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Viscumin

Bio-Assays **3 of 4 tests passed**

Product: Corticotrophin
Test method: Subcutaneuos injection
Test criterion: Mass of ascorbic acid
X-Units: unit/100g

An assay of corticotrophin by subcutaneous injection in rats

The standard preparation is administered at 0.25 and 1.0 unit per 100g of body mass. Two preparations to be examined are both assumed to have a potency of 1 unit per milligram and they are administered in the same quantities as the standard. The individual responses and means per treatment are given in the following tables.

Component: **Example 5.1.1**

Parallel-Line Model (Completely randomised design)

Data Input for Standard: Standard S [mg/100g]

Dose [unit/100g]	1,000	0,250
1	300	289
2	310	221
3	330	267
4	290	236
5	364	250
6	328	231
7	390	229
8	360	269
9	342	233
10	306	259

Data Input for Sample: Prepar. T [mg/100g]

Dose [unit/100g]	1,000	0,250
1	310	230
2	290	210
3	360	280
4	341	261
5	321	241
6	370	290
7	303	223
8	334	254
9	295	216
10	315	235

Data Input for Sample: Prepar. U [mg/100g]

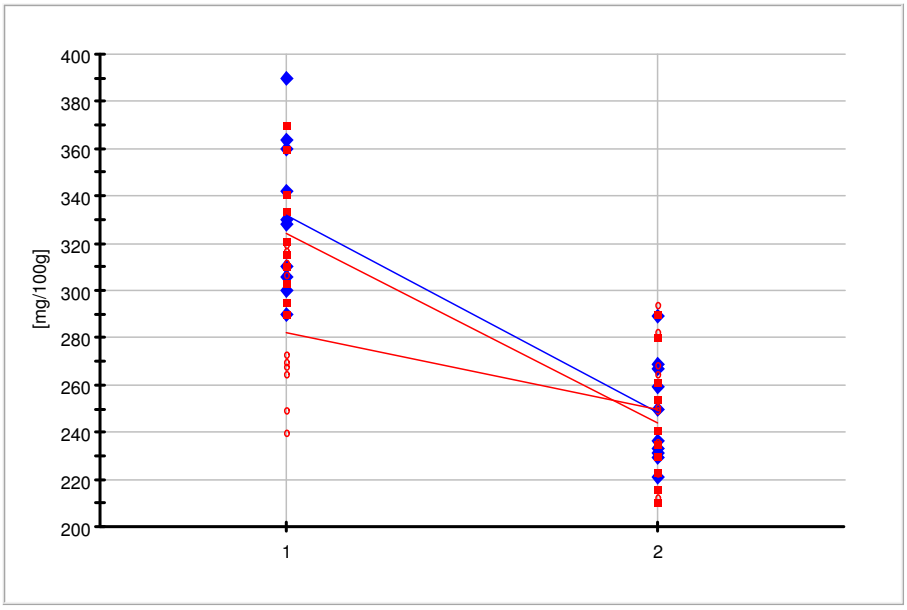
Dose [unit/100g]	1,000	0,250
1	250	236
2	268	213
3	273	283
4	240	269
5	307	251
6	270	294

7	317	223
8	312	250
9	320	216
10	265	265

Input Statistics

		Standard S		Prepar. T	
Number	Dose [unit/100g]	Mean [mg/100g]	CV [%]	Mean [mg/100g]	CV [%]
1	1,000	332,0000	9,65	323,9000	8,31
2	0,250	248,4000	8,86	244,0000	10,99

		Prepar. U	
Number	Dose [unit/100g]	Mean [mg/100g]	CV [%]
1	1,000	282,2000	10,36
2	0,250	250,0000	11,20



Test on normal distribution

√

According to the Shapiro-Wilk test, all doses are normally distributed.

Test for variance homogeneity

√

Value Chi-square for variance homogeneity acc. to Bartlett = 1,2810
Table value for Bartlett test = 11,0700 (5%-one-sided)
According to Bartlett-test variance homogeneity is given.

Testing criteria:
If Chi-square > 11,070, then there is no variance homogeneity.
If Chi-square <= 11,070, then variance homogeneity is given.

Value C for variance homogeneity according to Cochran test = 0,2235
Table value for Cochran test = 0,3682 (5%-one-sided)
According to Cochran-test variance homogeneity is given.

Testing criteria:
If C > 0,368, then there is no variance homogeneity.
If C <= 0,368, then variance homogeneity is given.

Analysis of variances

Source of variation	Sum of squares	Mean square	F-ratio	Probability α
Preparations	6256,6333	3128,3167		
Regression	63830,8167	63830,8167	83,3766	1,5424E-12
Non-paralellism	8218,2333	4109,1167	5,3674	0,01
Treatments	78305,6833			
Residual Error	41340,9000	765,5722		
Total	119646,5833			

Linear regression

√

The probability for linear regression is significant: 100,00%.

Parallelism

×

The Probability for non-parallelism of the preparations is 99,25%.
One or more preparations are not parallel to the standard.

Calculation of Potency

Calculation of potency is not valid, because not all of the preparations are parallel to the standard.

Common slope = -47,0559 $^{mg/100g}/_{unit/100g}$

Results for preparation: Prepar. T

Calculated Potency:	**1,1420 unit/100g**	
Lower limit of confidence interval:	0,7830 unit/100g	(5%-two-sided)
Upper limit of confidence interval:	1,6883 unit/100g	(5%-two-sided)

Results for preparation: Prepar. U

Calculated Potency:	**1,6689 unit/100g**	
Lower limit of confidence interval:	1,1473 unit/100g	(5%-two-sided)
Upper limit of confidence interval:	2,5575 unit/100g	(5%-two-sided)

Conclusion
The analysis confirms a highly significant linear regression. Departure from parallelism, however, is also significant which was to be expected from the graphical observation that preparation *U* is not parallel to the standard. This preparation is therefore rejected.

Bio-Assays (reduced) **4 of 4 tests passed**

Product: orticotrophin
Test method: Subcutaneuos injection
Test criterion: Mass of ascorbic acid
X-Units: unit/100g

(Reduced) assay of corticotrophin by subcutaneous injection in rats

The previous analysis is repeated without preparation *U*.

Component: **Example 5.1.1**

Parallel-Line Model (Completely randomised design)

Data Input for Standard: Standard S [mg/100g]

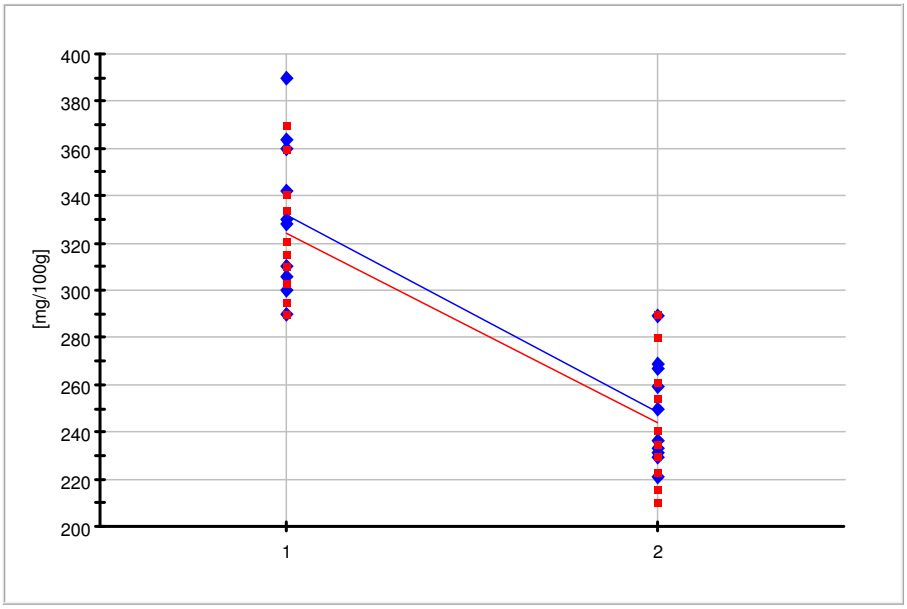
Dose [unit/100g]	1,000	0,250
1	300	289
2	310	221
3	330	267
4	290	236
5	364	250
6	328	231
7	390	229
8	360	269
9	342	233
10	306	259

Data Input for Sample: Prepar. T [mg/100g]

Dose [unit/100g]	1,000	0,250
1	310	230
2	290	210
3	360	280
4	341	261
5	321	241
6	370	290
7	303	223
8	334	254
9	295	216
10	315	235

Input Statistics

		Standard S		Prepar. T	
Number	Dose [unit/100g]	Mean [mg/100g]	CV [%]	Mean [mg/100g]	CV [%]
1	1,000	332,0000	9,65	323,9000	8,31
2	0,250	248,4000	8,86	244,0000	10,99



Test on normal distribution

√

According to the Shapiro-Wilk test, all doses are normally distributed.

Test for variance homogeneity

√

Value Chi-square for variance homogeneity acc. to Bartlett	=	1,1985
Table value for Bartlett test	=	7,8100 (5%-one-sided)

According to Bartlett-test variance homogeneity is given.

Testing criteria:
If Chi-square > 7,810, then there is no variance homogeneity.
If Chi-square <= 7,810, then variance homogeneity is given.

Value C for variance homogeneity according to Cochran test	=	0,3475
Table value for Cochran test	=	0,5017 (5%-one-sided)

According to Cochran-test variance homogeneity is given.

Testing criteria:
If C > 0,502, then there is no variance homogeneity.
If C <= 0,502, then variance homogeneity is given.

Analysis of variances

Source of variation	Sum of squares	Mean square	F-ratio	Probability α
Preparations	390,6250	390,6250		
Regression	66830,6250	66830,6250	90,4907	2,3198E-11
Non-paralellism	34,2250	34,2250	0,0463	0,83
Treatments	67255,4750			
Residual Error	26587,3000	738,5361		
Total	93842,7750			

Linear regression √

The probability for linear regression is significant: 100,00%.

Parallelism √

The Probability for non-parallelism of the preparations is 16,92%.
All preparations are parallel to the standard.

Calculation of Potency

Common slope = -58,9702 $^{mg/100g}/_{unit/100g}$

Results for preparation: Prepar. T

Calculated Potency:	**1,1118 unit/100g**	
Lower limit of confidence interval:	0,8232 unit/100g	(5%-two-sided)
Upper limit of confidence interval:	1,5170 unit/100g	(5%-two-sided)

Conclusion
The analysis without preparation *U* results in compliance with the requirements with respect to both regression and parallelism.

Test method: Antibiotic turbidimetric assay

Antibiotic turbidimetric assay
The assay is designed to assign a potency in international units per vial. The standard has an assigned potency of 670 IU/mg. The test preparation has an assumed potency of 20 000 IU/vial. On the basis of this information the stock solutions are prepared as follows. 16,7 mg of the standard is dissolved in 25 ml sovent and the contents of one vial of the test preparation are dissolved in 40 ml solvent. The final solutions are prepared by first diluting to 1/40 and further using a dilution ratio of 1,5.
The tubes are placed in a water-bath in a randomized block arrangement. Responses are listed in the following tables.

Component: **Example 5.1.3**

Parallel-Line Model (Randomised block design)

Data Input for Standard: Standard

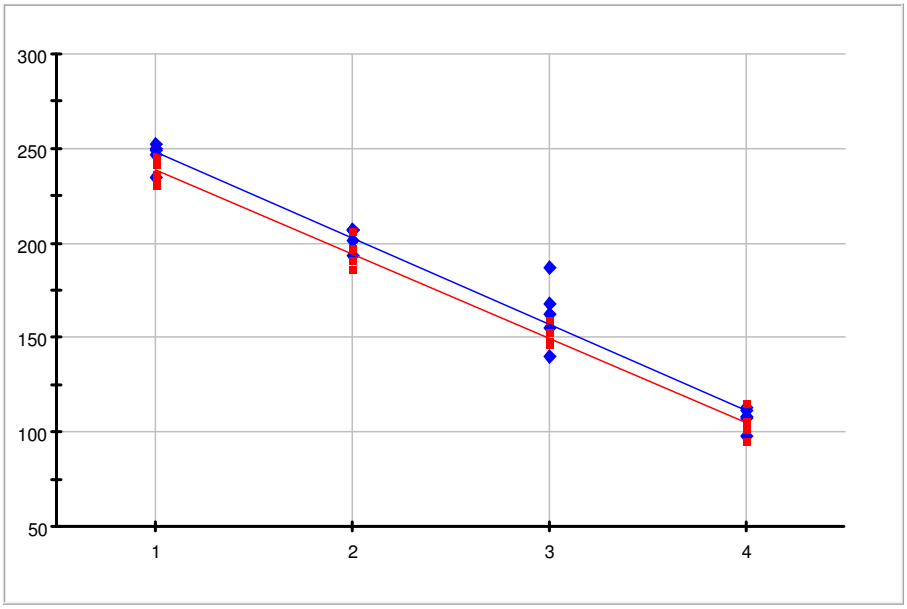
Dose	1,000	0,667	0,444	0,296
1	252	207	168	113
2	249	201	187	107
3	247	193	162	111
4	250	207	155	108
5	235	207	140	98

Data Input for Sample: Sample1

Dose	1,000	0,667	0,444	0,296
1	242	206	146	115
2	236	197	153	102
3	246	197	148	104
4	231	191	159	106
5	232	186	146	95

Input Statistics

		Standard		Sample1	
Number	Dose	Mean	CV [%]	Mean	CV [%]
1	1,000	246,6000	2,73	237,4000	2,72
2	0,667	203,0000	3,04	195,4000	3,84
3	0,444	162,4000	10,64	150,4000	3,72
4	0,296	107,4000	5,37	104,4000	6,93



Test on normal distribution

According to the Shapiro-Wilk test, dose 2 of preparation 1 is not normally distributed.

Value for Shapiro-Wilk	=	0,7606
Table value for Shapiro-Wilk	=	0,7620 (5%-one-sided)

Test for variance homogeneity

Value Chi-square for variance homogeneity acc. to Bartlett	=	9,7854
Table value for Bartlett test	=	14,0700 (5%-one-sided)

According to Bartlett-test variance homogeneity is given.

Testing criteria:
If Chi-square > 14,070, then there is no variance homogeneity.
If Chi-square <= 14,070, then variance homogeneity is given.

Value C for variance homogeneity according to Cochran test	=	0,5000
Table value for Cochran test	=	0,3910 (5%-one-sided)

According to Cochran-test there is no variance homogeneity.

Testing criteria:
If C > 0,391, then there is no variance homogeneity.
If C <= 0,391, then variance homogeneity is given.

Analysis of variances

Source of variation	Sum of squares	Mean square	F-ratio	Probability α
Preparations	632,0250	632,0250		
Regression	101745,6050	101745,6050	1887,1109	0,00
Non-paralellism	25,2050	25,2050	0,4675	0,50
Non-linearity	259,1400	64,7850	1,2016	0,3321
Treatments	102661,9750			
Blocks (rows)	876,7500	219,1875		
Residual Error	1509,6500	53,9161		
Total	105048,3750			

Linear regression √

The probability for linear regression is significant: 100,00%.

Parallelism √

The Probability for non-parallelism of the preparations is 50,02%.
All preparations are parallel to the standard.

Non-linearity √

The probability for non-linearity is not significant: 66,79%.

Calculation of Potency

Common slope = -111,2549

Results for preparation: Sample1

Calculated Potency:	**1,0741**	
Lower limit of confidence interval:	1,0291	(5%-two-sided)
Upper limit of confidence interval:	1,1214	(5%-two-sided)

Bio-Assays **4 of 5 tests passed**

Product: Hepatitis B vaccine
Test method: Optical Densities (Absorbance)
X-Units: pg protein/ml

An in-vitro assay of three hepatitis B vaccines against a standard

Three independent two-fold dilution series of five dilutions were prepared from each of the vaccines. After some additional steps in the assay procedure, absorbances were measured.
The logarithm of the optical densities are known to have a linear relationship with the logarithm of doses. The tables below show ln-transformed responses.

Component: **Example 5.1.4**

Parallel-Line Model (Completely randomised design)

Data Input for Standard: S

Dose [pg protein/ml]	0,063	0,125	0,250	0,500	1,000
1	-3,146555163	-2,375155786	-1,838851077	-1,262308381	-0,665532014
2	-3,101092789	-2,312635429	-1,870802677	-1,220779923	-0,632993258
3	-2,975929646	-2,501036032	-1,795767491	-1,016111067	-0,606969484

Data Input for Sample: T

Dose [pg protein/ml]	0,063	0,125	0,250	0,500	1,000
1	-2,333044300	-1,789761467	-1,117795108	-0,691149178	0,131028262
2	-2,333044300	-1,851509474	-1,035637490	-0,407968238	0,326421901
3	-2,364460497	-1,725971729	-1,064210862	-0,551647618	0,049742092

Data Input for Sample: U

Dose [pg protein/ml]	0,063	0,125	0,250	0,500	1,000
1	-2,453407983	-2,063568193	-1,283737773	-0,534435489	-0,043951888
2	-2,645075402	-1,924148657	-1,316768298	-0,715392790	-0,143870370
3	-2,617295838	-2,017406151	-1,313043899	-0,605136303	0,044016885

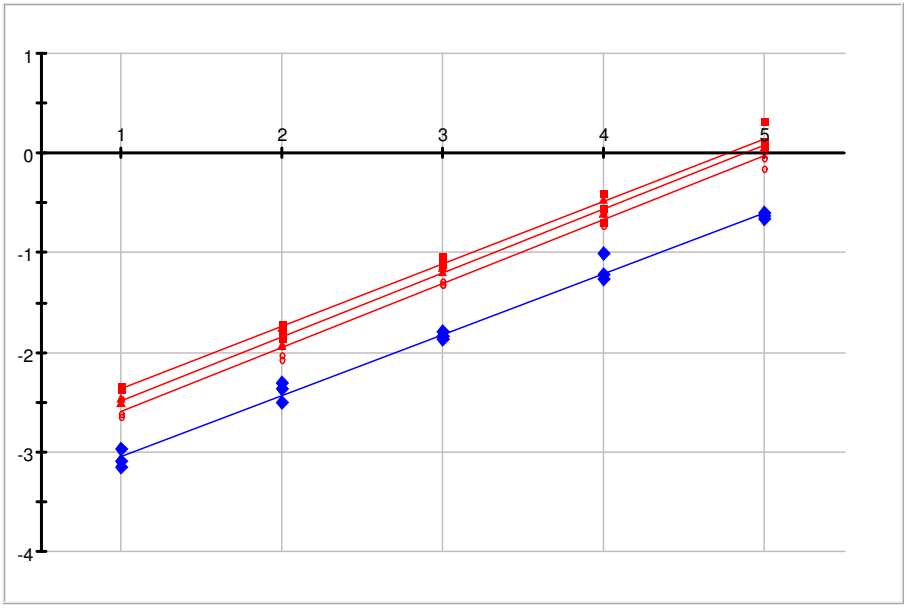
Data Input for Sample: V

Dose [pg protein/ml]	0,063	0,125	0,250	0,500	1,000
1	-2,501036032	-1,931021537	-1,145703896	-0,594207233	0,036331929
2	-2,501036032	-1,937941979	-1,184170177	-0,596020470	0,038258712
3	-2,453407983	-1,754463684	-1,152013065	-0,471604911	0,065787741

Input Statistics

Number	Dose [pg protein/ml]	S Mean	S CV [%]	T Mean	T CV [%]
1	0,063	-3,0745	-2,87	-2,3435	-0,77
2	0,125	-2,3963	-4,00	-1,7891	-3,51
3	0,250	-1,8351	-2,05	-1,0725	-3,89
4	0,500	-1,1664	-11,30	-0,5503	-25,73
5	1,000	-0,6352	-4,62	0,1691	84,11

Number	Dose [pg protein/ml]	U Mean	U CV [%]	V Mean	V CV [%]
1	0,063	-2,5719	-4,03	-2,4852	-1,11
2	0,125	-2,0017	-3,55	-1,8745	-5,55
3	0,250	-1,3045	-1,39	-1,1606	-1,78
4	0,500	-0,6183	-14,75	-0,5539	-12,87
5	1,000	-0,0479	-196,11	0,0468	35,22



Test on normal distribution ×

According to the Shapiro-Wilk test, dose 1 of preparation 2 is not normally distributed.
Value for Shapiro-Wilk = 0,7500
Table value for Shapiro-Wilk = 0,7670 (5%-one-sided)
According to the Shapiro-Wilk test, dose 1 of preparation 4 is not normally distributed.
Value for Shapiro-Wilk = 0,7500
Table value for Shapiro-Wilk = 0,7670 (5%-one-sided)
According to the Shapiro-Wilk test, dose 4 of preparation 4 is not normally distributed.
Value for Shapiro-Wilk = 0,7609
Table value for Shapiro-Wilk = 0,7670 (5%-one-sided)

Test for variance homogeneity √

Value Chi-square for variance homogeneity acc. to Bartlett = 25,6793
Table value for Bartlett test = 30,1400 (5%-one-sided)
According to Bartlett-test variance homogeneity is given.

Testing criteria:
If Chi-square > 30,140, then there is no variance homogeneity.
If Chi-square <= 30,140, then variance homogeneity is given.

Value C for variance homogeneity according to Cochran test = 0,1514
Table value for Cochran test = 0,2705 (5%-one-sided)
According to Cochran-test variance homogeneity is given.

Testing criteria:
If C > 0,271, then there is no variance homogeneity.
If C <= 0,271, then variance homogeneity is given.

Analysis of variances

Source of variation	Sum of squares	Mean square	F-ratio	Probability α
Preparations	4,4752	1,4917		
Regression	47,5841	47,5841	7125,8467	0,00
Non-paralellism	0,0187	0,0062	0,9327	0,43
Non-linearity	0,0742	0,0062	0,9264	0,5308
Treatments	52,1523			
Residual Error	0,2671	0,0067		
Total	52,4194			

Linear regression √

The probability for linear regression is significant: 100,00%.

Parallelism √

The Probability for non-parallelism of the preparations is 56,62%.
All preparations are parallel to the standard.

Non-linearity √

The probability for non-linearity is not significant: 46,92%.

Calculation of Potency

Common slope = 0,9085 $^{1}/_{\text{pg protein/ml}}$

Results for preparation: T
Calculated Potency: 2,1710 pg protein/ml
Lower limit of confidence interval: 2,0272 pg protein/ml (5%-two-sided)
Upper limit of confidence interval: 2,3270 pg protein/ml (5%-two-sided)

Results for preparation: U
Calculated Potency: 1,7581 pg protein/ml
Lower limit of confidence interval: 1,6435 pg protein/ml (5%-two-sided)
Upper limit of confidence interval: 1,8820 pg protein/ml (5%-two-sided)

Results for preparation: V

Calculated Potency:	**1,9701 pg protein/ml**	
Lower limit of confidence interval:	1,8406 pg protein/ml	(5%-two-sided)
Upper limit of confidence interval:	2,1103 pg protein/ml	(5%-two-sided)

Bio-Assays (rounded) **4 of 5 tests passed**

Product: Hepatitis B vaccine
Test method: Optical Densities (Absorbance)
X-Units: pg protein/ml

An in-vitro assay of three hepatitis B vaccines against a standard

Three independent two-fold dilution series of five dilutions were prepared from each of the vaccines. After some additional steps in the assay procedure, absorbances were measured.
The logarithm of the optical densities are known to have a linear relationship with the logarithm of doses. The tables below show ln-transformed responses rounded to three decimal places.

Component: **Example 5.1.4**

Parallel-Line Model (Completely randomised design)

Data Input for Standard: S

Dose [pg protein/ml]	0,625	1,250	2,500	5,000	10,000
1	-3,147	-2,375	-1,839	-1,262	-0,666
2	-3,101	-2,313	-1,871	-1,221	-0,633
3	-2,976	-2,501	-1,796	-1,016	-0,607

Data Input for Sample: T

Dose [pg protein/ml]	0,625	1,250	2,500	5,000	10,000
1	-2,333	-1,790	-1,118	-0,691	0,131
2	-2,333	-1,852	-1,036	-0,408	0,326
3	-2,364	-1,726	-1,064	-0,552	0,050

Data Input for Sample: U

Dose [pg protein/ml]	0,625	1,250	2,500	5,000	10,000
1	-2,453	-2,064	-1,284	-0,534	-0,044
2	-2,645	-1,924	-1,317	-0,715	-0,144
3	-2,617	-2,017	-1,313	-0,605	0,044

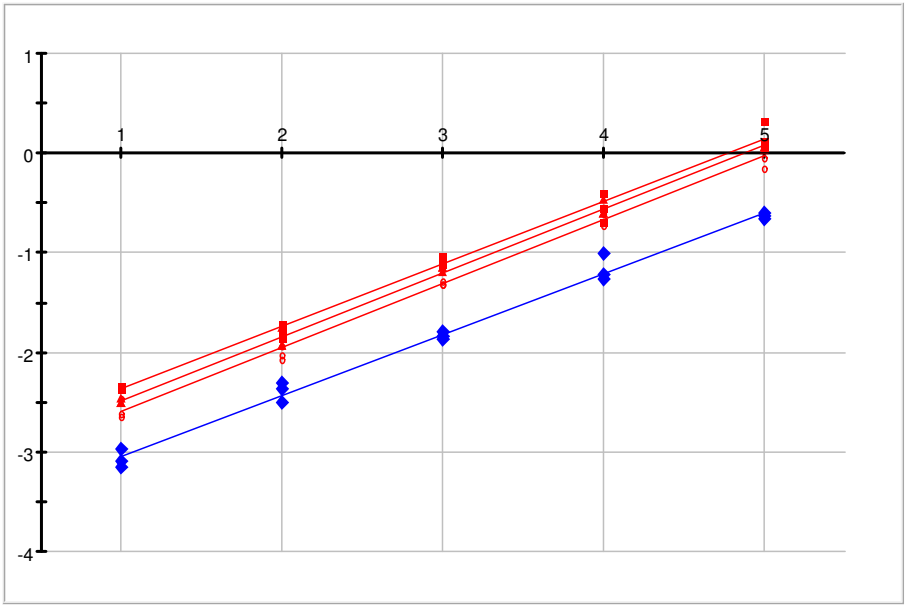
Data Input for Sample: V

Dose [pg protein/ml]	0,625	1,250	2,500	5,000	10,000
1	-2,501	-1,931	-1,146	-0,594	0,036
2	-2,501	-1,938	-1,184	-0,596	0,038
3	-2,453	-1,754	-1,152	-0,472	0,066

Input Statistics

Number	Dose [pg protein/ml]	S Mean	S CV [%]	T Mean	T CV [%]
1	0,625	-3,0747	-2,88	-2,3433	-0,76
2	1,250	-2,3963	-4,00	-1,7893	-3,52
3	2,500	-1,8353	-2,05	-1,0727	-3,89
4	5,000	-1,1663	-11,30	-0,5503	-25,71
5	10,000	-0,6353	-4,65	0,1690	83,95

Number	Dose [pg protein/ml]	U Mean	U CV [%]	V Mean	V CV [%]
1	0,625	-2,5717	-4,03	-2,4850	-1,12
2	1,250	-2,0017	-3,56	-1,8743	-5,56
3	2,500	-1,3047	-1,38	-1,1607	-1,76
4	5,000	-0,6180	-14,76	-0,5540	-12,82
5	10,000	-0,0480	-195,97	0,0467	35,94



Test on normal distribution ×

According to the Shapiro-Wilk test, dose 1 of preparation 2 is not normally distributed.
Value for Shapiro-Wilk = 0,7500
Table value for Shapiro-Wilk = 0,7670 (5%-one-sided)
According to the Shapiro-Wilk test, dose 1 of preparation 4 is not normally distributed.
Value for Shapiro-Wilk = 0,7500
Table value for Shapiro-Wilk = 0,7670 (5%-one-sided)
According to the Shapiro-Wilk test, dose 4 of preparation 4 is not normally distributed.
Value for Shapiro-Wilk = 0,7621
Table value for Shapiro-Wilk = 0,7670 (5%-one-sided)

Test for variance homogeneity √

Value Chi-square for variance homogeneity acc. to Bartlett = 25,6502
Table value for Bartlett test = 30,1400 (5%-one-sided)
According to Bartlett-test variance homogeneity is given.

Testing criteria:
If Chi-square > 30,140, then there is no variance homogeneity.
If Chi-square <= 30,140, then variance homogeneity is given.

Value C for variance homogeneity according to Cochran test = 0,1507
Table value for Cochran test = 0,2705 (5%-one-sided)
According to Cochran-test variance homogeneity is given.

Testing criteria:
If C > 0,271, then there is no variance homogeneity.
If C <= 0,271, then variance homogeneity is given.

Analysis of variances

Source of variation	Sum of squares	Mean square	F-ratio	Probability α
Preparations	4,4763	1,4921		
Regression	47,5789	47,5789	7125,0923	0,00
Non-paralellism	0,0186	0,0062	0,9292	0,44
Non-linearity	0,0745	0,0062	0,9296	0,5278
Treatments	52,1483			
Residual Error	0,2671	0,0067		
Total	52,4154			

Linear regression √

The probability for linear regression is significant: 100,00%.

Parallelism √

The Probability for non-parallelism of the preparations is 56,45%.
All preparations are parallel to the standard.

Non-linearity √

The probability for non-linearity is not significant: 47,22%.

Calculation of Potency

Common slope = 0,9084 $^{1}/_{\text{pg protein/ml}}$

Results for preparation: T
Calculated Potency: 2,1712 pg protein/ml
Lower limit of confidence interval: 2,0274 pg protein/ml (5%-two-sided)
Upper limit of confidence interval: 2,3272 pg protein/ml (5%-two-sided)

Results for preparation: U
Calculated Potency: 1,7586 pg protein/ml
Lower limit of confidence interval: 1,6439 pg protein/ml (5%-two-sided)
Upper limit of confidence interval: 1,8825 pg protein/ml (5%-two-sided)

Results for preparation: V

Calculated Potency:	**1,9704 pg protein/ml**	
Lower limit of confidence interval:	1,8409 pg protein/ml	(5%-two-sided)
Upper limit of confidence interval:	2,1106 pg protein/ml	(5%-two-sided)

Product: Viscumin

Component: **Viscumin**

Parallel-Line Model (Completely randomised design)

Data Input for Standard: Viscum album

Dose	0,100	0,050	0,025	0,013	0,006
1	0,003	0,031	0,128	0,279	0,409
2	0,005	0,030	0,122	0,294	0,408
3	0,005	0,038	0,122	0,249	0,392
4	0,003	0,035	0,123	0,263	0,390
5	0,004	0,034	0,133	0,281	0,392

Dose	0,003	0,002	0,001	0,000
1	0,490	0,485	0,477	0,484
2	0,456	0,447	0,481	0,484
3	0,470	0,470	0,471	0,457
4	0,465	0,470	0,471	0,471
5	0,475	0,466	0,485	0,490

Data Input for Sample: MLA

Dose	0,100	0,050	0,025	0,013	0,006
1	-0,001	0,033	0,130	0,277	0,379
2	0,003	0,034	0,134	0,272	0,395
3	0,004	0,029	0,142	0,266	0,358
4	0,000	0,035	0,145	0,262	0,443
5	0,000	0,039	0,132	0,265	0,394

Dose	0,003	0,002	0,001	0,000
1	0,438	0,480	0,441	0,434
2	0,403	0,439	0,467	0,497
3	0,429	0,446	0,437	0,479
4	0,418	0,430	0,471	0,499
5	0,430	0,453	0,475	0,488

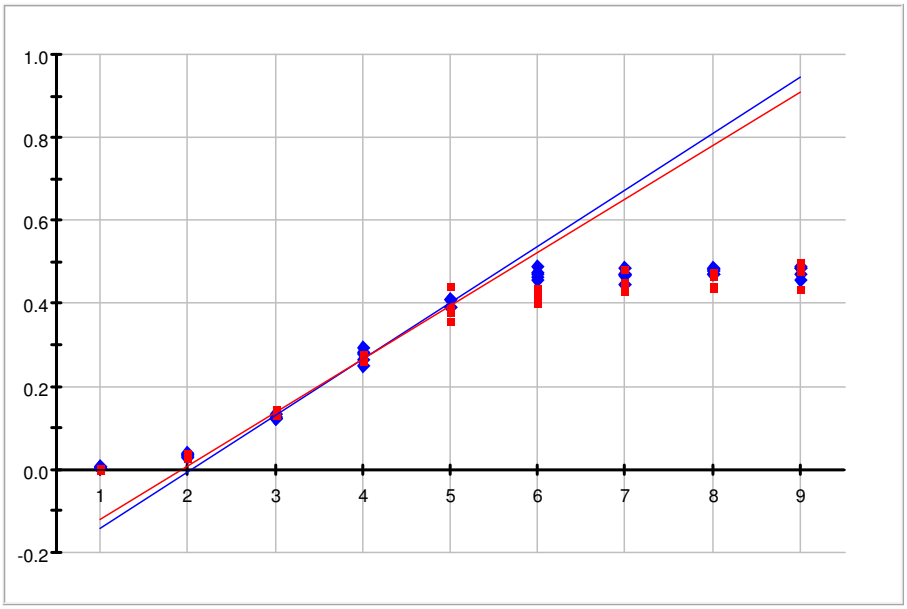
Data Input for Controls: Control

Number	Value
1	0,448
2	0,465
3	0,483
4	0,476
5	0,468
6	0,440
7	0,435
8	0,459
9	0,444
10	0,450
11	0,446

12	0,431	
13	0,430	
14	0,432	
15	0,455	
16	0,345	
17	0,418	
18	0,422	
19	0,427	
20	0,417	
Mean		0,440*
CV [%]		6,6*

Input Statistics

		Viscum album		MLA	
Number	Dose	Mean	CV [%]	Mean	CV [%]
1	0,100	0,0040	25,00	0,0012	180,66
2	0,050	0,0336	9,55	0,0340	10,60
3	0,025	0,1256	3,84	0,1366	4,79
4	0,013	0,2732	6,38	0,2684	2,24
5	0,006	0,3982	2,37	0,3938	7,95
6	0,003	0,4712	2,68	0,4236	3,20
7	0,002	0,4676	2,91	0,4496	4,23
8	0,001	0,4770	1,29	0,4582	3,89
9	0,000	0,4772	2,78	0,4794	5,55



Test on normal distribution

According to the Shapiro-Wilk test, dose 5 of preparation 1 is not normally distributed.

Value for Shapiro-Wilk = 0,7619

Table value for Shapiro-Wilk = 0,7620 (5%-one-sided)

Test for variance homogeneity

Value Chi-square for variance homogeneity acc. to Bartlett = 69,7590

Table value for Bartlett test = 27,5900 (5%-one-sided)

According to Bartlett-test there is no variance homogeneity.

Testing criteria:
If Chi-square > 27,590, then there is no variance homogeneity.
If Chi-square <= 27,590, then variance homogeneity is given.

Value C for variance homogeneity according to Cochran test = 0,2699
Table value for Cochran test = 0,2090 (5%-one-sided)
According to Cochran-test there is no variance homogeneity.

Testing criteria:
If C > 0,209, then there is no variance homogeneity.
If C <= 0,209, then variance homogeneity is given.

Analysis of variances

Source of variation	Sum of squares	Mean square	F-ratio	Probability α
Preparations	0,0019	0,0019		
Regression	2,7146	2,7146	13448,6679	8,4134E-17
Non-paralellism	0,0008	0,0008	3,9540	0,05
Non-linearity	0,3369	0,0241	119,2311	6,3962E-44
Treatments	3,0543			
Residual Error	0,0145	0,0002		
Total	3,0688			

Analysis of variances for reduced number of doses (dose 3 to 5)

Source of variation	Sum of squares	Mean square	F-ratio	Probability α
Preparations	0,0000	0,0000		
Regression	0,3509	0,3509	1425,7774	5,8601E-17
Non-paralellism	0,0003	0,0003	1,2047	0,28
Non-linearity	0,0005	0,0002	0,9342	0,4067
Treatments	0,3516			
Residual Error	0,0059	0,0002		
Total	0,3575			

Linear regression √

The probability for linear regression is significant: 100,00%.

Parallelism √

The Probability for non-parallelism of the preparations is 71,67%.
All preparations are parallel to the standard.

Non-linearity √

The probability for non-linearity is not significant: 59,33%.

Calculation of Potency

Common slope = 0,1911

Results for preparation: MLA

Calculated Potency:	**1,0031**	
Lower limit of confidence interval:	0,9429	(5%-two-sided)
Upper limit of confidence interval:	1,0673	(5%-two-sided)

Well Layout (D = dose, N = injection, Std = standard, Smp = sample)

	1	2	3	4	5	6	7	8	9	10	11	12
A		Ctrl N 15	Std D 4 N 3	Smp D 2 N 1	Std D 9 N 2	Smp D 2 N 3	Ctrl N 5	Smp D 4 N 1	Smp D 6 N 3	Std D 1 N 2	Ctrl N 13	Smp D 5 N 1
B		Smp D 5 N 3	Ctrl N 8	Std D 5 N 3	Std D 5 N 5	Ctrl N 16	Smp D 3 N 5	Ctrl N 6	Smp D 8 N 4	Std D 9 N 1	Std D 2 N 2	Std D 3 N 1
C		Smp D 2 N 4	Ctrl N 19	Smp D 5 N 2	Smp D 7 N 3	Std D 2 N 4	Smp D 7 N 1	Ctrl N 3	Smp D 6 N 1	Smp D 3 N 4	Std D 3 N 3	Smp D 6 N 2
D		Std D 7 N 3	Std D 6 N 2	Ctrl N 2	Ctrl N 11	Std D 3 N 4	Smp D 1 N 3	Std D 7 N 4	Smp D 3 N 1	Std D 4 N 1	Ctrl N 12	Ctrl N 9
E		Smp D 3 N 2	Std D 4 N 2	Std D 2 N 1	Std D 5 N 2	Smp D 9 N 2	Smp D 5 N 5	Smp D 3 N 3	Ctrl N 18	Std D 8 N 2	Std D 7 N 5	Std D 8 N 4
F		Std D 1 N 1	Smp D 4 N 3	Std D 4 N 4	Ctrl N 14	Smp D 9 N 4	Std D 6 N 3	Smp D 7 N 4	Std D 6 N 1	Smp D 6 N 5	Smp D 5 N 4	Std D 9 N 4
G		Smp D 8 N 2	Std D 7 N 2	Smp D 8 N 5	Smp D 4 N 2	Std D 2 N 5	Ctrl N 1	Smp D 8 N 3	Smp D 9 N 1	Smp D 1 N 1	Std D 3 N 2	Std D 1 N 5
H		Ctrl N 7	Smp D 7 N 5	Std D 5 N 1	Smp D 4 N 5	Std D 9 N 5	Std D 2 N 3	Smp D 7 N 2	Std D 7 N 1	Std D 8 N 3	Ctrl N 20	Smp D 2 N 5
I		Std D 8 N 5	Std D 1 N 3	Ctrl N 4	Std D 6 N 5	Smp D 9 N 3	Std D 9 N 3	Std D 4 N 5	Std D 3 N 5	Smp D 9 N 5	Smp D 1 N 4	Smp D 6 N 4
J		Ctrl N 10	Std D 5 N 4	Std D 1 N 4	Ctrl N 17	Smp D 4 N 4	Smp D 1 N 2	Std D 6 N 4	Smp D 8 N 1	Smp D 2 N 2	Std D 8 N 1	Smp D 1 N 5

Biological Assay Analysis

Biological methods are used when the potency of a preparation cannot be adequately assured by chemical or physical analysis. A standard is compared to one or more preparations in various solutions or concentrations. All measurements are carried out simultaneously under identical conditions. The potency of all preparations in relation to the standard is to be calculated.

Symbols:

Symbol	Description
h	Number of preparations (includes standard)
d	Number of doses per preparation
n	Number of experimental units per dose and preparation
x_{kji}	Response of the *i*-th replicate of dose *j* of preparation *k*
$\bar{x}_{kj}$	Mean of responses of dose *j* of preparation *k*
s_{kj}^2	Variance of response of dose *j* of preparation *k*
P_k	Total preparation
L_k	Linear contrast
R_i	Sum of row *i* in randomized block designs
H_P	Construction variable for analysis of variances
H_L	Construction variable for analysis of variances
K	Construction variable for analysis of variances
SS_{prep}	Sum of squares: preparations
SS_{reg}	Sum of squares: linear regression
SS_{par}	Sum of squares: non-parallelism
SS_{lin}	Sum of squares: non-linearity
SS_{treat}	Sum of squares: treatments
SS_{block}	Sum of squares: blocks
SS_{res}	Sum of squares: residual error
SS_{tot}	Sum of squares: Total
MS_*	Mean square corresponding to a sum of squares above
F_*	F-ratio corresponding to a sum of squares above
p_*	Probability for F-ratio F_*
l	Logarithm of the ratio between adjacent doses (≠0)
b	Common slope
M'_k	Logarithm of the potency of preparation *k*
$t_{(f,\alpha)}$	Value t-distribution for probability α and f degrees of freedom
C	Construction variable for the confidence limits of the potency
V	Construction variable for the confidence limits of the potency

Basic statistics

Mean of treatments: $$\bar{x}_{kj} = \frac{\sum_{i=1}^{n} x_{kji}}{n}$$

Variance of treatments: $s_{kj}^2 = \dfrac{\sum_{i=1}^{n}(x_{kji} - \bar{x}_{kj})^2}{n-1}$

Sum of block: $R_i = \sum_{k=1}^{h}\sum_{j=1}^{d} x_{kji}$

Coefficient of variance (in percent) of treatments: $CV\%_{kj} = \dfrac{\sqrt{s_{kj}^2}}{|\bar{x}_{kj}|} \cdot 100\%$

Variance analysis

Construction variables:

$$P_k = \sum_{j=1}^{d} \bar{x}_{kj}$$

$$L_k = \left(\sum_{j=1}^{d} j\bar{x}_{kj}\right) - \frac{1}{2}(d+1)P_k$$

$$H_P = \frac{n}{d}$$

$$H_L = \frac{12n}{d^3 - d}$$

$$K = \frac{n(\sum_{k=1}^{h} P_k)^2}{hd}$$

Sum of squares:

(n_* are the corresponding degrees of freedom)

$$SS_{prep} = H_P\left(\sum_{k=1}^{h} P_k^{\,2}\right) - K, \qquad n_{prep} = h-1$$

$$SS_{reg} = \frac{1}{h} H_L\left(\sum_{k=1}^{h} L_k\right)^2, \qquad n_{reg} = 1$$

$$SS_{par} = H_L\left(\sum_{k=1}^{h} L_k^{\,2}\right) - SS_{reg}, \quad n_{par} = h-1$$

$$SS_{lin} = SS_{treat} - SS_{prep} - SS_{reg} - SS_{par}, \qquad n_{lin} = h(d-2)$$

$$SS_{treat} = n\left(\sum_{k=1}^{h}\sum_{j=1}^{d} \bar{x}_{kj}^{\,2}\right) - K, \quad n_{treat} = hd-1$$

$$SS_{block} = hd\left(\sum_{i=1}^{n} R_i^2\right) - K, \qquad n_{block} = n-1$$

Completely randomized design:

$$SS_{res} = SS_{tot} - SS_{treat}, \qquad n_{res} = hd(n-1)$$

Randomized block design:

$$SS_{res} = SS_{tot} - SS_{treat} - SS_{block}, \; n_{res} = (hd-1)(n-1)$$

$$SS_{tot} = \sum_{k=1}^{h}\sum_{j=1}^{d}\sum_{i=1}^{n}\left(x_{kji} - \bar{x}\right)^2, \quad n_{tot} = nhd - 1$$

F-ratio: $$F_* = \frac{SS_*}{n_* SS_{res}} \quad \left(= \frac{MS_*}{SS_{res}}\right)$$

Mean squares: $$MS_* = \frac{SS_*}{n_*}$$

Potency

Common slope: $$b = \frac{H_L\left(\sum_{k=1}^{h} L_k\right)}{Inh}$$

Potency of preparation *k:* $\exp(M'_k) = \exp\left(\frac{P_k - P_1}{db}\right)$, where P_1 is the preparation of the standard

Construction variables for confidence limits:

$$C = \frac{SS_{reg}}{SS_{reg} - SS_{res} t^2_{(n_{res},\alpha)}}$$

$$V = \frac{SS_{reg}}{b^2 dn}$$

Confidence interval for potency of preparation *k*:

$$\exp\left(CM'_k \pm \sqrt{(C-1)(CM'^2_k + 2V)}\right)$$

Shapiro-Wilk-test for normal distribution

Construction variables:

$$Q_{kj} = \sum_{i=1}^{n}\left(x_{kji} - \bar{x}_{kj}\right) = (n-1)s^2_{kj}$$

$$b_{kj} = \sum_{i=1}^{m} a_i \left(x_{kj[n-i+1]} - x_{kj[i]}\right), \; m = \left\lfloor \frac{n}{2} \right\rfloor$$

Test value:

$$W_{kj} = \frac{b^2_{kj}}{Q_{kj}}$$

Results

Normal distribution is not given, if $W_{kj} < W_{n;\alpha}$.

Cochran-Test for homogeneity of variances

If $s^2_{[hd]}$ is the largest variance of all *hd* responses. Homogeneity of variances is not given, if

$$\frac{s^2_{[hd]}}{\sum_{k=1}^{h}\sum_{j=1}^{d} s^2_{kj}} > g_{hd,f;1-\alpha}\ ,\ f = n-1.$$

Bartlett-test for homogeneity of variances

Construction variables:
As all treatment groups have the same size *n,* calculation is simplified to:

$$c = 1 + \frac{1}{3(hd-1)}\left(\frac{hd}{f} - \frac{1}{hdf}\right),\ f = n-1$$

$$s^2 = \frac{\sum_{k=1}^{h}\sum_{j=1}^{d} s^2_{kj}}{hd}$$

Results

Homogeneity of variances is not given, if $-\frac{f}{c}\sum_{k=1}^{h}\sum_{j=1}^{d}\ln\frac{s^2_{kj}}{s^2} > \chi^2_{hd-1;1-\alpha}$.

Parameters of Configuration: BioAssay-Demo

Outlier tests

Dixon:	5%-one-sided
Grubbs:	5%-one-sided

Trend tests

Neumann:	5%-one-sided

Precision

t-values for r, u, CI:	5%-two-sided
Test on normal distribution (Shapiro-Wilk):	5%-one-sided
Stability t-test:	5%-one-sided

Test for calibration function

Partial F-test:	5%-one-sided
t-test (confidence interval):	5%-two-sided

Homogeneity Test

Cochran:	5%-one-sided
Bartlett:	5%-one-sided
F-Test (2 rows):	5%-one-sided

Test for robustness

F-test:	5%-one-sided
t-test:	5%-two-sided
Factor simplified robustness:	4

Precision (robustness, ring experiment)

t-values for r, R:	5%-two-sided
u_r, u_R:	5%-two-sided
Confidence interval:	5%-two-sided

Limit of detection and quantification

t for critical value y_k:	1%-one-sided
t for support value D:	1%-two-sided
Factor detection limit:	3,3
Factor quantification limit:	10

Nominal/actual comparison

t-test:	5%-two-sided
Wilcoxon-Test:	5%-two-sided

Method comparison

t-test for joined samples:	5%-two-sided
Comparison of mean values t-test:	5%-two-sided
Comparison of mean values F-test:	5%-one-sided

Systematic errors

t-test selectivity:	5%-two-sided
t-test extended spiking method:	5%-two-sided

Standard addition

Variance homogeneity F-test:	5%-one-sided
Slope difference t-test:	5%-two-sided

Linearity

t-test (confidence interval):	5%-two-sided

Biological Assay Analysis

Confidence interval:	5%-two-sided
Significance limit linear regression:	5%
Significance limit parallelity:	5%
Significance limit linearity:	5%
Test on normal distribution (Shapiro-Wilk):	5%-one-sided
Cochran:	5%-one-sided
Bartlett:	5%-one-sided

Other variable values

Standard number of samples:	6
Limit of detection and quantification (h):	3 (not adjustable)
Ring experiments:	DIN ISO 5725

Whiskers in box-& whisker-plots show smallest and largest value (range).