

# 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:2)  
Document Type: Quantitative Response Assay/14  
Last Modified: 02.06.2014 12:09:16  
Database: PLA 3.0 Customer Support  
Database GUID: 83d38d90-634b-4dc2-ac0b-15697a1768d2

## Calculation

Calculation performed: 02.06.2014 12:09:16, Matthias Schmitt (PLA 3.0.0 Build 623, nbmsc03, 10014)  
Report generated: 02.06.2014 12:09:31, Matthias Schmitt (PLA 3.0.0 Build 623, nbmsc03, 10014)

## 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

## Signatures

Responsibility

Approval

Review



DOCUMENT-22



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## Overview

### General Properties

Analysis	General
Response Adjustment	None
Response Transformation	Logarithmic
Model	Linear Model
Multiplex Assay	Yes
Potency Estimation Confidence Interval	95.0%
Calculate mean potency estimate of test samples	No
ANOVA Model	ANOVA (Pure Error Separation)
ANOVA with consideration of additional factors	Yes
Logarithm Base	Natural logarithm (base e)
Invert Potency	No

### Documentation

General	
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, 2, 3, 4, 5

Dose Value	0.00100	5.00000E-04	0.00025	0.00013	6.25000E-05
Dose Step	1	2	3	4	5
Response 1	<b>0.514</b>	<b>0.283</b>	<b>0.159</b>	<b>0.093</b>	<b>0.043</b>
Response 2	<b>0.531</b>	<b>0.295</b>	<b>0.154</b>	<b>0.099</b>	<b>0.045</b>
Response 3	<b>0.545</b>	<b>0.362</b>	<b>0.166</b>	<b>0.082</b>	<b>0.051</b>
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, 2, 3, 4, 5

Dose Value	0.00100	5.00000E-04	0.00025	0.00013	6.25000E-05
Dose Step	1	2	3	4	5
Response 1	<b>1.14</b>	<b>0.501</b>	<b>0.327</b>	<b>0.167</b>	<b>0.097</b>
2	<b>1.386</b>	<b>0.665</b>	<b>0.355</b>	<b>0.157</b>	<b>0.097</b>
3	<b>1.051</b>	<b>0.576</b>	<b>0.345</b>	<b>0.178</b>	<b>0.094</b>
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, 2, 3, 4, 5

Dose Value	0.00100	5.00000E-04	0.00025	0.00013	6.25000E-05
Dose Step	1	2	3	4	5
Response 1	<b>0.957</b>	<b>0.586</b>	<b>0.277</b>	<b>0.127</b>	<b>0.086</b>
2	<b>0.866</b>	<b>0.489</b>	<b>0.268</b>	<b>0.146</b>	<b>0.071</b>
3	<b>1.045</b>	<b>0.546</b>	<b>0.269</b>	<b>0.133</b>	<b>0.073</b>
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, 2, 3, 4, 5

Dose Value	0.00100	5.00000E-04	0.00025	0.00013	6.25000E-05
Dose Step	1	2	3	4	5
Response 1	<b>1.037</b>	<b>0.552</b>	<b>0.318</b>	<b>0.145</b>	<b>0.082</b>
2	<b>1.039</b>	<b>0.551</b>	<b>0.306</b>	<b>0.144</b>	<b>0.082</b>
3	<b>1.068</b>	<b>0.624</b>	<b>0.316</b>	<b>0.173</b>	<b>0.086</b>
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		
Preparation	3	4.47522	1.49174		
Regression	1	47.58413	47.58413	7125.84668	1.08443E-46
Non-Parallelism	3	0.01869	0.00623	0.93274	0.43382
Non-Linearity (LoF)	12	0.07423	0.00619	0.92637	0.53078
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	5.71347	0.09172	r <sup>2</sup> 0.99780
T Intercept	6.41770	0.09172	r <sup>2</sup> adjusted 0.99760
U Intercept	6.22609	0.09172	
V Intercept	6.32949	0.09172	
Common Slope	0.90848	0.01076	
Unrestricted Regression			
Parameter	Estimate	Error	Quality of Regression
S Intercept	5.48792	0.17977	r <sup>2</sup> 0.99792
S Slope	0.88128	0.02152	r <sup>2</sup> adjusted 0.99760
T Intercept	6.37808	0.17977	
T Slope	0.90370	0.02152	
U Intercept	6.38676	0.17977	
U Slope	0.92785	0.02152	
V Intercept	6.43400	0.17977	
V Slope	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	2	0	0	0	0
Overall Test Result	Passed				

### Sample Suitability Tests

F-Test (Hypothesis Test): Significance of Non-Linearity S, T, U, V		Passed
Test Severity	Warning	
F <sub>critical</sub> (95.0%) F	2.00346 0.92637	
F-Test (Hypothesis Test): Significance of Non-Parallelism S, T, U, V		Passed
Test Severity	Warning	
F <sub>critical</sub> (95.0%) F	2.83875 0.93274	

## 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%)	
<b>Stock Solution</b>			
Assumed/Assigned 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%)	
<b>Stock Solution</b>			
Assumed/Assigned 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%)	
<b>Stock Solution</b>			
Assumed/Assigned 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%)	

## Graphics

