Europ. Pharm., 5th Ed. (2005), Ch. 5.3, 5.1.4.a - Five-dose multiple assay with completely randomised design - An in-vitro assay of three hepatitis B vaccines against a standard

Document-Id:	Document-22 (Revision:12)
Document type:	Quantitative Response Assay/26
Last modified:	08.05.2019 10:33:46
Database:	Bioassay Package 26 (PLA 3.0.4)
Database GUID:	a23398ab-7853-4e3d-aa1a-5bedeec03463

Calculation

Calculation performed:	08.05.2019 10:33:45, Dr. Matthias Schmitt (PLA 3.0.4 Build 762, nbmsc03, 20100)
Report generated:	08.05.2019 10:34:14, Dr. Matthias Schmitt (PLA 3.0.4 Build 762, nbmsc03, 20100)

Signatures

Responsibility	Review	Approval

Comments

Comment by Matthias Schmitt (17.03.2014 12:16:18)

European Pharmacopoeia, 5th Edition (2005), Chapter 5.3
5.1.4 Five-dose multiple assay with completely randomised design - An in-vitro assay of three hepatitis B vaccines against a standard

Remarks: Multiplex analysis

DOCUMENT-22

A23398AB-7853-4E3D-AA1A-5BEDEEC03463

Overview

General properties

Property	Value
Response adjustment	None
Response transformation	Logarithmic
Model	Linear model
Simultaneous regression	Yes
Potency estimation confidence interval	95.00%
Calculate mean potency estimate of test samples	No
ANOVA model	ANOVA (pure error separation)
ANOVA with additional factors	Yes
Logarithm base	Natural logarithm (base e)
Invert potency	No
Potency confidence interval calculation method	Based on ANOVA error
Test exception handling	Informal

Documentation

Date	25.02.2014 11:26:42

Assay

Setup

Sample setup

Setup	Standard sample: S	Test sample: T	Test sample: U
Preparation scheme	PreparationScheme-1	PreparationScheme-1	PreparationScheme-1
Step count	5	5	5
Replicate count	3	3	3
Potency definition	by stock solution	by stock solution	by stock solution
Assigned/assumed potency	20 µg protein/ml	20 µg protein/ml	20 µg protein/ml
Dilution scale	n-fold sequence	n-fold sequence	n-fold sequence
Factor	0.001	0.001	0.001
Base	2.0	2.0	2.0
Analysis	**Standard sample: S**	**Test sample: T**	**Test sample: U**
Data selection scheme	DataSelectionScheme-1	DataSelectionScheme-1	DataSelectionScheme-1
Outlier detection	None	None	None
Range	Full	Full	Full
Optimization	No	No	No

Setup	Test sample: V
Preparation scheme	PreparationScheme-1
Step count	5
Replicate count	3
Potency definition	by stock solution
Assigned/assumed potency	20 µg protein/ml
Dilution scale	n-fold sequence
Factor	0.001
Base	2.0
Analysis	**Test sample: V**
Data selection scheme	DataSelectionScheme-1
Outlier detection	None
Range	Full
Optimization	No

Observations

Response values in bold are used for analysis as a result of your configuration.

Standard sample: S

Selected steps: 1-5

Dose value		1.000 E-03	5.000 E-04	2.500 E-04	1.250 E-04	6.250 E-05
Dose step		1	2	3	4	5
Response	1	**0.514**	**0.283**	**0.159**	**0.093**	**0.043**
	2	**0.531**	**0.295**	**0.154**	**0.099**	**0.045**
	3	**0.545**	**0.362**	**0.166**	**0.082**	**0.051**
Mean		0.53000	0.31333	0.15967	0.09133	0.04633
SD		0.01552	0.04257	0.00603	0.00862	0.00416
CV[%]		2.92909	13.58665	3.77519	9.43979	8.98561

Test sample: T

Selected steps: 1-5

Dose value		1.000 E-03	5.000 E-04	2.500 E-04	1.250 E-04	6.250 E-05
Dose step		1	2	3	4	5
Response	1	**1.14**	**0.501**	**0.327**	**0.167**	**0.097**
	2	**1.386**	**0.665**	**0.355**	**0.157**	**0.097**
	3	**1.051**	**0.576**	**0.345**	**0.178**	**0.094**
Mean		1.19233	0.58067	0.34233	0.16733	0.09600
SD		0.17352	0.08210	0.01419	0.01050	0.00173
CV[%]		14.55325	14.13884	4.14485	6.27727	1.80422

Test sample: U

Selected steps: 1-5

Dose value		1.000 E-03	5.000 E-04	2.500 E-04	1.250 E-04	6.250 E-05
Dose step		1	2	3	4	5
Response	1	**0.957**	**0.586**	**0.277**	**0.127**	**0.086**
	2	**0.866**	**0.489**	**0.268**	**0.146**	**0.071**
	3	**1.045**	**0.546**	**0.269**	**0.133**	**0.073**
Mean		0.95600	0.54033	0.27133	0.13533	0.07667
SD		0.08950	0.04875	0.00493	0.00971	0.00814
CV[%]		9.36236	9.02177	1.81802	7.17675	10.62330

Test sample: V

Selected steps: 1-5

Dose value		1.000 E-03	5.000 E-04	2.500 E-04	1.250 E-04	6.250 E-05
Dose step		1	2	3	4	5
Response	1	**1.037**	**0.552**	**0.318**	**0.145**	**0.082**
	2	**1.039**	**0.551**	**0.306**	**0.144**	**0.082**
	3	**1.068**	**0.624**	**0.316**	**0.173**	**0.086**
Mean		1.04800	0.57567	0.31333	0.15400	0.08333
SD		0.01735	0.04186	0.00643	0.01646	0.00231
CV[%]		1.65547	7.27172	2.05184	10.68966	2.77128

Result

Analysis of variance (ANOVA)

Total number of observations: 60

Source of variation	d.f.	Sum of squares	Mean squares	F-ratio	Probability
Treatments	19	52.15227	2.74486	411.04934	7.527 E-40
Preparation	3	4.47522	1.49174	223.39203	5.163 E-25
Regression	1	47.58413	47.58413	7125.8467	1.084 E-46
non-Parallelism	3	0.01869	0.00623	0.93274	0.43382
non-Linearity (LoF)	12	0.07423	0.00619	0.92637	0.53078
S	3	0.01703	0.00568	0.85022	0.47474
T	3	0.02826	0.00942	1.41043	0.25385
U	3	0.01775	0.00592	0.88625	0.45648
V	3	0.01119	0.00373	0.55859	0.64544
Residual (pure) error	40	0.26711	0.00668		
Total	59	52.41937	0.88846		

Regression

Restricted model (common slope and asymptotes) Parameter	Estimate	Error		Quality of regression
S Intercept (linear models)	5.71347	0.09172	r^2	0.99780
T Intercept (linear models)	6.41770	0.09172	r^2 adjusted	0.99760
U Intercept (linear models)	6.22609	0.09172		
V Intercept (linear models)	6.32949	0.09172		
Common Slope (linear models)	0.90848	0.01076		
Unrestricted regression Parameter	Estimate	Error		Quality of regression
S Intercept (linear models)	5.48792	0.17977	r^2	0.99792
S Slope (linear models)	0.88128	0.02152	r^2 adjusted	0.99760
T Intercept (linear models)	6.37808	0.17977		
T Slope (linear models)	0.90370	0.02152		
U Intercept (linear models)	6.38676	0.17977		
U Slope (linear models)	0.92785	0.02152		
V Intercept (linear models)	6.43400	0.17977		
V Slope (linear models)	0.92108	0.02152		

Validity tests

Overview:

	Passed	Failed (rejected)	Failed (warning)	Passed (info)	Not calculated
Assay suitability	0	0	0	0	0
Sample suitability	0	0	0	0	0
Overall test result	Passed (no tests available)				

Potency estimation

Relative potency	T	S
Potency ratio	2.17098	
95% Confidence interval	2.02724 - 2.32698	
Relative confidence interval	93.38% - 107.19% (13.81%)	

Stock solution		
Assigned/assumed potency	20.00000 µg protein/ml	20.00000 µg protein/ml
Factor rel. estimated sample potency	2.17098	2.17098
Estimated sample potency (stock solution)	43.41962 µg protein/ml	
95% Confidence interval	40.54479 - 46.53966 µg protein/ml	
Relative confidence interval	93.38% - 107.19% (13.81%)	

Relative potency	U	S
Potency ratio	1.75815	
95% Confidence interval	1.64349 - 1.88202	
Relative confidence interval	93.48% - 107.05% (13.57%)	

Stock solution		
Assigned/assumed potency	20.00000 µg protein/ml	20.00000 µg protein/ml
Factor rel. estimated sample potency	1.75815	1.75815
Estimated sample potency (stock solution)	35.16298 µg protein/ml	
95% Confidence interval	32.86981 - 37.64049 µg protein/ml	
Relative confidence interval	93.48% - 107.05% (13.57%)	

Relative potency	V	S
Potency ratio	1.97008	
95% Confidence interval	1.84063 - 2.11029	
Relative confidence interval	93.43% - 107.12% (13.69%)	

Stock solution		
Assigned/assumed potency	20.00000 µg protein/ml	20.00000 µg protein/ml
Factor rel. estimated sample potency	1.97008	1.97008
Estimated sample potency (stock solution)	39.40168 µg protein/ml	
95% Confidence interval	36.81254 - 42.20575 µg protein/ml	
Relative confidence interval	93.43% - 107.12% (13.69%)	

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