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edqm
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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

Indirect dilution assay

Response observed at various doses

R positive wells
out of N = 10 wells

5 doses (IU) per
preparation

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

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

Statistical regression models
needed to estimate EDs
and their uncertainty

Indirect dilution assay

Common structure

★X = several preparations & doses

★Y = single or repeated measurements

Regression models
in CombiStats

$Y = f(X)$

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

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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	+	+	+	+	+	-

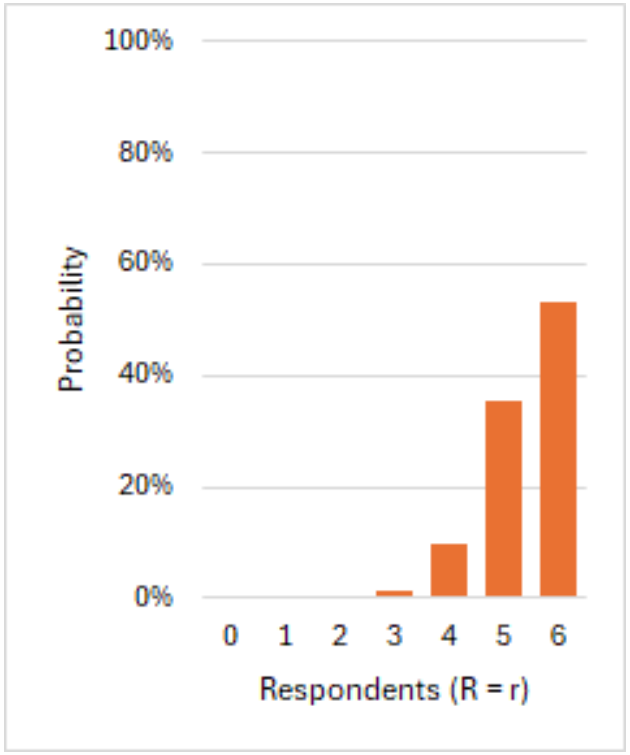
$$P(r) = C_n^r \cdot \pi^r \cdot (1 - \pi)^{n-r}$$

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

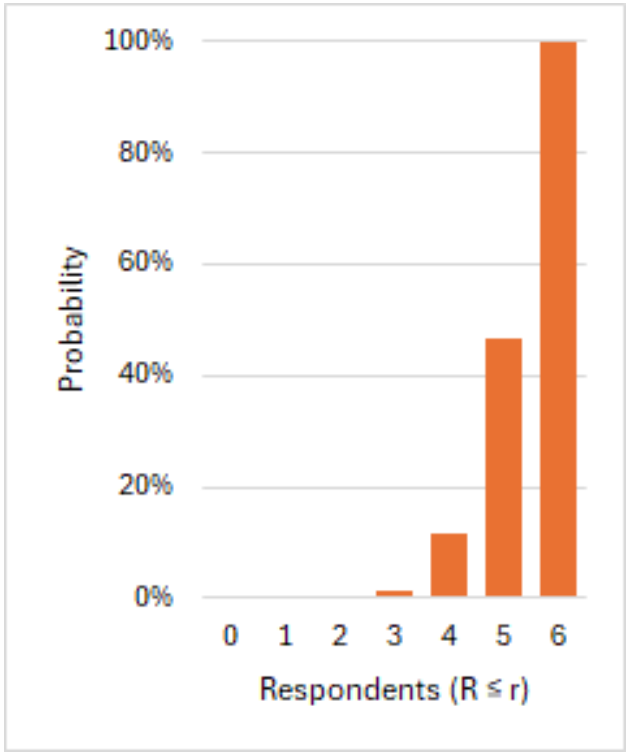


r	P(R = r)	P(R ≤ r)
0	0%	0%
1	0%	0%
2	0%	0%
3	1%	2%
4	10%	11%
5	35%	47%
6	53%	100%

π = 90%

5 positive wells out of 6: 35% chance

★ Cumulative probabilities



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)

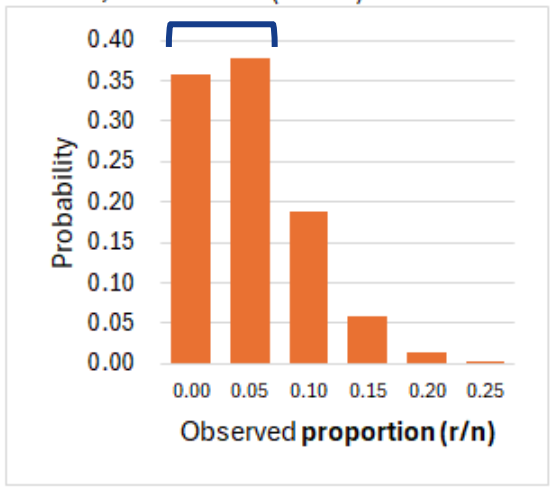
Var = p(1-p)/n

The variance depends on the mean

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

Dose: 1 IU

π = 5%, var = 0.24% (n = 20)

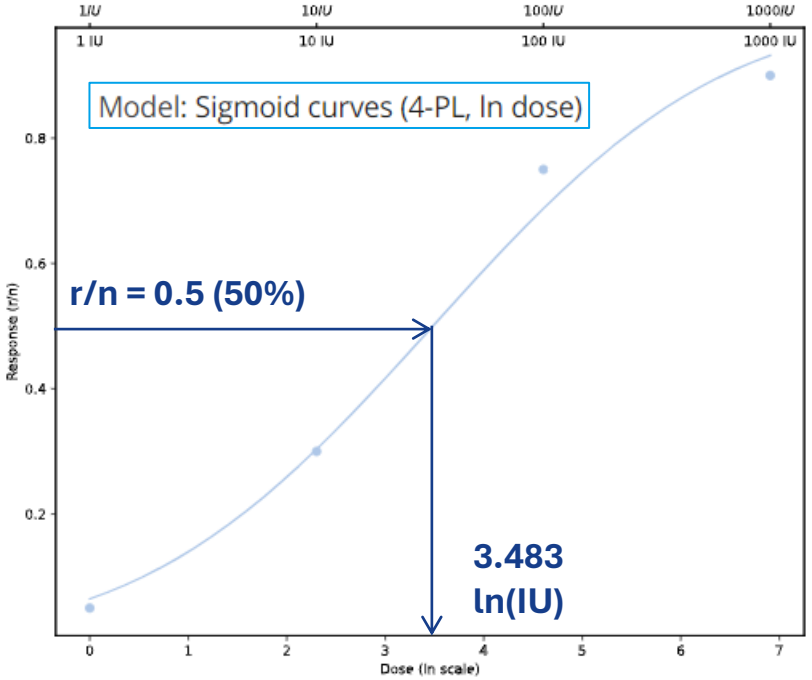


r/n = 0/20 and 1/20 are most likely

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**

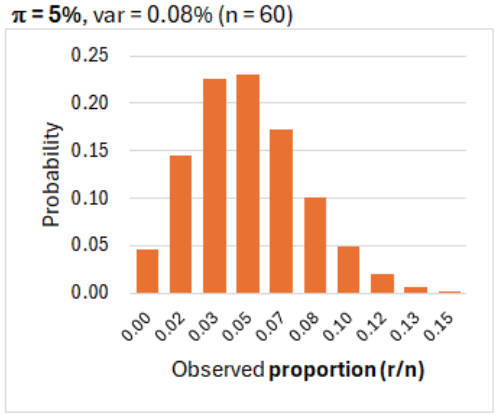
How to improve precision?

★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

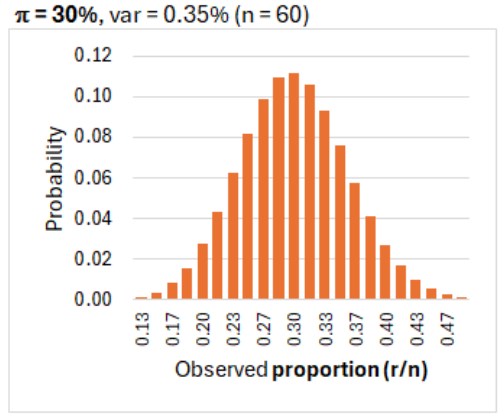
Order	ED50	Order	ED50	Order	ED50
1	32.6	13	31.5	25	31.5
2	34.2	14	33.9	26	31.8
3	31.1	15	34.2	27	28.9
4	31.4	16	31.1	28	33.9
5	33.8	17	33.8	29	36.8
6	32.7	18	32.9	30	33.5
7	33.0	19	30.0	31	30.4
8	30.0	20	30.2	32	32.5
9	35.5	21	32.7	33	35.2
10	32.3	22	35.5	34	34.2
11	32.6	23	32.3	35	31.1
12	31.3	24	35.1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

Dose 1 IU



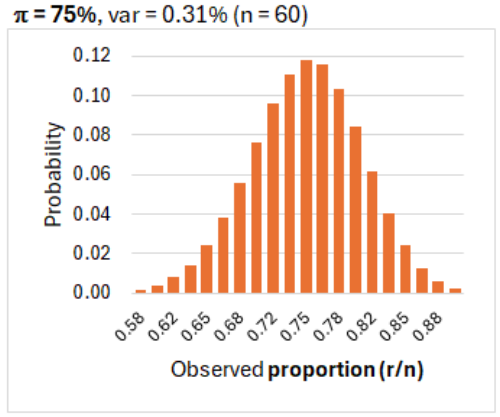
r/n = 2/60 and 3/60 are most likely

Dose 10 IU



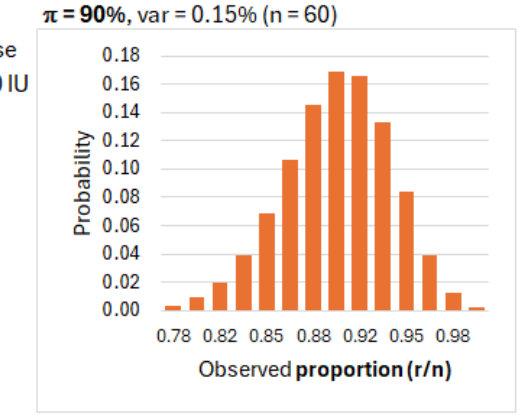
r/n = 17/60, 18/60 and 19/60 are most likely

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)

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Content

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

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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*

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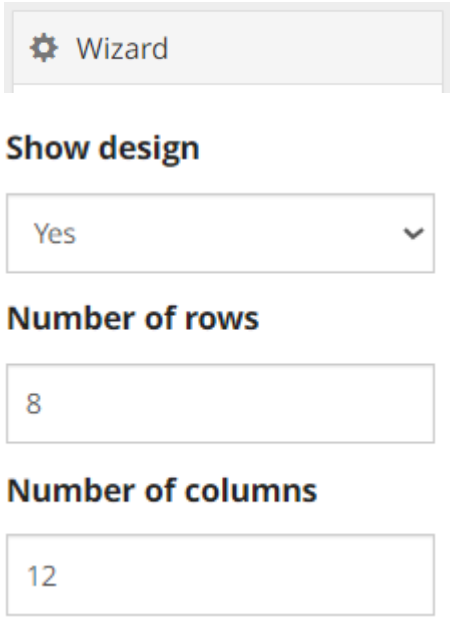
“Show design” option

★E.g. 96-well plate



Prep|Dose|Rep coordinates

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 +

Individual results

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

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Indirect dilution assay

★Rates observed at fixed doses (dilutions)

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

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

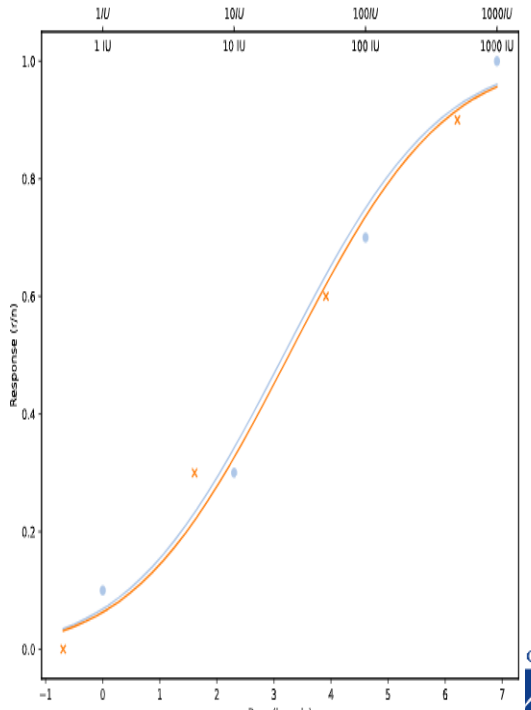
Standard: ED_{50} between 10 and 100 IU

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

Regression model ➔ to

estimate EDs & their precision

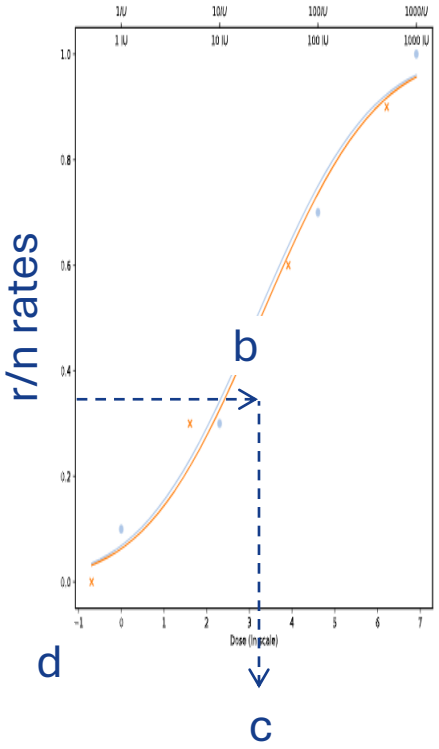
Shape	Model
Sigmoid curve	4-PL



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

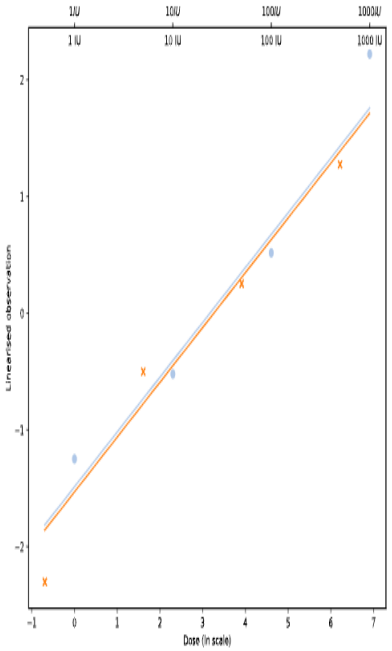
Upper asymptote: a = 1

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

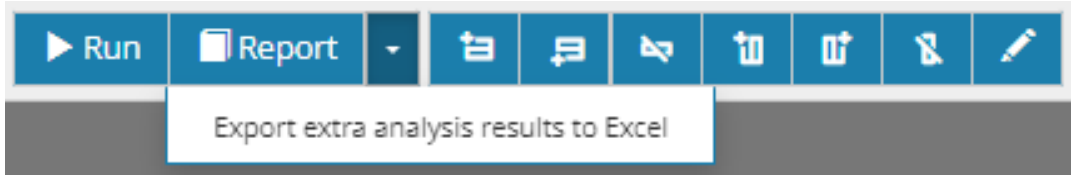
95% confidence level

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!)

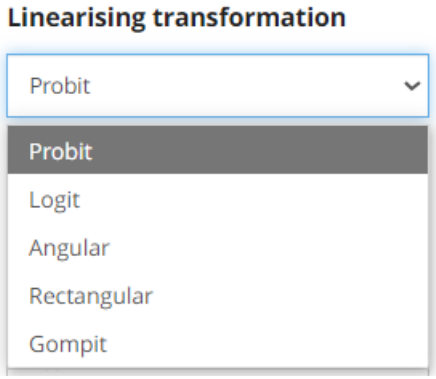
Assessment (see next section)

Option 1: significance test

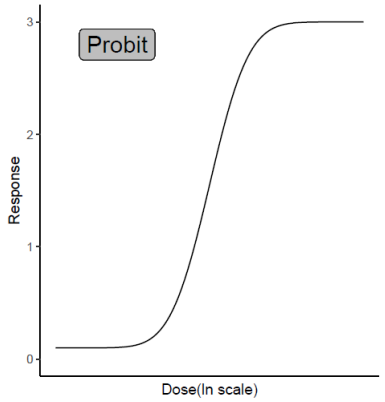
Option 2: equivalence test

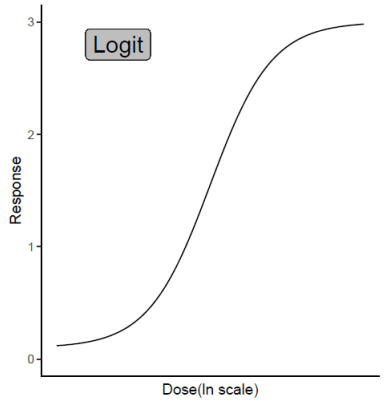
Any other proposal?

Linearising transformation: options



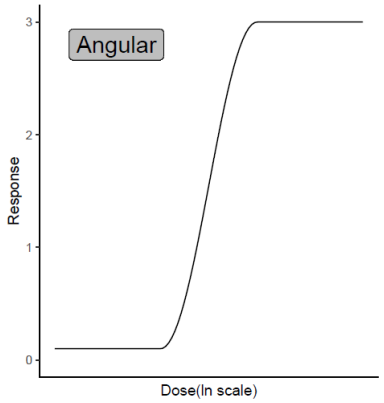
Probit and Logit are most frequently used

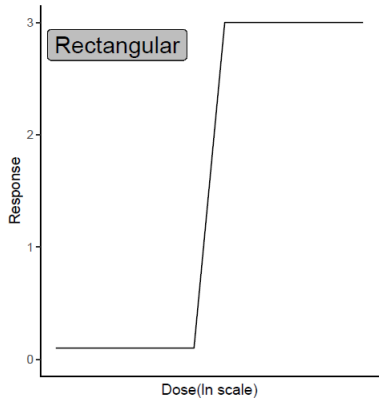


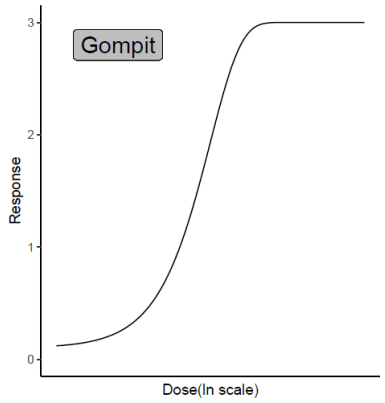


Probit: symmetrical curves with short tails (asymptotes reached rapidly)

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

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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

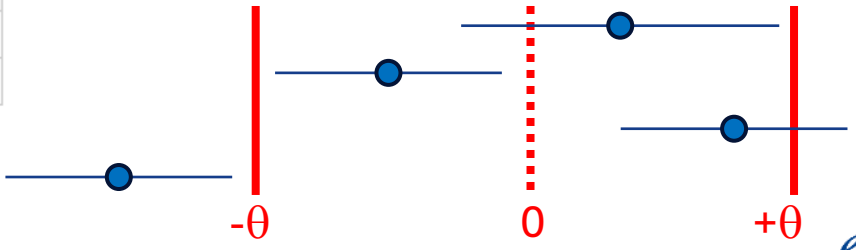
Preparation	Slope	Difference with Standard	Ratio with Standard
Standard: S	0.821108 (0.368129, 1.27409)	0.000000	1.00000
Sample 1: T	0.775419 (0.320032, 1.23081)	-0.0456893 (-0.584736, 0.493358)	0.944357 (0.436873, 1.96713)

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

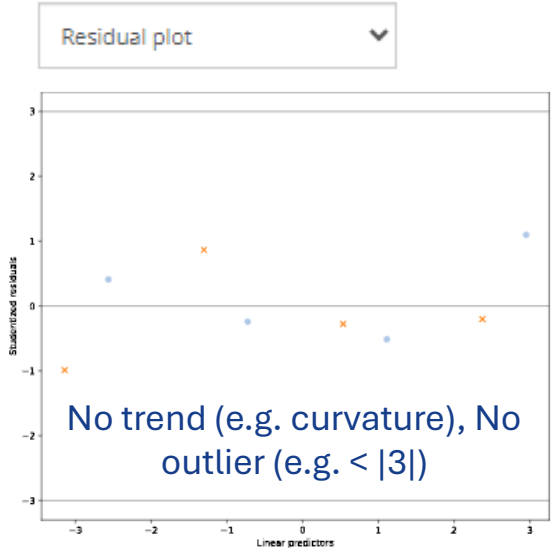
Coefficient of determination > X%

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

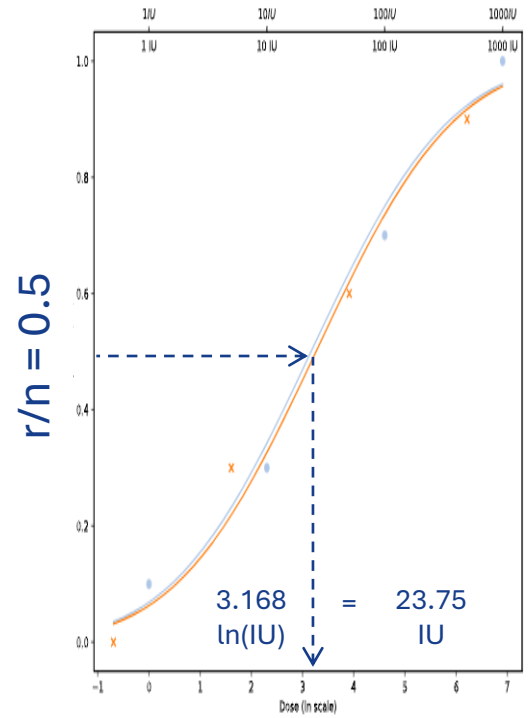
		Effective Dose (ED)		Relative To Estimate (%)	
Preparation	**Units**	**Estimate**	**(LCL, UCL)**	**Rel. To Est.**	**(LCL, UCL)**
Standard: S	IU/ED50	23.7484	(7.70907, 71.7567)	100	(32.46, 302.15)
Sample 1: T	IU/ED50	26.1042	(8.67929, 81.2479)	100	(33.25, 311.24)

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

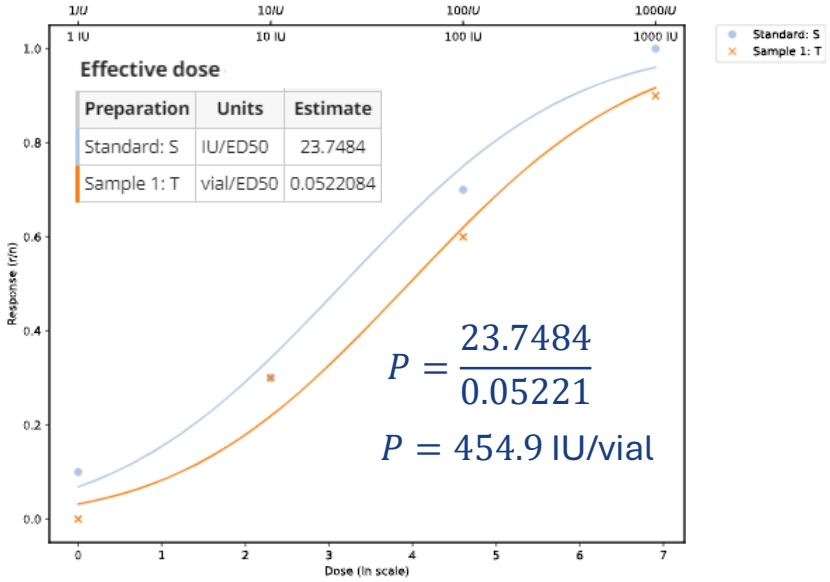
ED_{90} (r/n = 90%): 364 IU

Potency estimates

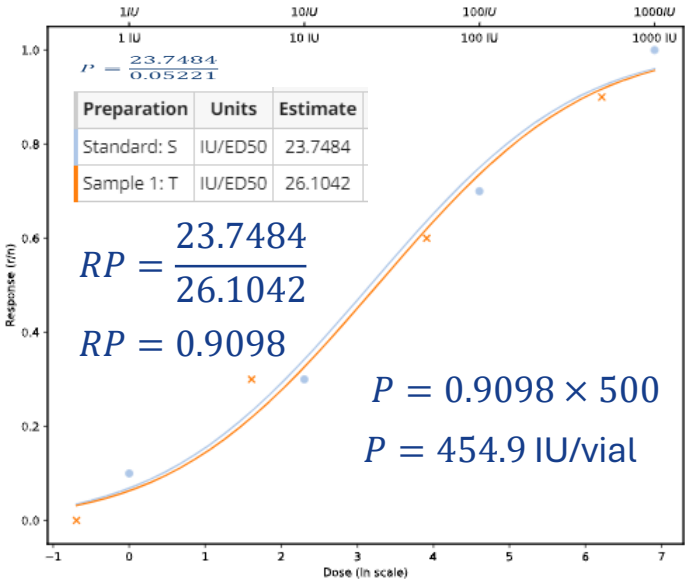
Table 1	
Preparation	Standard
ID	S
Potency	Assigned
Potency value	1000 IU/vial
Dose	**Rep.1**
1 IU	1/10
10 IU	3/10
100 IU	7/10
1000 IU	10/10

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/via	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

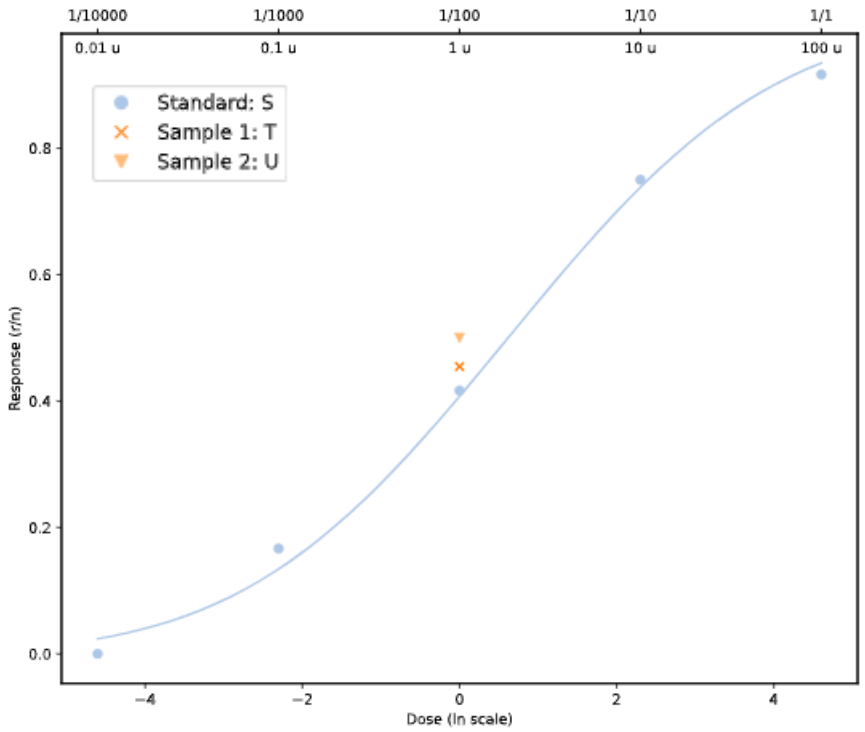
The regression outputs are those of the standard...

	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**

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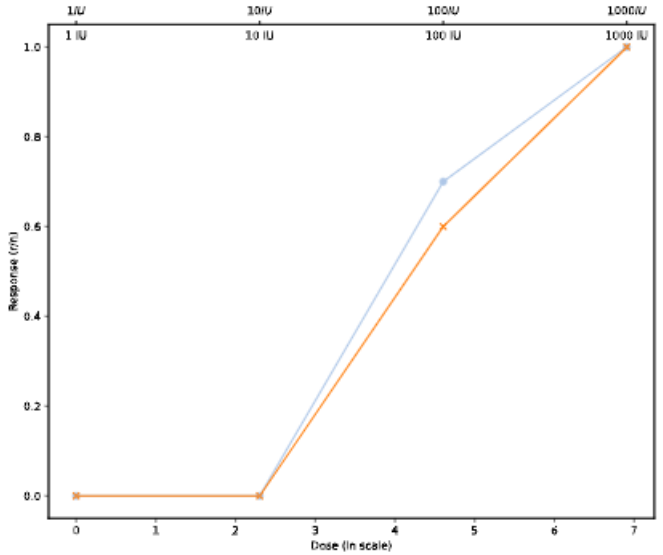
Empirical method (no regression analysis)

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

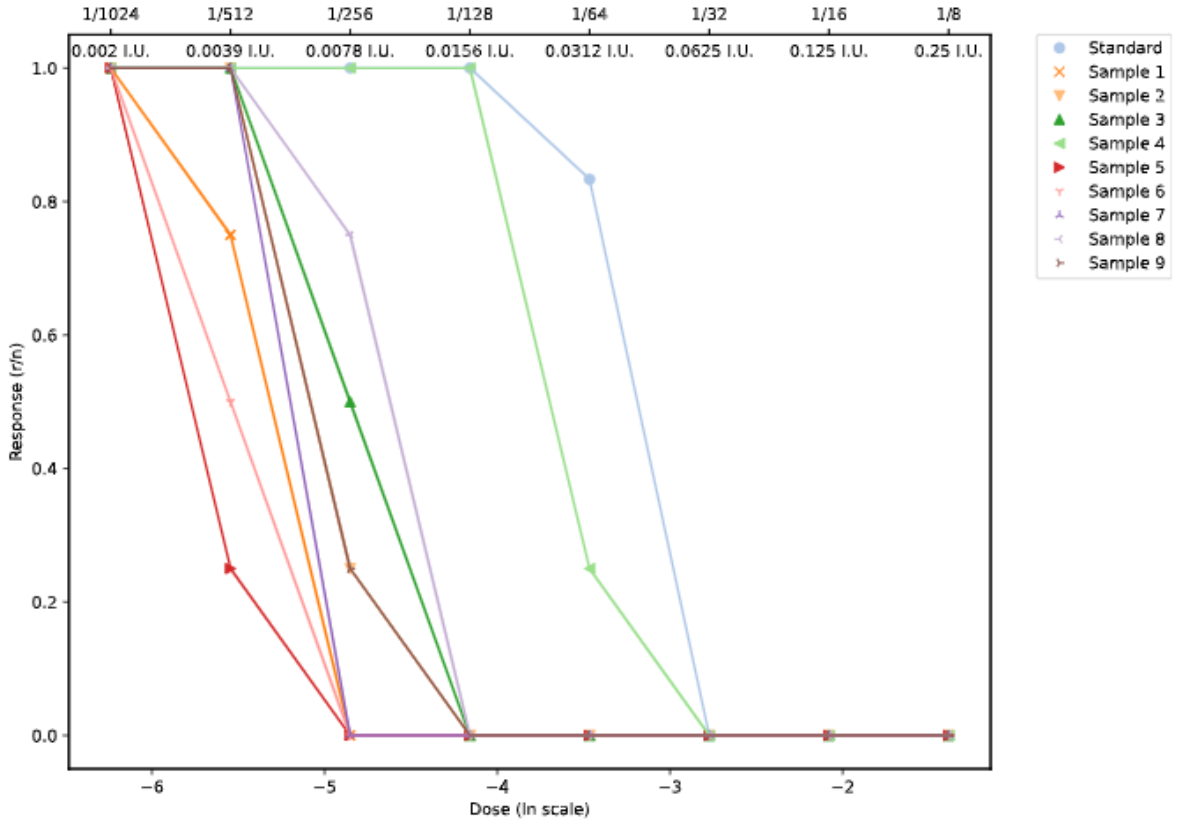
Table 2	
Preparation	Sample 1
ID	
Potency	Assumed
Potency value	? I.U./Dosis
Dose	**Rep.1**
1/8	0/4
1/16	0/4
1/32	0/4
1/64	0/4
1/128	0/4
1/256	0/4
1/512	3/4
1/1024	4/4

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/

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