THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

edqm

European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE

CONSEIL DE L'EUROPE

CombiStats online
Training module 2

Quantal data
e.g. pass/fail results

Content

- Quantal data definition
- Data entry: aggregated/individual data
- Regression analysis: the 4PL model
- Output statistics and tables
- Spearman-Kaerber method
- Q&A

Indirect dilution assay

Response observed at various doses

R positive wells
out of N = 10 wells

5 doses (IU)
per preparation

Ref. Preparation			Test Preparation		
Dose	N	R	Dose	N	R
45	10	10	67.5	10	9
30	10	7	45	10	8
20	10	4	30	10	5
13.3	10	1	20	10	2
8.9	10	0	13.3	10	0

Fictitious data

Prep.	ED_{100}	ED_{50}
Ref.	About 45 IU	In-between 20-30 IU
Test	Greater than 67.5 IU	About 30 IU

Statistical regression models
needed to estimate EDs
and their uncertainty

Indirect dilution assay

Common structure

- X = several preparations & doses
- Y = single or repeated measurements

$$Y = f(X)$$

Regression models
in CombiStats

Quantal responses

Y = Proportion of respondents

E.g. *in-vivo* & *in-vitro* assay

Doses	(1)	(2)	(3)	(4)	(5)	(6)
1 IU	-	-	-	-	-	-
1.6 IU	-	-	-	+	-	-
2.5 IU	-	+	+	-	-	+
4.0 IU	+	+	+	-	+	+

→

Doses	(1)
1 IU	0/6
1.6 IU	1/6
2.5 IU	3/6
4.0 IU	5/6

Raw data: **pos./neg.**
Binary

Aggregated
Proportions

Ph. Eur. Chapter 5.3 Statistical analysis of results of biological assays and tests

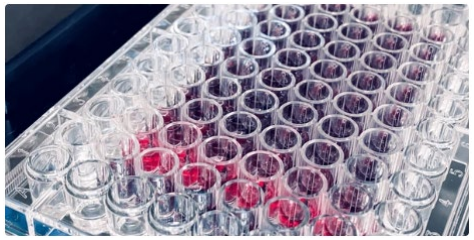
1. introduction
2. randomisation and independence of individual treatments
3. assays depending upon **quantitative responses**
   - 3.2. the parallel-line model
   - 3.3. the slope-ratio model
   - 3.4. extended sigmoid dose-response curves
4. assays depending upon **quantal responses**
   - 4.2. the probit method
   - 4.3. the logit method
   - 4.5. the median effective dose
5. examples
6. **combination of assay results**
   - 6.2. combination of independent assay results
   - 6.3. unweighted combination of assay results
7. beyond this annex
8. tables and generating procedures
9. glossary of symbols
10. literature

Quantal data

- 2 possible outcomes, e.g. positive/negative

➔ Binary, dichotomous, pass/fail results

Binomial distribution: probability of r respondents out of n tested (r/n) given a true rate π



Well	1	2	3	4	5	6
Seq.1	-	+	+	+	+	+
Seq.2	+	-	+	+	+	+
Seq.3	+	+	-	+	+	+
Seq.4	+	+	+	-	+	+
Seq.5	+	+	+	+	-	+
Seq.6	+	+	+	+	+	-

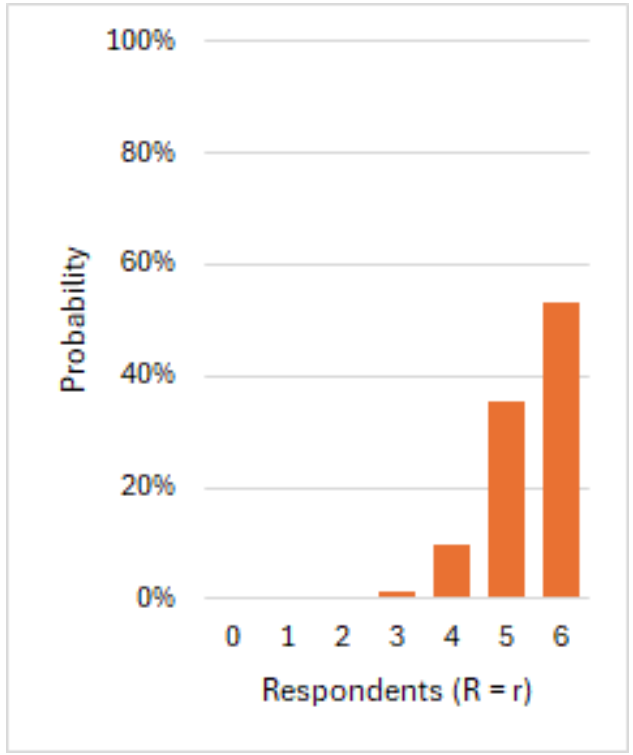
$$P(r) = C_n^r \cdot \pi^r \cdot (1 - \pi)^{n-r}$$

Probability of r = 5 positive wells out of n = 6, given π = 90%

$$P(5) = \underbrace{C_6^5}_{\text{At the bench, 6 sequences of 5 positive wells out of 6 are possible}} \cdot \underbrace{0.90^5}_{\text{Proba of 5 consecutive positive wells}} \cdot \underbrace{0.10^{6-5}}_{\text{Proba of 1 negative well}} = 0.35 \text{ (35\% chance)}$$

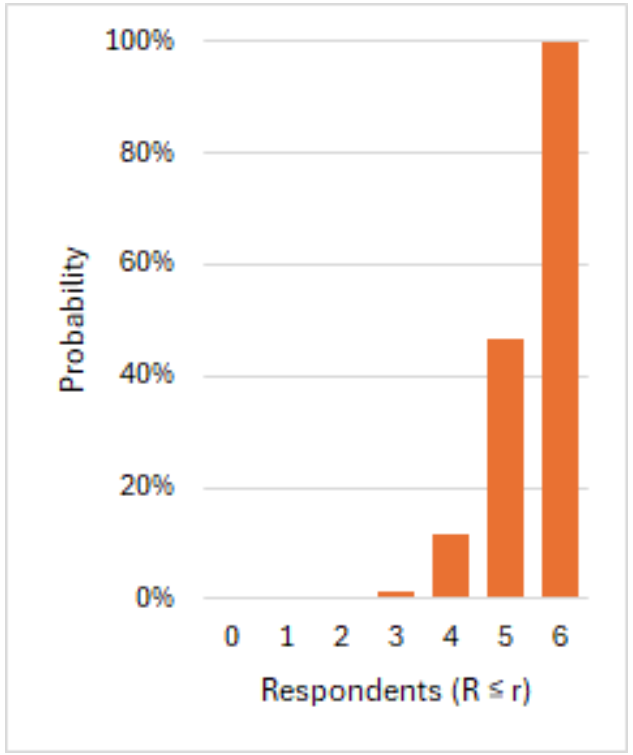
Binomial distribution

- Individual probabilities
- Cumulative probabilities



r	P(R = r)	P(R ≤ r)
0	0%	0%
1	0%	0%
2	0%	0%
3	1%	2%
4	10%	11%
5	35%	47%
6	53%	100%

π = 90%



5 positive wells out of 6:
35% chance

0 to 4 positive wells: 11% chance
More than 4 positive wells: 89% chance

Distribution parameters

- **Mean** (location)

p = r/n

"observed proportion"

- **Variance** (dispersion)

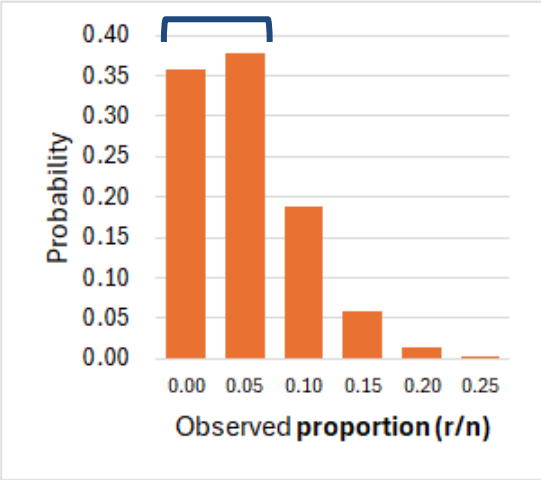
Var = p(1-p)/n

The variance depends on the mean

→ weighted regression analysis ($w_i = 1/var_i$)

Dose: 1 IU

π = 5%, var = 0.24% (n = 20)

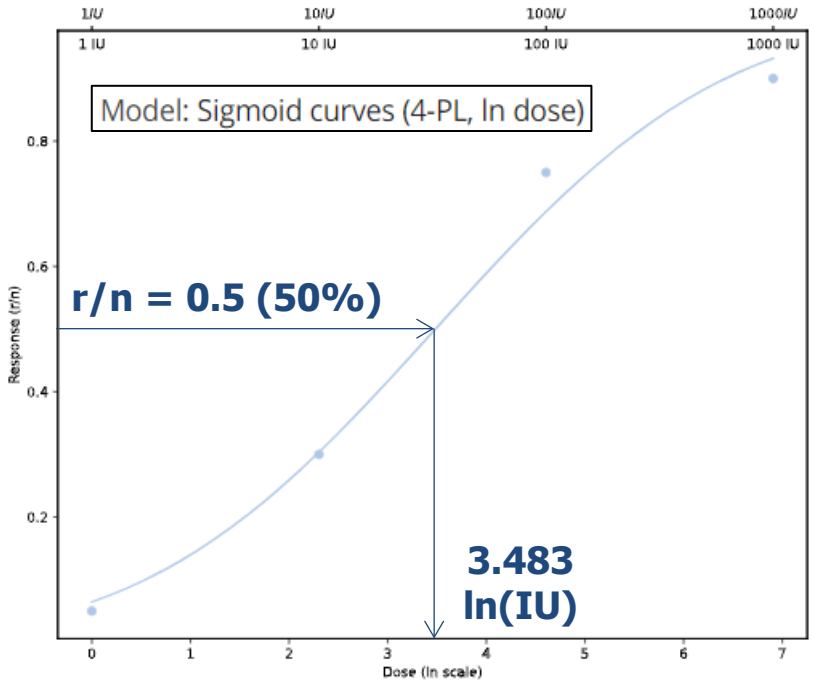


r/n = 0/20 and 1/20 are most likely

Dose-response curve

- **Using most probable rates**

Table 1	
Preparation	Standard
ID	S
Potency	Assigned
Potency value	1000 IU/vial
Dose	**Rep.1**
1 IU	1/20
10 IU	6/20
100 IU	15/20
1000 IU	18/20



Effective dose estimates

		Effective Dose (ED)		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Standard: S	IU/ED50	32.5578	(14.2583, 74.5349)	100	(43.79, 228.93)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Dose	Most probable rates (r/n)
1 IU	0/20 - 1/20
10 IU	5/20 - 6/20 - 7/20
100 IU	14/20 - 15/20 - 16/20
1000 IU	18/20 - 19/20
	36 r/n combinations

Order	ED50	Order	ED50	Order	ED50
1	32.6	13	29.7	25	29.6
2	37.9	14	36.9	26	30.4
3	28.3	15	36.4	27	22.8
4	29.2	16	38.2	28	36.7
5	36.4	17	28.3	29	47.2
6	33.0	18	33.8	30	35.3
7	34.0	19	25.4	31	26.6
8	25.4	20	26.0	32	32.5
9	42.4	21	42.1	33	41.1
10	31.6	22	31.6	34	37.6
11	32.7	23	33.2	35	28.3
12	29.1	24	40.7	36	32.9

Min 22.8 Max 47.2 Rge **24.4**

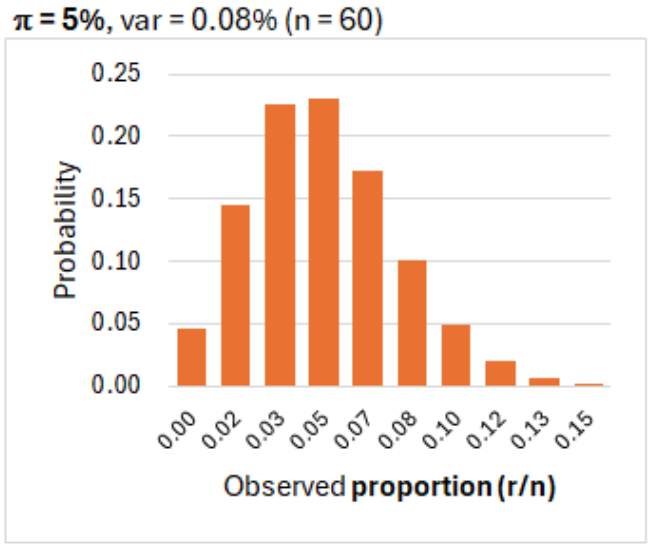
How to improve precision?

- **Increase sample size**

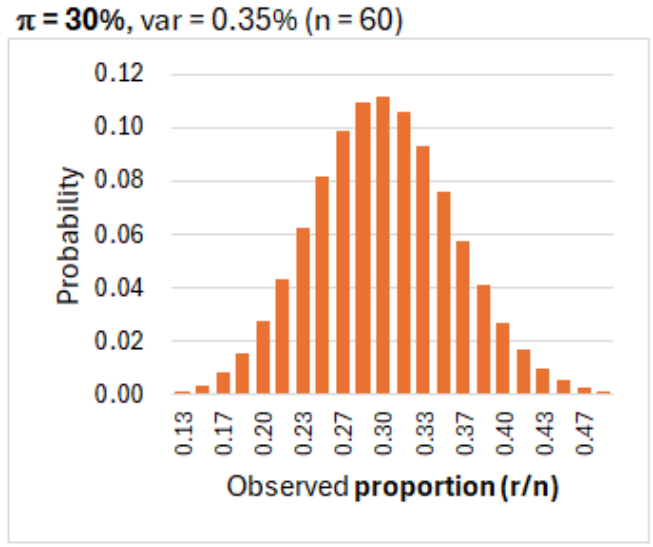
Dose	Most probable rates (r/n)
1 IU	2/60 - 3/60
10 IU	17/60 - 18/60 - 19/60
100 IU	44/60 - 45/60 - 46/60
1000 IU	54/60 - 55/60
	36 r/n combinations

Order	ED50	Order	ED50	Order	ED50
1	32.6	13	31.5	25	31.5
2	34.2	14	33.9	26	31.8
3	31.1	15	34.2	27	28.9
4	31.4	16	31.1	28	33.9
5	33.8	17	33.8	29	36.8
6	32.7	18	32.9	30	33.5
7	33.0	19	30.0	31	30.4
8	30.0	20	30.2	32	32.5
9	35.5	21	32.7	33	35.2
10	32.3	22	35.5	34	34.2
11	32.6	23	32.3	35	31.1
12	31.3	24	35.1	36	32.6
Min	28.9	Max	36.8	Rge	**7.9**

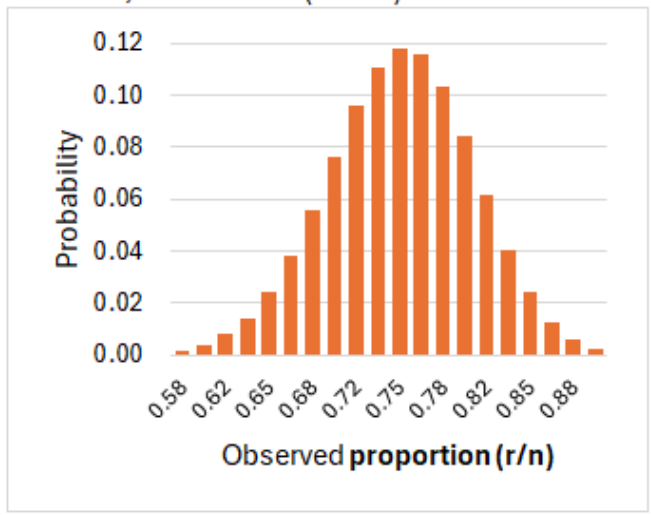
Dose 1 IU



Dose 10 IU

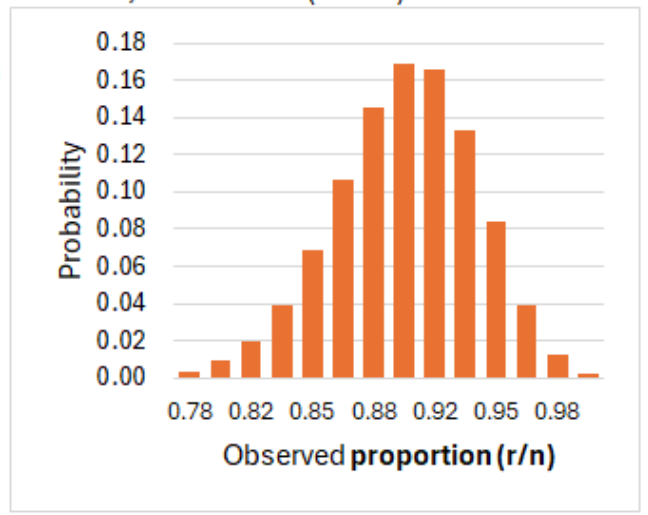


Dose 100 IU



r/n = 44/60, 45/60 and 46/60 are most likely

Dose 1000 IU



r/n = 54/60 and 55/60 are most likely

How to improve precision?

- **Steep slope**
  - Assay development > optimal conditions for routine analyses
- **Appropriate dose range**
  - Response rates between 0.05 and 0.95 (probit), 0.10 and 0.90 (logit)
  - $Dose_{Test} = Dose_{Std} - \ln(R_0)$ (R_0 = guessed value of relative potency)
- **Equal division of N subjects** between preparations/doses
- **Proper randomisation** (deviation from linearity is likely, otherwise)
- **Block design** (e.g. mice from the same litter are more likely to vary less in their individual responses than are mice from different litters ➔ litters = blocks)

Content

- Quantal data definition
- **Data entry**
- Regression analysis: the 4PL model
- Output statistics and tables
- Spearman-Kaerber method
- Q&A

Data tables

- Aggregated results (r/n)

Raw data

Table 1	
Preparation	Standard
ID	S
Potency	Assigned
Potency value	1000 IU/vial
Dose	**Rep.1**
1 IU	1/10
10 IU	3/10
100 IU	7/10
1000 IU	10/10

Table 2	
Preparation	Sample 1
ID	T
Potency	Assumed
Potency value	500 IU/vial
Dose	**Rep.1**
1/1000	0/10
1/100	3/10
1/10	6/10
1/1	9/10

- Individual results (0/1 or -/+)

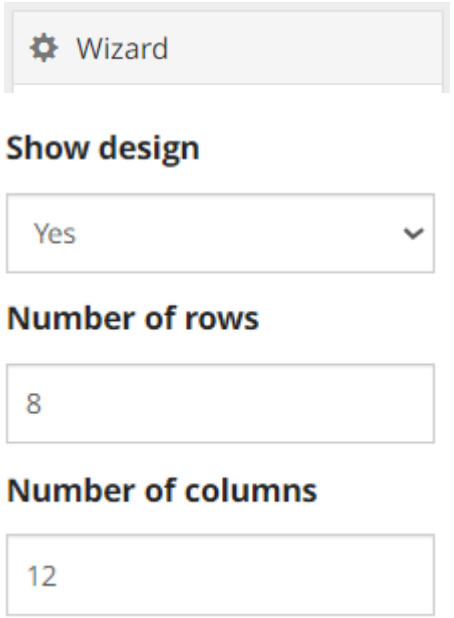
Raw data

Table 1				
Preparation	Standard			
ID	S			
Potency	Assigned			
Potency value	1000 IU/vial			
Dose	1 IU	10 IU	100 IU	1000 IU
Rep.1	0	0	1	1
Rep.2	0	0	0	1
Rep.3	0	1	1	1
Rep.4	0	0	1	1
Rep.5	1	0	0	1
Rep.6	0	0	1	1
Rep.7	0	1	0	1
Rep.8	0	0	1	1
Rep.9	0	0	1	1
Rep.10	0	1	1	1
r/n	*1/10*	*3/10*	*7/10*	*10/10*

Table 2				
Preparation	Sample 1			
ID	T			
Potency	Assumed			
Potency value	500 IU/vial			
Dose	1/1000	1/100	1/10	1/1
Rep.1	0	0	1	1
Rep.2	0	1	1	1
Rep.3	0	0	0	1
Rep.4	0	0	1	0
Rep.5	0	1	0	1
Rep.6	0	0	0	1
Rep.7	0	0	1	1
Rep.8	0	0	1	1
Rep.9	0	0	1	1
Rep.10	0	1	0	1
r/n	*0/10*	*3/10*	*6/10*	*9/10*

“Show design” option

- E.g. 96-well plate



Prep|Dose|Rep coordinates

Individual results

Assay layout

Design	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12
r1	Blank	1|1|1	1|1|2	1|1|3	1|1|4	1|1|5	1|1|6	1|1|7	1|1|8	1|1|9	1|1|10	Ctrl -
r2	Blank	1|2|1	1|2|2	1|2|3	1|2|4	1|2|5	1|2|6	1|2|7	1|2|8	1|2|9	1|2|10	Ctrl -
r3	Blank	1|3|1	1|3|2	1|3|3	1|3|4	1|3|5	1|3|6	1|3|7	1|3|8	1|3|9	1|3|10	Ctrl -
r4	Blank	1|4|1	1|4|2	1|4|3	1|4|4	1|4|5	1|4|6	1|4|7	1|4|8	1|4|9	1|4|10	Ctrl -
r5	Blank	2|1|1	2|1|2	2|1|3	2|1|4	2|1|5	2|1|6	2|1|7	2|1|8	2|1|9	2|1|10	Ctrl +
r6	Blank	2|2|1	2|2|2	2|2|3	2|2|4	2|2|5	2|2|6	2|2|7	2|2|8	2|2|9	2|2|10	Ctrl +
r7	Blank	2|3|1	2|3|2	2|3|3	2|3|4	2|3|5	2|3|6	2|3|7	2|3|8	2|3|9	2|3|10	Ctrl +
r8	Blank	2|4|1	2|4|2	2|4|3	2|4|4	2|4|5	2|4|6	2|4|7	2|4|8	2|4|9	2|4|10	Ctrl +

Observ.	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12
r1		0	0	0	0	1	0	0	0	0	0	0
r2		0	0	1	0	0	0	1	0	0	1	0
r3		1	0	1	1	0	1	0	1	1	1	0
r4		1	1	1	1	1	1	1	1	1	1	0
r5		0	0	0	0	0	0	0	0	0	0	1
r6		0	1	0	0	1	0	0	0	0	1	1
r7		1	1	0	1	0	0	1	1	1	0	1
r8		1	1	1	0	1	1	1	1	1	1	1

Content

- Quantal data definition
- Data entry
- **Regression analysis**
- Output statistics and tables
- Spearman-Kaerber method
- Q&A

Indirect dilution assay

- Rates observed at fixed doses (dilutions)

Resp.	Dose scale	X-axis
Quantal	Fold-ratio	Ln(Dose)

Standard: ED_{50} between 10 and 100 IU

Sample: ED_{50} between dil. 1/10 and 1/100

Table 1

Preparation	Standard
ID	S
Potency	Assigned
Potency value	1000 IU/vial

Dose	Rep.1
1 IU	1/10
10 IU	3/10
100 IU	7/10
1000 IU	10/10

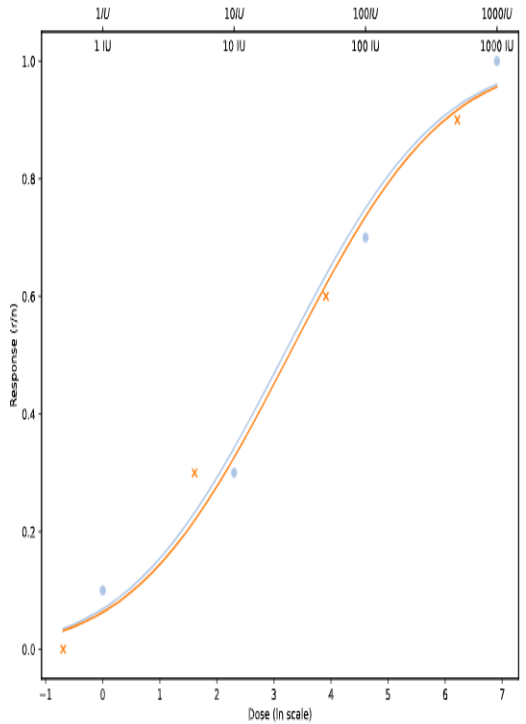
Table 2

Preparation	Sample 1
ID	T
Potency	Assumed
Potency value	500 IU/vial

Dose	Rep.1
1/1000	0/10
1/100	3/10
1/10	6/10
1/1	9/10

Regression model ➔ to estimate EDs & their precision

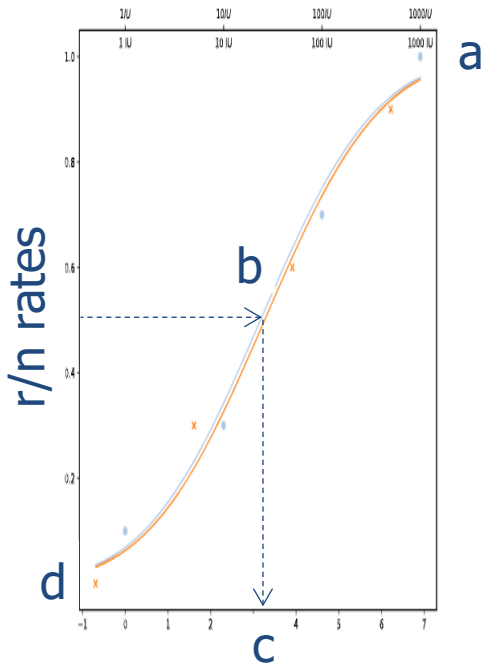
Shape	Model
Sigmoid curve	4-PL



Regression approach

CombiStats applies a linearising transformation to the 4-PL equation, fits linear regression lines and back transform relevant/useful statistics

Model plot



4-parameter logistic model

$$y = d + \frac{a - d}{1 + (x/c)^b} + \varepsilon$$

Lower asymptote: d = 0

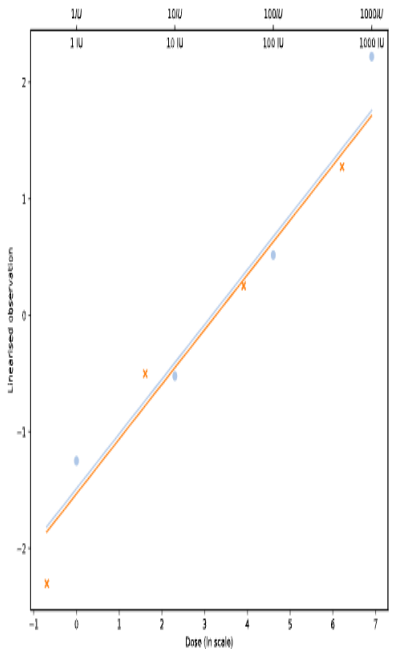
Upper asymptote: a = 1

Inflexion point (ED_{50}): c

Slope factor (Hill's slope): b

x: ln(dose), y:r/n, ε: error term

Model plot (linearised)



Linear regression lines

The calculated slope corresponds to the Hill's slope of the 4-PL model

	Common Slope
Estimated value	0.469493
Lower conf. Limit	0.303044
Upper conf. Limit	0.635941

95% confidence level

Effective doses are reported in a separate table

		Effective Dose (ED)	
Preparation	Units	Estimate	(LCL, UCL)
Standard: S	IU/ED50	23.7484	(7.70907, 71.7567)
Sample 1: T	IU/ED50	26.1042	(8.67929, 81.2479)

Processed data

Table 1

Dose	Rep.1
1 IU	1/10
10 IU	3/10
100 IU	7/10
1000 IU	10/10

			Rates (r/n)		Linearised (e.g. probit)		Residuals		
			observed	calculated	observed	calculated	working	standardized	studentized
Table	Flag	Dose	NLinObs	NLinPred	LinObs	LinPred	WorkRes	StandRes	StudRes
1	1	0.000	0.10	0.07	-1.25	-1.49	0.24	0.47	0.48
1	1	2.303	0.30	0.34	-0.52	-0.41	-0.12	-0.37	-0.37
1	1	4.605	0.70	0.75	0.52	0.67	-0.16	-0.47	-0.47
1	1	6.908	1.00	0.96	2.22	1.76	0.46	1.08	0.99
2	1	-0.693	0.00	0.03	-2.30	-1.86	-0.45	-0.94	-0.88
2	1	1.609	0.30	0.22	-0.50	-0.78	0.27	0.77	0.79
2	1	3.912	0.60	0.62	0.25	0.31	-0.05	-0.17	-0.17
2	1	6.215	0.90	0.92	1.27	1.39	-0.11	-0.25	-0.25
			Model plot (sigmoid)		Model plot (linear reg.)		Residual plot		

Flag = 0 if data is excluded

Dose => ln(dose)

Linearising transformation: added value

➔ Parallelism between regression lines can be assessed

Two products are similar if they act as dilution of the same substance, i.e. implies parallelism on log(Dose)

Lack of parallelism may suggest changes in:

- Performance of the method, and/or
- Manufacturing process (product has changed!)

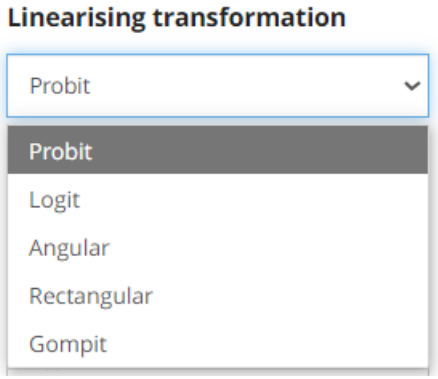
Assessment (see next section)

Option 1: significance test

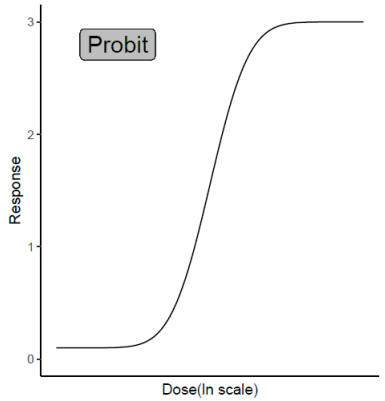
Option 2: equivalence test

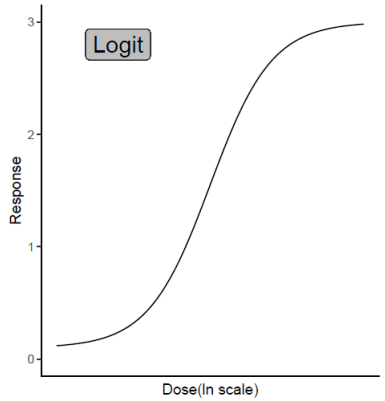
Any other proposal?

Linearising transformation: options



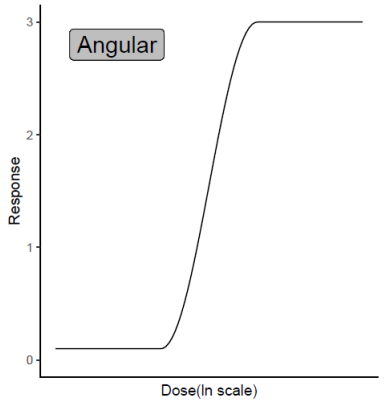
Probit and Logit are most frequently used

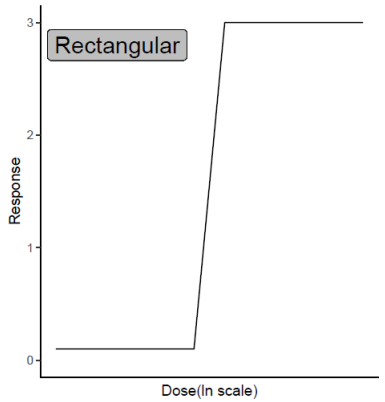


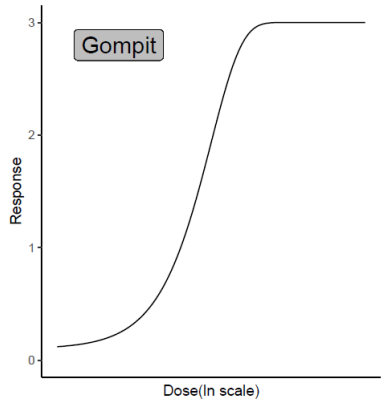


Probit: symmetrical curves with short tails (asymptotes reached rapidly)

Logit: symmetrical curves with long tails (asymptotes reached slowly)







Angular and rectangular: symmetrical curves with very short tails (asymptotes reached very rapidly)

Gompit: asymmetrical curves with a shorter lower tail and longer upper tail

Content

- Quantal data definition
- Data entry
- Regression analysis
- **Output statistics and tables**
- Spearman-Kaerber method
- Q&A

Common slope model

Used to calculate output results (e.g. EDs, potencies)

➔**Validity criterion**: no difference between individual slopes

Regression parameters

Global model: convergence reached

R^2 Standard: convergence reached

	Common Slope
Estimated value	0.798385
Lower conf. Limit	0.477232
Upper conf. Limit	1.11954

95% confidence level

Option 1: equality of slopes (any **statistically significant** difference?)

Source of variation	Degrees of freedom	Probability	Level of significance
Preparations	1	0.874636	
Regression	1	0.000001	***
Non-parallelism	1	0.889121	

p-value

0.89 (>0.05)

No significant difference between individual slopes

Option 2: equivalence of slopes (any difference of **practical relevance**?)

Equivalence of slopes

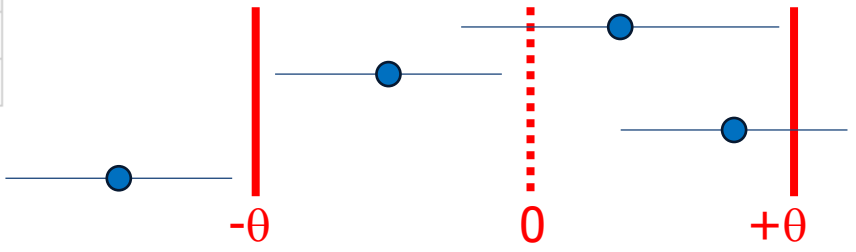
Preparation	Slope	Difference with Standard	Ratio with Standard
Standard: S	0.821108 (0.368129, 1.27409)	0.000000	1.00000
Sample 1: T	0.775419 (0.320032, 1.23081)	-0.0456893 (-0.584736, 0.493358)	0.944357 (0.436873, 1.96713)

Slopes: confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Differences and ratios of slopes: confidence limits (in brackets) calculated for a 90% confidence level.

Equivalence margins (±Θ) to be set prior to do the test

Assessment using differences or ratios of slopes (not both)



Equivalent

Equivalent

Inconclusive

Non-equivalent

Other validity criteria (cf. SOP)

Assay

Source of variation	Degrees of freedom	Probability	Level of significance
Preparations	1	0.874636	
Regression	1	0.000001	***
Non-parallelism	1	0.889121	
Non-linearity	4	0.781511	
Non-linearity Table 1	2	0.665302	
Non-linearity Table 2	2	0.626394	
Treatments	7	0.000609	***

weighted	
R^2 All	0.930685
R^2 Standard	0.932548

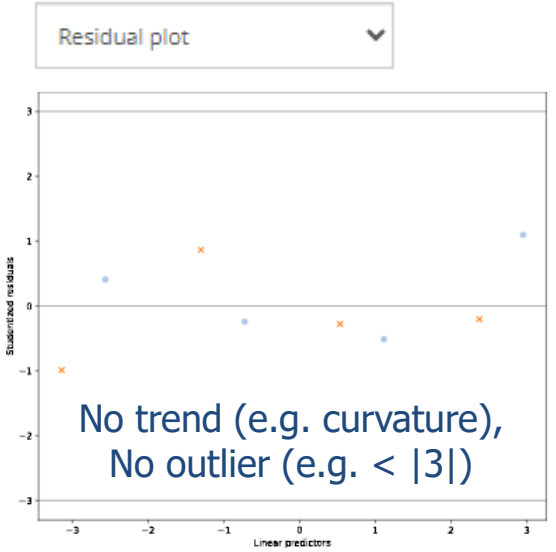
Coefficient of determination > X%

Significant common slope ($p \leq 0.05$)

Non-significant deviation from parallelism ($p > 0.05$)

Non-significant deviation from linearity ($p > 0.05$)

Pos/neg control, control charts, …



Potency results

Precise enough?

On target?

Potency estimates

		Potency		Relative To Estimate (%)		Relative To Assumed/Assigned (%)	
Preparation	Units	Estimate	(LCL, UCL)	Rel. To Est.	(LCL, UCL)	Rel. To Ass.	(LCL, UCL)
Sample 1: T	IU/vial	485.178	(89.5996, 2505.70)	100	(18.47, 516.45)	97.04	(17.92, 501.14)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Preparations

		Information	Potency	
Table	Preparation	ID	Potency	Value
1	Standard	S	Assigned	1000 IU/vial
2	Sample 1	T	Assumed	500 IU/vial

Pharm. Eur.

R^2. *The coefficient of determination calculated for the reference standard dose-response curve (R2) is not less than XX.*

Precision. *Unless otherwise stated in the monograph, the confidence limits (P = 0.95) are not less than XX per cent and not more than XX per cent of the estimated potency.*

Recovery. *The mean recovery must not be lower than XX per cent or above XX per cent.*

The amount is not less than XX per cent and not greater than XX per cent of the intended content.

Effective doses

Advanced options

PREDICTED VALUES

Effective dose

50 %

Reported as

Container / Effective Dose

Y values

0.1;0.5;0.9

You can specify up to 6 response values, separated by semicolons.

Reported as "Container/ED": ED_{50} = 23.75 IU

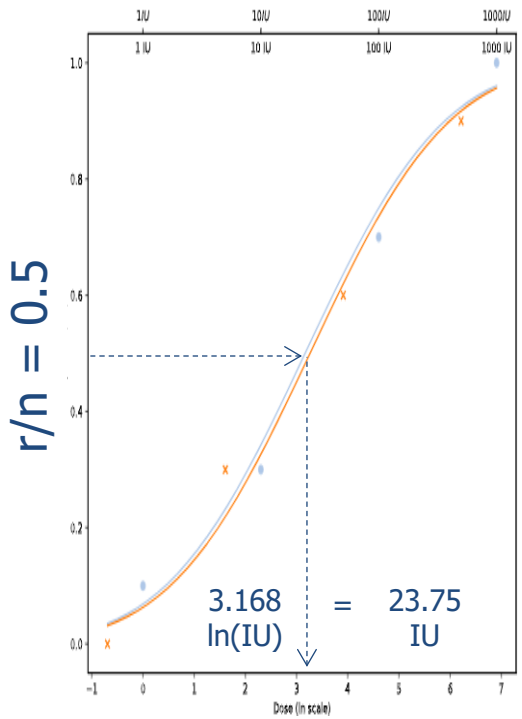
		Effective Dose (ED)		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Standard: S	IU/ED50	23.7484	(7.70907, 71.7567)	100	(32.46, 302.15)
Sample 1: T	IU/ED50	26.1042	(8.67929, 81.2479)	100	(33.25, 311.24)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Reported as "ED/Container": 1 vial is equivalent to 42 ED_{50}

		Effective Dose (ED)		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Standard: S	ED50/vial	42.1080	(13.9360, 129.717)	100	(33.10, 308.06)
Sample 1: T	ED50/vial	19.1540	(6.15401, 57.6084)	100	(32.13, 300.76)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).



Inverse predictions

		y-value(s)					
		0.1		0.5		0.9	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Estimate**	**(LCL, UCL)**	**Estimate**	**(LCL, UCL)**
Standard: S	IU	1.54939	(0.211241, 5.08503)	23.7484	(7.70907, 71.7567)	364.006	(113.050, 2519.91)
Sample 1: T	IU	1.70309	(0.251095, 5.45337)	26.1042	(8.67929, 81.2479)	400.114	(120.812, 3005.95)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

ED_{10} (r/n = 10%): 1.55 IU

ED_{50} (r/n = 50%): 23.75 IU

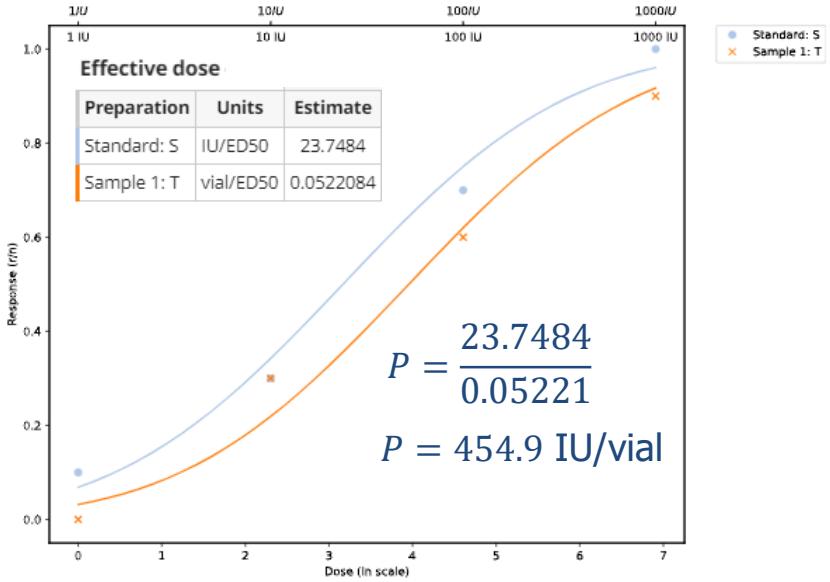
ED_{90} (r/n = 90%): 364 IU

Potency estimates

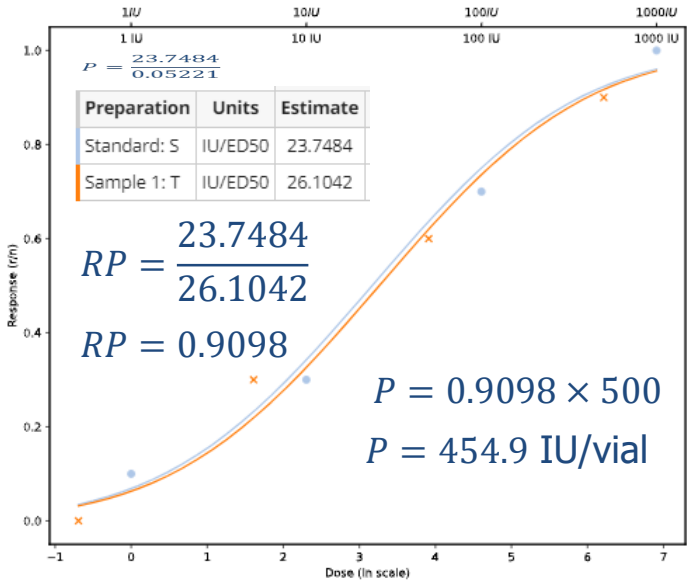
Table 1	
Preparation	Standard
ID	S
Potency	Assigned
Potency value	1000 IU/vial
Dose	**Rep.1**
1 IU	1/10
10 IU	3/10
100 IU	7/10
1000 IU	10/10

Table 2	
Preparation	Sample 1
ID	T
Potency	Assumed
Potency value	? IU/vial
Dose	**Rep.1**
1/1000	0/10
1/100	3/10
1/10	6/10
1/1	9/10

		Information	Potency	
Table	**Preparation**	**ID**	**Potency**	**Value**
1	Standard	S	Assigned	1000 IU/vial
2	Sample 1	T	Assumed	? IU/vial



		Information	Potency	
Table	**Preparation**	**ID**	**Potency**	**Value**
1	Standard	S	Assigned	1000 IU/vial
2	Sample 1	T	Assumed	500 IU/vial



Potency estimates

Precision

Recovery

		Potency		Relative To Estimate (%)		Relative To Assumed/Assigned (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**	**Rel. To Ass.**	**(LCL, UCL)**
Sample 1: T	IU/vial	454.878	(91.1866, 2150.69)	100	(20.05, 472.81)	90.98	(18.24, 430.14)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Multiple-dose standard only

Table 1	
Preparation	Standard
ID	S
Potency	Assigned
Potency value	100 u/d
Dose	**Rep.1**
1/1	11/12
1/10	9/12
1/100	5/12
1/1000	2/12
1/10000	0/12

Table 2	
Preparation	Sample 1
ID	T
Potency	Assumed
Potency value	? u/d
Dose	**Rep.1**
1/100	5/11

Table 3	
Preparation	Sample 2
ID	U
Potency	Assumed
Potency value	? u/d
Dose	**Rep.1**
1/100	6/12

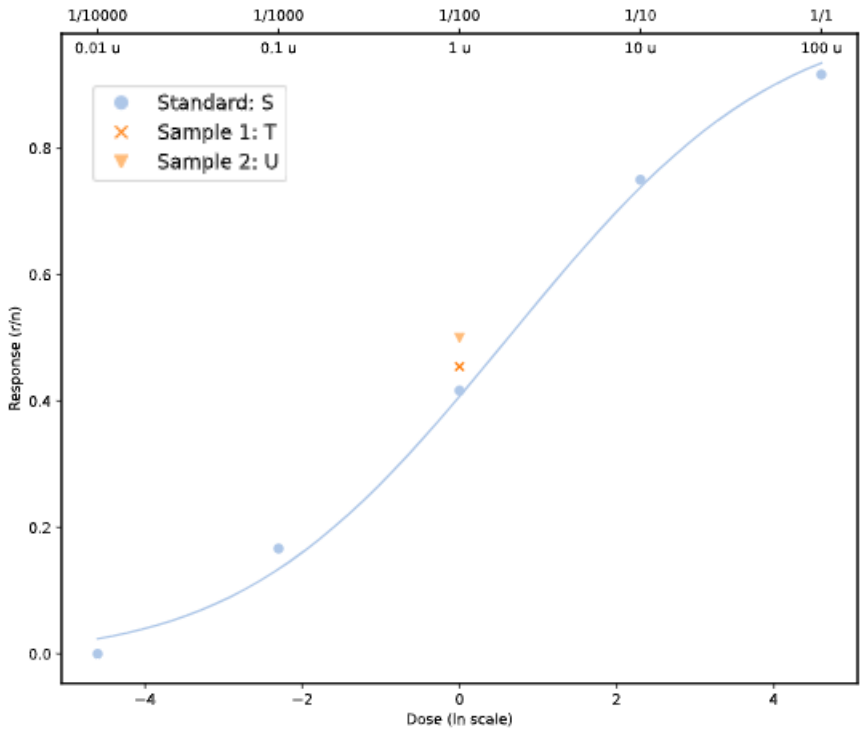
The regression outputs are those of the standard…

	Slope		weighted
Estimated value	0.378897	R^2 Standard	0.979272
Lower conf. Limit	0.222646		
Upper conf. Limit	0.535148		

Anova table

Normal

Source of variation	Degrees of freedom	Probability	Level of significance
Regression	1	0.000002	***
Non-linearity	3	0.923667	
Treatments	4	0.000123	***
Theoretical variance			
Total	4		



Single dose estimates

		Single-dose		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Sample 1: T	u/d	137.280	(41.3280, 427.834)	100	(30.10, 311.65)
Sample 2: U	u/d	185.562	(59.4401, 614.946)	100	(32.03, 331.40)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Content

- Quantal data definition
- Data entry
- Regression analysis
- Output statistics and tables
- **Spearman-Kaerber method**
- Q&A

Empirical method (no regression analysis)

Used when no slope can be estimated

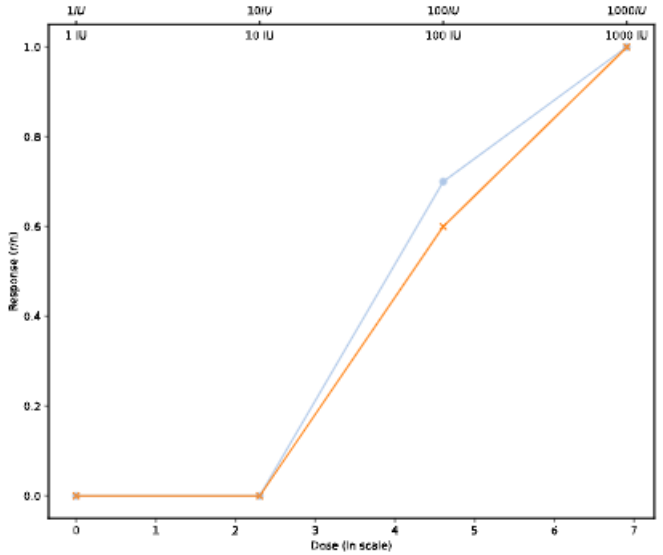
Example: (quasi)separation (not enough intermediate r/n rates)

Table 1

Preparation	Standard
ID	S
Potency	Assigned
Potency value	1000 IU/vial
Dose	**Rep.1**
1 IU	0/10
10 IU	0/10
100 IU	7/10
1000 IU	10/10

Table 2

Preparation	Sample 1
ID	T
Potency	Assumed
Potency value	? IU/vial
Dose	**Rep.1**
1/1000	0/10
1/100	0/10
1/10	6/10
1/1	10/10



Analysis options

Assay: Multiple-dose

Response: Quantal (e.g. pass/fail)

~~Model: Sigmoid curves (4-PL, ln-dose)~~

~~Design: Completely randomised~~

~~Linearising transformation: Probit~~

Most analysis options do not apply

Note: Spearman-Kaerber method used (no inverse prediction)

Potency estimates

		Potency		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Sample 1: T	IU/vial	794.328	(304.950, 2069.05)	100	(38.39, 260.48)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Effective dose estimates

		Effective Dose (ED)		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Standard: S	IU/ED50	63.0957	(32.8076, 121.346)	100	(52.00, 192.32)
Sample 1: T	vial/ED50	0.0794328	(0.0394788, 0.159822)	100	(49.70, 201.20)

Confidence limits (in brackets) calculated for a 95% confidence level (advanced options).

Example: SNT rabies mouse sera

Table 1	
Preparation	Standard
ID	
Potency	Assigned
Potency value	2 I.U./Dosis
Dose	**Rep.1**
1/8	0/6
1/16	0/6
1/32	0/6
1/64	5/6
1/128	6/6
1/256	6/6
1/512	6/6
1/1024	6/6

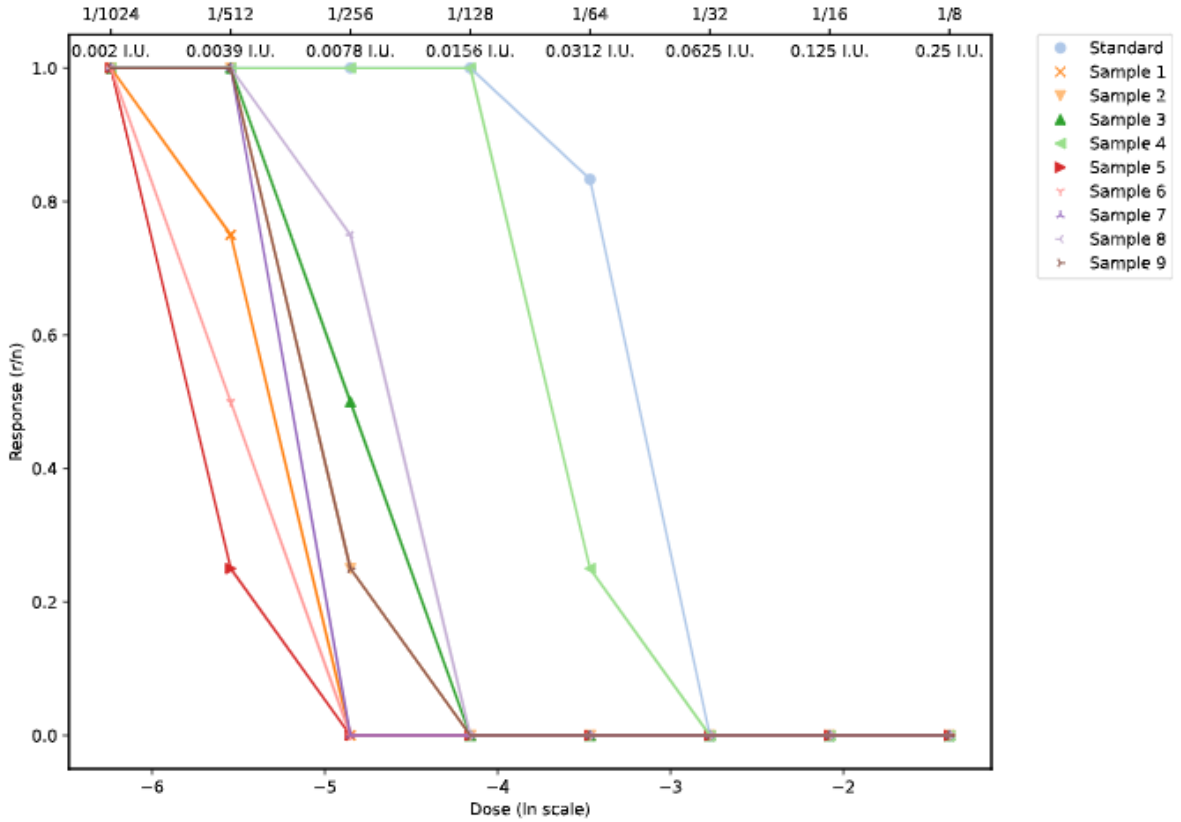
Table 2	
Preparation	Sample 1
ID	
Potency	Assumed
Potency value	? I.U./Dosis
Dose	**Rep.1**
1/8	0/4
1/16	0/4
1/32	0/4
1/64	0/4
1/128	0/4
1/256	0/4
1/512	3/4
1/1024	4/4

Table 3	
Preparation	Sample 2
ID	
Potency	Assumed
Potency value	? I.U./Dosis
Dose	**Rep.1**
1/8	0/4
1/16	0/4
1/32	0/4
1/64	0/4
1/128	0/4
1/256	1/4
1/512	4/4
1/1024	4/4

Table 4	
Preparation	Sample 3
ID	
Potency	Assumed
Potency value	? I.U./Dosis
Dose	**Rep.1**
1/8	0/4
1/16	0/4
1/32	0/4
1/64	0/4
1/128	0/4
1/256	2/4
1/512	4/4
1/1024	4/4

Potency estimates Note: Spearman-Kaerber method used

		Potency		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Sample 1	I.U./Dosis	16.9514	(11.8326, 24.2847)	100	(69.80, 143.26)
Sample 2	I.U./Dosis	11.9865	(8.36688, 17.1719)	100	(69.80, 143.26)
Sample 3	I.U./Dosis	10.0794	(6.77272, 15.0004)	100	(67.19, 148.82)
Sample 4	I.U./Dosis	2.99661	(2.09172, 4.29297)	100	(69.80, 143.26)
Sample 5	I.U./Dosis	23.9729	(16.7338, 34.3438)	100	(69.80, 143.26)
Sample 6	I.U./Dosis	20.1587	(13.5454, 30.0008)	100	(67.19, 148.82)
Sample 7	I.U./Dosis	14.2544	(11.5926, 17.5273)	100	(81.33, 122.96)
Sample 8	I.U./Dosis	8.47570	(5.91628, 12.1424)	100	(69.80, 143.26)
Sample 9	I.U./Dosis	11.9865	(8.36688, 17.1719)	100	(69.80, 143.26)



"If the transition occurs only in very few steps, the Spearman Kaerber method is applied automatically"

Requirements

- **Doses should be equidistant.** If not, CombiStats uses the smallest distance between adjacent doses giving unequal responses
- **Doses should cover 0% and 100% rates.** If not, the previous or next dose, although not tested, is assumed to be 0% or 100%
- **Rates should be monotonic** (e.g. increasing). See SOP for guidance, otherwise

Requirements: met or not met?

Table 1	
Preparation	Standard
ID	S
Potency	Assigned
Potency value	1000 IU/vial
Dose	**Rep.1**
1 IU	0/10
10 IU	0/10
100 IU	7/10
1000 IU	9/10

Table 2	
Preparation	Sample 1
ID	T
Potency	Assumed
Potency value	? IU/vial
Dose	**Rep.1**
1/1000	1/10
1/100	0/10
1/10	6/10
1/1	10/10

Table 3	
Preparation	Sample 2
ID	U
Potency	Assigned
Potency value	1000 IU/vial
Dose	**Rep.1**
1 IU	1/10
10 IU	2/10
500 IU	7/10
1000 IU	9/10

Table 4	
Preparation	Sample 3
ID	V
Potency	Assumed
Potency value	? IU/vial
Dose	**Rep.1**
1/1000	0/10
1/100	1/10
1/10	5/10
1/1	10/10

Useful links

- Helpdesk

  https://helpdesk.edqm.eu/servicedesk/customer/user/login?destination=portals

- Institutional website

  https://www.edqm.eu/en/lp-combistats

- FAQs, privacy, security notices

  https://combistats.edqm.eu/help/

- User guide (sign in first)

  https://combistats.edqm.eu/user-manuals/combistats_user_guide.pdf/

Thank you for your attention

Stay connected with the EDQM

EDQM Newsletter: **https://go.edqm.eu/Newsletter**
LinkedIn: **https://www.linkedin.com/company/edqm/**
X: **@edqm_news**
Facebook: **@EDQMCouncilofEurope**