Development and Validation of a Serological Potency Test for the Release of *Leptospira* Vaccines - Requirements in the European Union

ICCVAM-Workshop on Leptospirosis, 19 – 21 September 2012

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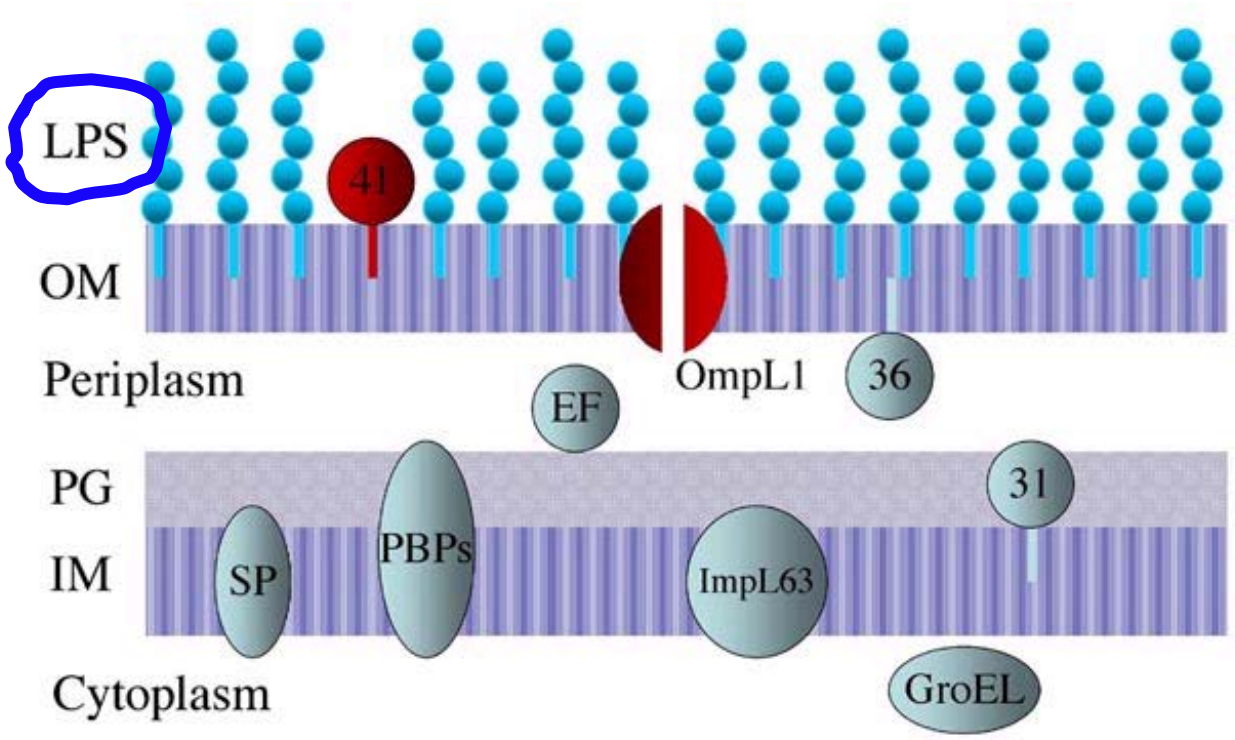
Licensed vaccines

- **Canine vaccines**
  - ***L. interrogans***
    - **Serogroup**
      - **Icterohaemorrhagiae**
      - **Canicola**
      - **Australis**
  - ***L. kirschneri***
    - **Serogroup**
      - **Grippotyphosa**

- **Bovine vaccine**
  - ***L. borgpetersenii***
    - **Serovar**
      - **hardjo (type hardjobovis)**

Leptospiral membrane protein architecture



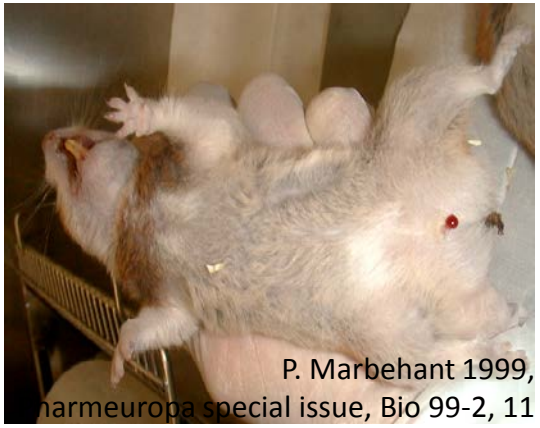
Zuerner et al., 2000, J Mol Microbiol Biotechnol, 2(4), 455

LPS

- **Target for agglutinating and opsonizing antibodies**
- **Immunity mostly serovar-specific**
- **Correlates with levels of agglutinating LPS-specific antibodies in transferred sera**
- **LPS-specific mabs passively protect naive animals from leptospirosis**
- **Purified LPS can stimulate active immunity**

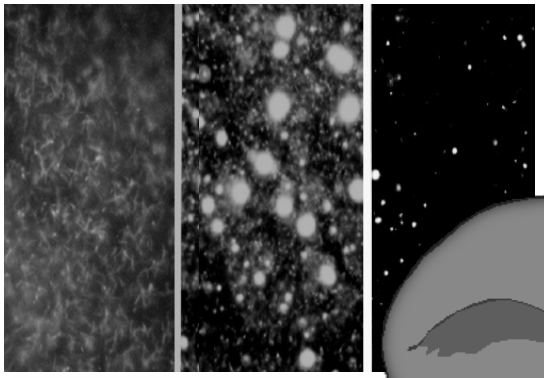
Batch potency

- **Hamster challenge**

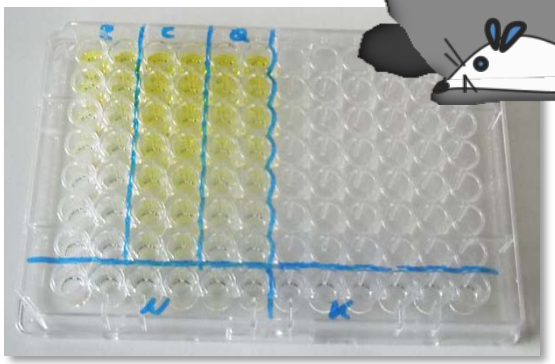


P. Marbehant 1999,
harmeuropa special issue, Bio 99-2, 11

- **Microscopic agglutination-test (MAT)**



- **Antigen quantification (pabs/mabs)**

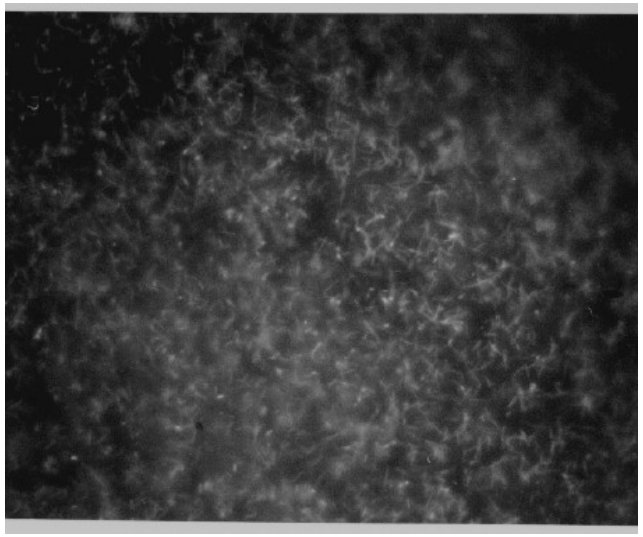


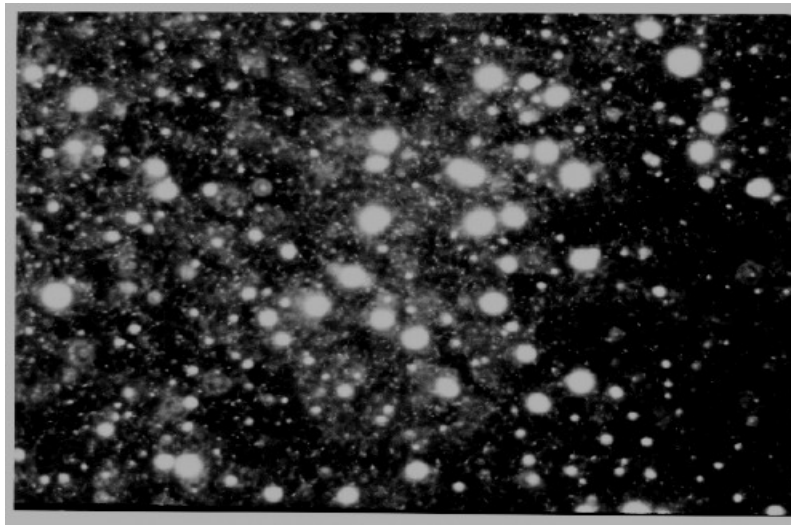
Microscopic Agglutination-Test (MAT)

(Martin and Pettit, 1918)

- **Serial dilution of serum plus equal volume of leptospirosis**
- **Estimating 50% agglutination as the end point titre**
- **Paired serum samples**
- **Most important: antigen density/definition of significant titres**
- **Inactivation without agglutination in case of very low titres**
- **Titres serovar and vaccine (components, adjuvant) dependent**
- **Reactivity of animals weight dependent**
- **Transferability poor**

(Goddard et al. 1986, J Biol Stand, 14, 337; Ebert 1999, Pharmeuropa special issue Bio 99-2, 102; Ebert et al. 2000, ECVAM project, contract no. 12992-97-06 F1ED ISP D, Study 2)





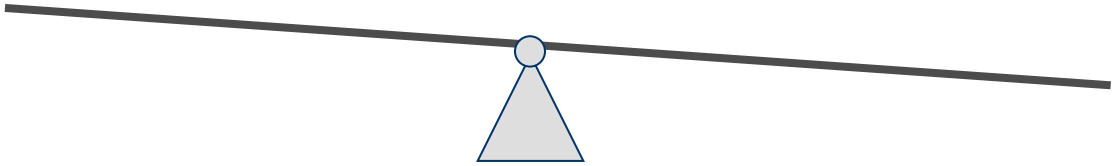
MAT

Strengths

- Specificity
- Detection of group-specific antibodies
- Detection of protective antibodies (Challa et al., 2011, Vaccine 29, 4431)
- Titres reflect reaction to entire vaccine (no further vaccine processing required)
- Titres reflect vaccine dose/vaccination scheme
- Suitable for testing of non-lethal strains and stability testing

Weaknesses

- Requires animal testing (ethics, costs, time, extrapolation of data between species)
- Maintenance of live reference strains (contamination, mislabelling, switching of strains, hazardous)
- Standardization and transfer difficult
- No differentiation of IgM and IgG (as compared to ELISA)
- Might not be suitable for all vaccines



Validation

- **Technical guide for the elaboration of monographs, Chapter III, Analytical Validation, 6th ed. (2011)**
  http://www.edqm.eu/medias/fichiers/technical_guide_for_the_elaboration_of_monographs_.pdf
- **VICH Guideline 1 (1998)**
  Validation of analytical procedures: Definition and terminology
  http://www.vichsec.org/pdf/gl01_st7.pdf
- **VICH Guideline 2 (1998)**
  Validation: Methodology
  http://www.vichsec.org/pdf/gl02_st7.pdf
- **Hendriksen et al. (1998)**
  Validation of alternative methods for the potency testing of vaccines (ATLA, 26, 747– 761)
  http://staging-ecvam.jrc.it/publication/WorkshopReport31.pdf

Validation/test validity criteria for routine quality control

Type of test / Criteria	Identity	Purity		Content/ Potency
		quant.	qual.	
Specificity	+	+	+	+
Accuracy	-	+	-	+
Precision	-	+	-	+
Linearity	-	+	-	+
Detect. limit	-	-	+	-
Quant. limit	-	+	-	-
Range	-	+	-	+

Technical guide for the elaboration of monographs.
Chapter III, Analytical validation, 6th edition, 2011,

Specificity

- Ability to **assess unequivocally the analyte** in the presence of other antigenic components/excipients/residuals/degradants.
  - Veterinary vaccine preparations are not purified preparations in most cases.
  - For multivalent vaccines, it is necessary to test the specificity of the response for each component in the vaccine.

⇨ During validation and each time a critical reagent is changed

Specificity of serological assays

- **Clinical relevance**
  - Correlation to efficacy/in vivo potency (passive protection studies; vaccination-challenge tests)
  - Dose/response (titration) studies (fraction dose preparations/placebo vaccine)
- **Immunorelevance/Immunodominance**
  - Epitope(s) detected by vaccinated/challenged animals
  - Epitope(s) not detected by naive animals
  - specific/related/unrelated antigens
    - ELISA/Agglutination assay/Western blot
    - “Growth Inhibition Test” (in vitro)

Specificity vs. Consistency

PARADIGM SHIFT 2

Acceptance of the consistency approach will imply a major shift in current thinking. Current alternative approaches are too often characterized by the replacement of individual quality control tests (1 by 1 repla… in the established practice of th… unique product with emphasis … th…

« In vitro tests do not provide and do not have to provide the same information as in vivo tests »

Co…sistency testing starts from… su…sequent lots produced can b… an earlier (reference) lot (clini… cal…t) which is thoroughly cha… regard to q…lity, safety and e… qu…es consistent p…ductio… t… co…trol and strict applicatio… f… lity A…surance. Lot release is then… valence with …e reference lot. This shift in… paradigm implies the acceptance that in vi… provide and do not have to provide the sa… as in vivo tests.

INTERNATIONAL SUPPORT

Since the majority of EU vaccines productio… outside the EU, representatives of EU man… ghlighted the need for convergence at th… level. Representatives of the EDQM high… interest in the project, explained EDQM … and suggested conditions and mechanis… forward. Authorities from US and Canada… their interest in participation.

4 FOCUSED APPROACH

In conclusion, participants agreed to embark on this challenging project, diversifying activities between vaccines for human and for veterinary use. Authorities and representatives of vaccine ma… issue guidance and advice on prio… …nce criteria and to select the pilot …

…stablishment of the proof of concept … fic tests to the level necessary for … Standardisation Programme of EDQM … tion process. It will avoid duplication … anizations.

…tage on:
…ves (prevent duplication)
…xisting initiatives (quick wins)
… (hard wins)

…effort from manufacturers and …pwise globally harmonized action. …pecifically dedicated expert consul-…

…cal Standardisation …r …g Plough …ion Joint Research Centre … Vaccine Institute …ogicals …accines and Diagnostics …t), …or)

epaa
The European Partnership
for Alternative Approaches to Animal Testing

For further information:

Visit http://www.ec.europa.eu/enterprise/epaa/index_en.htm

Contact entr-epaa@ec.europa.eu

http://ec.europa.eu/enterprise/epaa/3_events/3_3_workshops/flash-report-vaccines-workshop-april-2011.pdf

Accuracy

Closeness of agreement between conventional true value and value found (recognize/eliminate systematic errors)

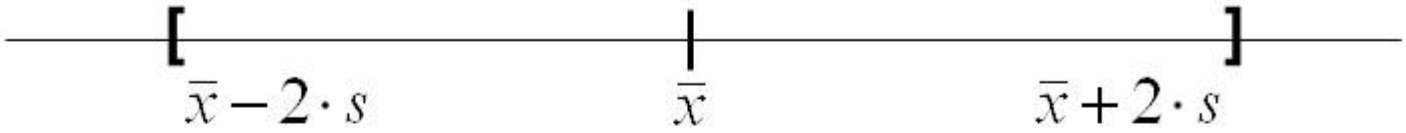
min. 9 determinations

- **quantitative accuracy**
  - Usually expressed as agreement of mean value (incl. confidence interval) and specification of respective test signal (e.g. x 2 SD) set beforehand.

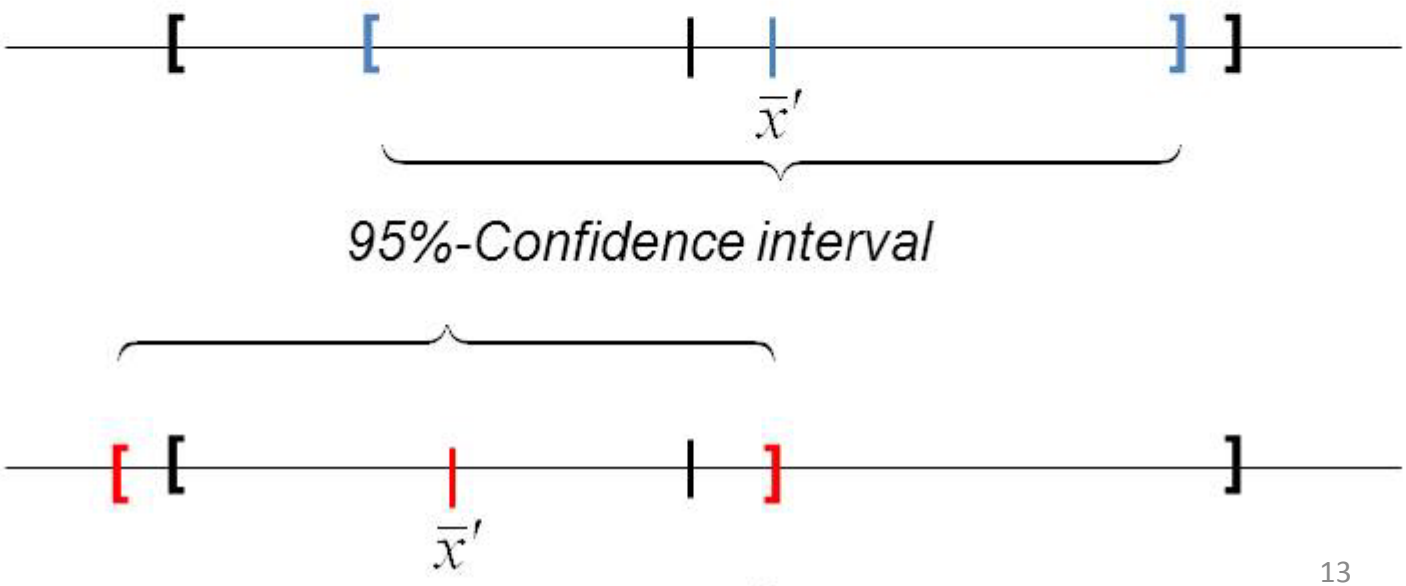
- **validated alternative procedure**

Accuracy

1) **Trials to fix the specification of relevant test parameters (e.g. mean +/- 2 s)**



2) **Validation successful, if mean value including 95% confidence interval fall completely within specification set beforehand.**

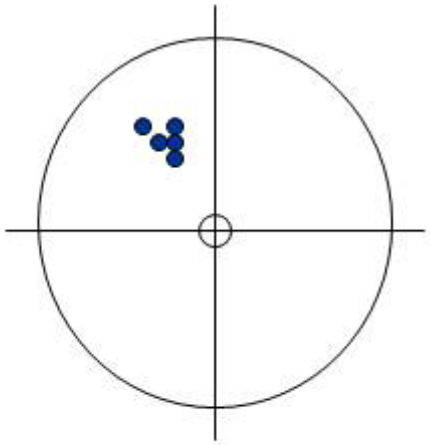


Precision

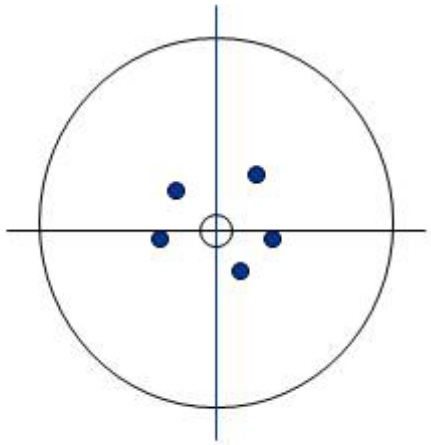
Closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (recognise/eliminate random errors)

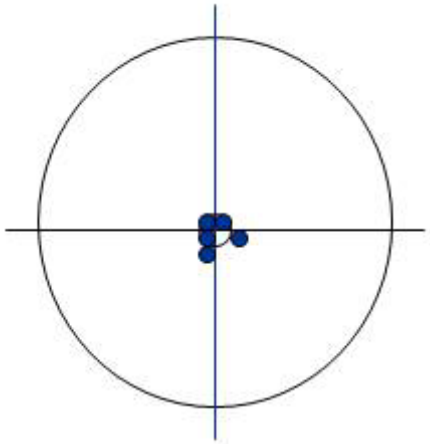
- Usually expressed as variance, standard deviation or coefficient of variation of a series of measurements (min. 6 determinations)
- 3 Levels:
  - Repeatability
  - Intermediate precision
  - Reproducibility

A-, P+

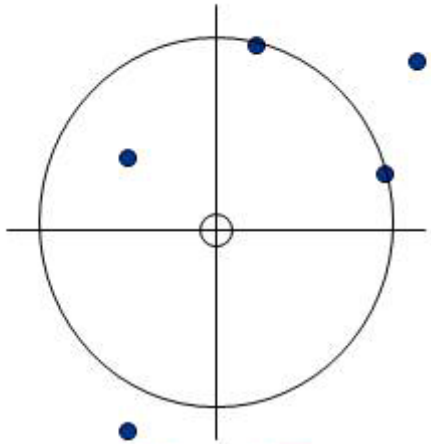


A+, P-





A+, P+



A-, P-

A - accuracy, P - precision, “+” - acceptable, “-“ - not acceptable

Linearity

- **Test result (within a given range) is proportional to the concentration/amount of analyte**
- A linear relationship should be evaluated across the range of an analytical procedure
- In some cases data may need to be subjected to mathematical transformation prior to regression analysis
- For the establishment of linearity a minimum of 5 concentrations is recommended

The batch release value (OD, antigen content, titre) must fall within the linear part of the titration curve

Range

- Interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a **suitable level of precision, accuracy and linearity.**

Assessment of batch potency
I. Relative potency

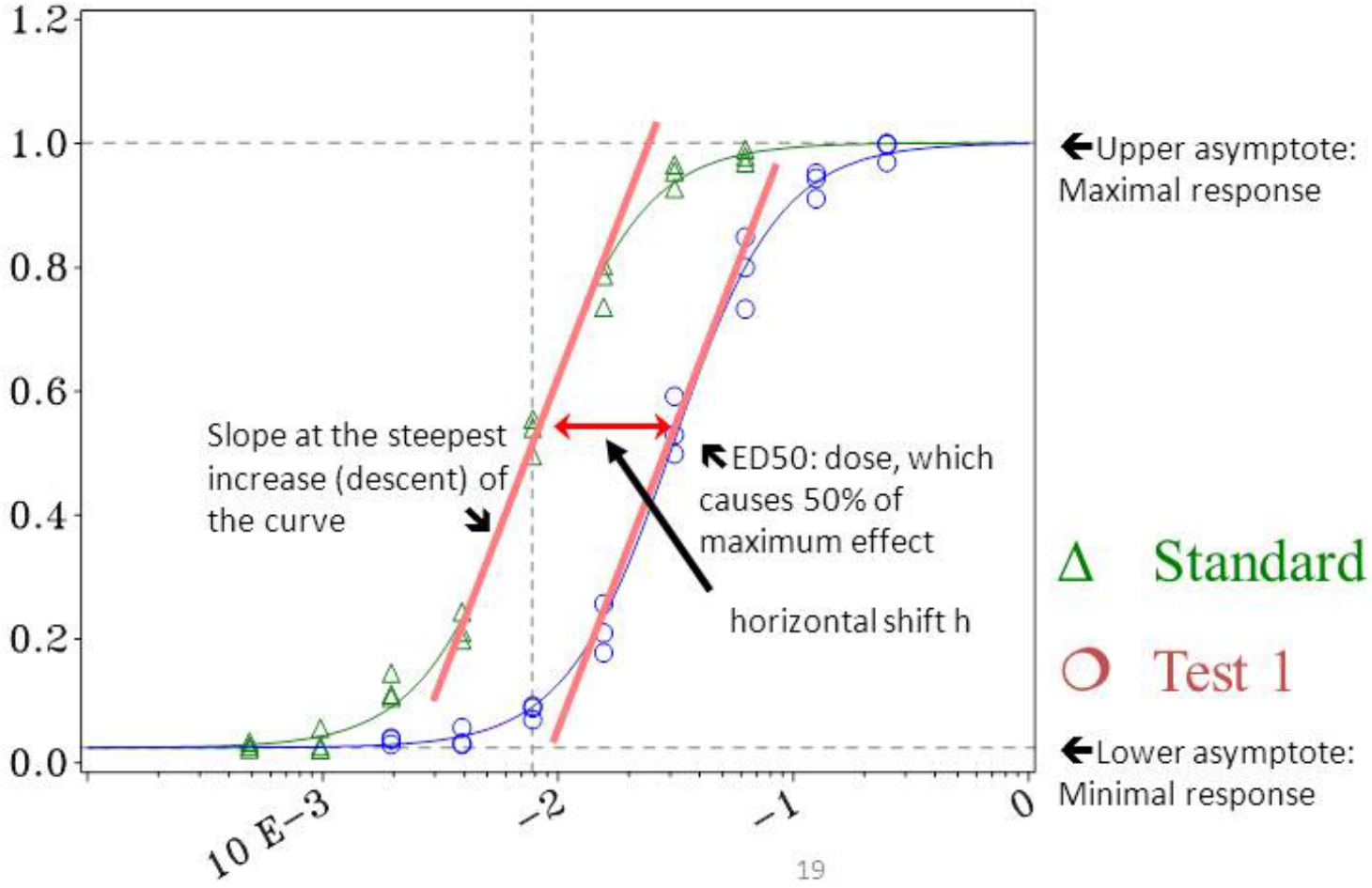
Ph. Eur. 5.3:
STATISTICAL ANALYSIS OF RESULTS OF BIOLOGICAL ASSAYS AND TESTS

…The principle applied wherever possible throughout these assays is that of comparison with a standard preparation so as to determine how much of the substance to be examined produces the same biological effect as a given quantity, the *Unit*, of the standard preparation…

- standard vaccine shown to be efficacious in target species
- standard serum derived thereof (advantageous in terms of 3Rs)

Assessment of batch potency
I. Relative potency

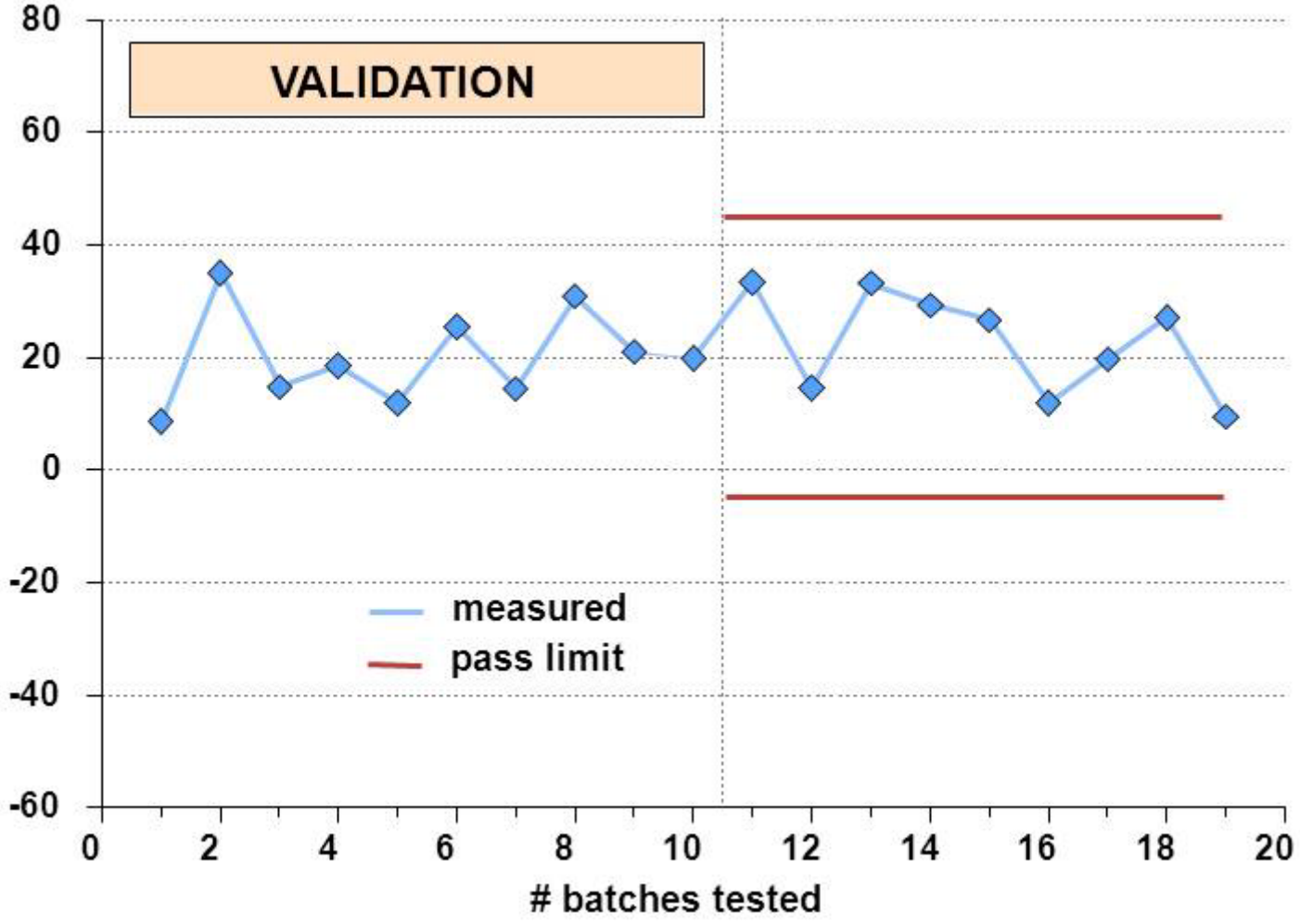
- Parallel line assay
- Four-parameter logistic curve model



Assessment of batch potency
II. Fixed acceptance criteria

- **Release limit** (mean + 3 SD of sub-standard batch)
- **Reference interval** (Mean ± 2 (3)SD of batches with 100% antigen)
  - covers 95.4 % (99. 7 %) of the population
- **Tolerance interval**
  - Interval that cover percentiles of the population
  - Interval that cover percentiles of the population with a certain probability

Assessment of batch potency
II. Fixed acceptance criteria



Assessment of batch potency
II. Fixed acceptance criteria
Detection of sub-standard batches

- **Sub-standard batch still efficacious in target animal species**
- **Will not pass batch potency test**

⇨ **analytical sensitivity („Discriminative power“) of potency test (slope of dose-response curve)**

⇨ **Sero-response may be antigen specific**

⇨ **There may be need for additional testing in the target species or adjustment of antigen content.**

Thank you for your attention

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