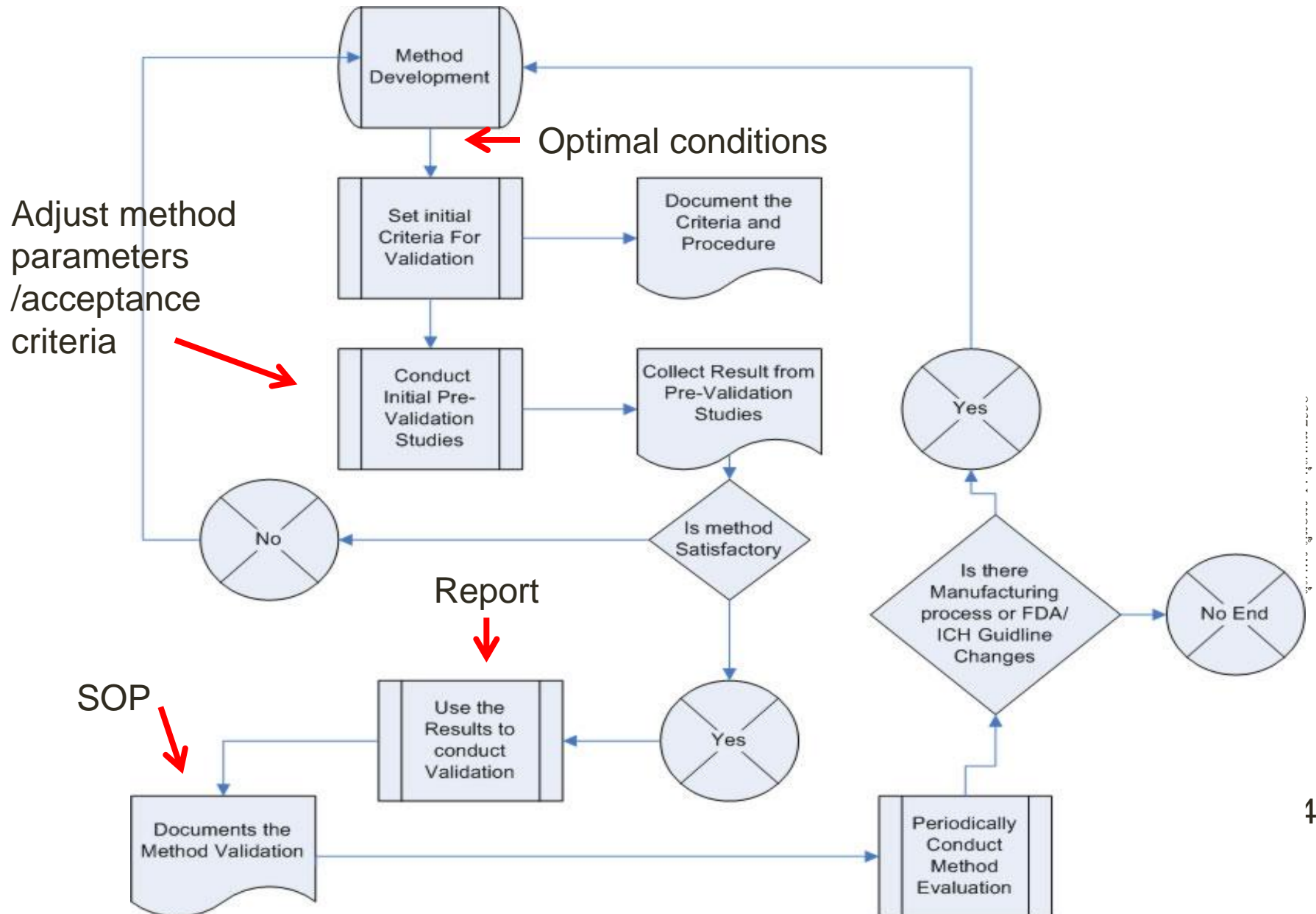
(from: Tim Schofield, 2012)

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e.g. procedures used to measure biological activity include:

- Animal-based biological assays, which measure an organism's biological response to the product
- Cell culture-based biological assays, which measure biochemical or physiological response at the cellular level
- Biochemical assays, which measure biological activities such as enzymatic reaction rates or biological responses induced by immunological interactions
  (from: Tim Schofield, 2012)

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Method development-Validation life cycle

Optimal conditions

Adjust method parameters /acceptance criteria

Report

SOP

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What is the purpose of analytical method validation?

- <u>Identification</u> of sources and quantitation of <u>potential errors</u>
- To assure method is <u>acceptable for intended use</u> (reliable, reproducible and accurate)
- Establish <u>proof that a method can be used for decision making</u>
- Satisfy FDA/EMEA requirements

Why do we need method validation?

A method that is valid in one situation could be invalid in another.

Common Misconceptions

Method Validation ≠
Method Optimization ≠
Method Qualification

Validation vs. Verification

Non-compendial Methods vs. Compendial

Compendial methods -<u>Verification</u>
Regulatory analytical procedure in USP/EU/WHO etc.
 <u>Partial Validation</u>

Non-compendial methods -<u>Validation</u>
Alternative analytical procedure proposed by the applicant for use instead of the regulatory analytical procedure

e.g.

A customer wants lab A to perform potency testing of influenza vaccine, however lab A has no method for this test. Lab A has only potency testing of varicella vaccine.

Lab A will use the method of potency testing of varicella vaccine for testing of live influenza vaccine.

What does lab A has to be done?

e.g.

A customer wants laboratory A to do potency testing of influenza vaccine by using standard method, however lab A has not done the test before.

What does lab A has to be done?

Method verification

e.g. when a method developed in Lab A is transferred to Lab B, after implementation the test should be run as good as it was validated in Lab A
(even if both labs are located within the same institute)

Method verification

when a method that has not been in use for a while is started up again, it may perform merely a method verification using the same criteria that were previously defined.

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Method verification

- Method verification is also typically applicable for commercial assays (kits)
- Validated by the manufacturer
- No need to repeat all validation experiments
- Need to verify that assay in the lab is running according to the manufacturer's specifications

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Assay validation parameters

- ✓ Accuracy
- ✓ Precision (repeatability, reproducibility)
- ✓ Limit of Detection
- ✓ Limit of Quantitation
- ✓ Specificity/selectivity
- ✓ Linearity and range
- ✓ Ruggedness
- ✓ Robustness

? System suitability

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Accuracy

- the measure of exactness of an analytical method
- the <u>closeness</u> of agreement between the measured value
- the value that is accepted as a conventional <u>true value</u> or an <u>accepted reference value</u>
- % recovery (assay value/true valuex100)

Accuracy

- Usually requires a " <u>gold standard</u>"
- An accepted method to which a new method can be compared

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Precision

- The <u>degree</u> of agreement among individual test results
- The procedure is applied repeatedly to multiple samplings of a homogeneous sample
- <u>Without</u> the availability of <u>a gold standard</u>
- The <u>scatter of the data</u> rather than the exactness of the reported result

Precision Industry Standards

Coefficient of variation (CV or RSD)

- Enzyme based assay   <10%
- Binding (ELISA)   10-20%
- Cell based assay   avg. 25%
- *In vivo*   20-50%
- Virus titer assay   330%
      (0.5 log)

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Limit of Detection

- A method may be defined as the concentration of analyte which gives rise to a signal that is significantly different from the negative control or blank

- The <u>lowest concentration</u> of analyte that can be distinguished from background

Limit of Detection

- The result obtained at the LOD are not necessarily precision or accuracy
- End points dilution titre
- Dilutional sensitivity

e.g. ELISA

- Mean + 3SD
- Cut off

Mean of negative control (blank control)

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Limit of Quantitation

- The <u>lowest</u> concentrations of analyte in a sample or specimen that can be measured with an <u>acceptable level of accuracy</u> and <u>precision</u>

e.g. ELISA

- Mean + 10SD
- Lowest conc.

Mean of negative control (blank control)

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Specificity

- The ability of the method to measure the analyte of interest to the <u>exclusion of other relevant components</u>
- The term specific generally refers to a method that produces a response for a single analyte only

Selectivity

- Refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture.

- The method should provide response that is distinguished from all other responses.

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Exp. Monoclonal antibody
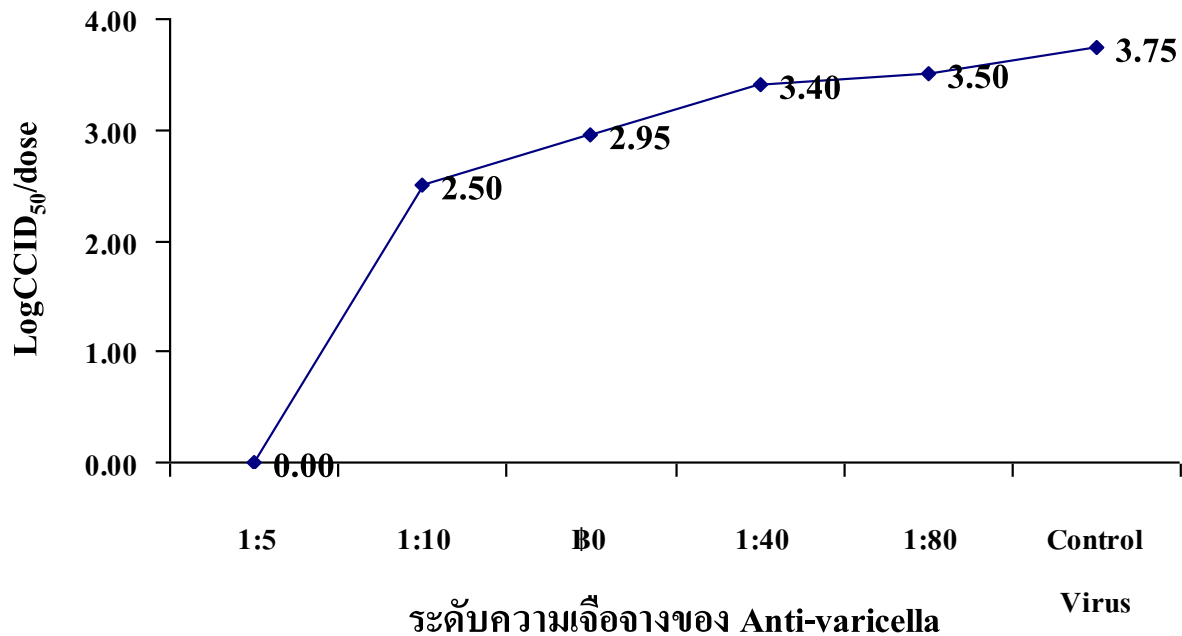
Ruggedness

- Typical parameters

  It provides an estimate of experimental reproducibility **unavoidable** changes or error

  - different laboratories
  - different machines
  - different operators
  - different reagent lots
  - different analysis days

Robustness

- A measure of the assay capacity to remain unaffected by small but <u>deliberate changes</u> in test conditions

- It provides an indication of the ability of the assay to perform under normal usage

Robustness

- The effect of <u>deliberate changes</u>

  - effect of freeze/thaw

  - incubation times

  - incubation temperatures

  - sample preparation

  - sample storage

  - cell passage number

Robustness

- lots of drugs

- variability between serum from different animals

- variability between patients

Robustness

- Robustness for cell based assays

    - cell bank (beginning, middle and end of freeze)

    - cell passage level

    - cell seeding density

    - cell stock density (how many days in culture)

    - cell age in flask

Robustness

- Robustness for cell based assays
  - incubation time
  - different plates
  - lots of serum
  - source of reagents

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System suitability

- Test for parallelism

- When plotting the log dose versus the response, serial dilutions of the reference and serial dilutions of the samples should give rise to parallel curves

System suitability
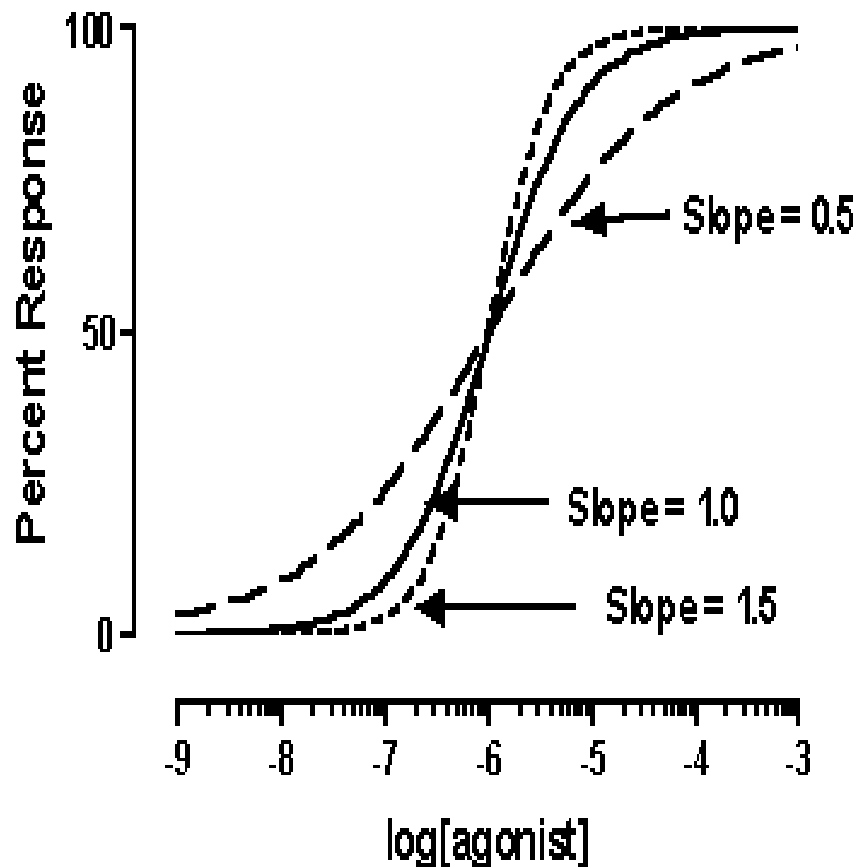
When plotting the log dose versus the response, serial dilutions of the reference and serial dilutions of the samples should give rise to parallel curves

System suitability

Validity criteria: Parallel line assay
1. The response to each treatment (dose) group are normally distributed.
2. The variances of the responses to each treatment group are homogeneous.
3. The overall assay dose-response is significant.
4. There are no significant deviations from parallelism.

5. There are no significant deviation from linearity.

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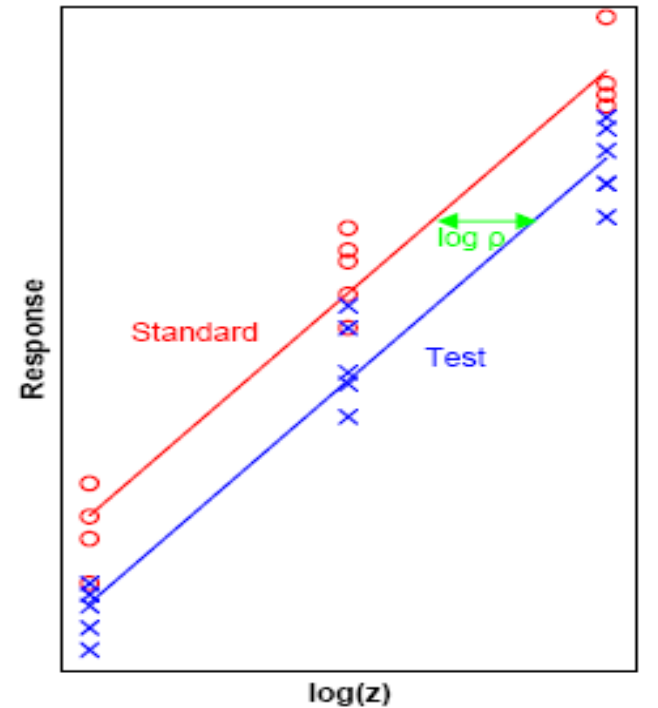
Assay validation protocol acceptance criteria

- You must have acceptance criteria specified in the protocol for each parameter

- e.g. acceptance criteria

  - Enzyme based assay   <10%
  - Binding (ELISA)   10-20%
  - Cell based assay   avg. 25%
  - *In vivo*   20-50%
  - Virus titer assay   330%

               (0.5 log)

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Assay validation protocol acceptance criteria

- The acceptance criteria will be different for different assays
- The acceptance criteria must be set prospectively

- Full validation for non- compendial method

    - It depends on test method and a purpose of the method.

    - Method is fit for purpose/ fit for its intended use.

- Partial validation for compendial method

  -It depends a purpose of the method.

  -It requires <u>at least precision (repeatability and intermediate precision)</u>.

<u>Accuracy is acceptable if standard material is available</u>.

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Remark:

If an assay run is not valid, the result is skipped completely, and the run is repeated. If more than one run is invalid, this indicates something is not in control. The validation should be postponed and the reason for the failure should be investigated. The assay may not be robust enough and may need further development or optimisation. After the method is optimised, the validation is repeated including three new runs.

References:

1. "Validation of compendial methods" USP 23 (1225) USPC Rockville Maryland USA 1994.
2. International Conference on Harmonization "Guideline on validation of Analytical Procedures.Q2A and Q6B
3. Schofield TL. Assay development. In: Chow SC, ed. Encyclopedia of Biopharmaceutical Statistics. 2nd ed. New York: Marcel Dekker; 2003

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